

**IMPACTS OF *HIPPODAMIA VARIEGATA* (COL.: COCCINELLIDAE)
PREDATION AND *APHIDIUS COLEMANI* (HYM.: BRACONIDAE)
PARASITISM ON *MYZUS PERSICAE NICOTIANAE* (HEM.: APHIDIDAE)**

By

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Declaration

I hereby declare that this thesis is my own original work and has not been submitted for a degree in any other university.

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Date

We as supervisors confirm that the work reported in this thesis was carried out by the candidate under our supervision. The thesis was examined and we approved it for final submission.

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Dedication

To my loving parents and sisters who gave me the support I always needed for the completion of my studies.

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I am sincerely grateful to my supervisor, Dr P. Chinwada, for his guidance and support provided throughout this research. All work which appeared tough was smoothened by his encouragement and ideas to work with the limited available resources which led to the successful completion of this study. I also appreciate the support provided by my co-supervisor, Dr M. Zimba during the course of my research. Lastly, I am grateful to Mr G. Ashely for providing all resources used in my research and to Mr S. Ndoma for his willingness to support me whenever I needed assistance.

Abstract

The tobacco aphid (*Myzus persicae nicotianae*) is a cosmopolitan pest and one of the major pests of tobacco. Due to its damaging potential, particularly as a vector of virus diseases, management of *M. persicae nicotianae* relies heavily on chemical control. However, due to insecticide resistance development and ever-stringent regulations on plant protection products in external markets in the First World, it is now clear that an integrated pest management approach which encourages natural enemy activity is needed. This study evaluated the effectiveness of two of the most commonly found tobacco aphid natural enemies in the country, the ladybird beetle, *Hippodamia variegata* (Coleoptera: Coccinellidae) and the parasitoid, *Aphidius colemani* (Hymenoptera: Braconidae) as biological control agents of the tobacco aphid. The biology of *H. variegata* and its aphid predation rates, *A. colemani* parasitism rate, intraguild predation of the parasitoid by the ladybird, and the effects of some common aphicide sprays on the ladybird were studied. The egg and larval-pupal periods of *H. variegata* were 2.6 and 12-17 days, respectively. *Hippodamia variegata* 1st instar larvae could not survive on tobacco leaves due to impeded movement by sticky exudates from leaf trichomes making them less effective in controlling the tobacco aphid. Adult ladybirds, however, proved to be the most effective. A female ladybird had a daily predation rate of 30.2 aphids compared to the male which consumed 5.5 aphids per day. The adult male + female and adult female + 3rd instar larva combinations were equally capable of suppressing tobacco aphid populations as was the female alone. *Aphidius colemani* developmental period ranged from 12 to 15 days with a mean percent adult emergence of 48.3% and a sex ratio of 1:0.9 (male: female). Adult female lifespan averaged 1.8 days with daily parasitism rate of 10.8 aphids on day 1 and 5 aphids on day 2. Ladybird predation of *A. colemani*-parasitized aphids occurred during the early stages of parasitoid development but significantly decreased with time after parasitization. Aphicides used for tobacco aphid control

differed in their effects on *H. variegata*. Dimethoate 40 EC caused the highest ladybird mortality (100% on both larvae and adults) followed by Acephate 75 SP (52% adult mortality and 22% larval mortality). Acetamiprid applied at 15 g/100 litres did not induce any ladybird mortality. From the findings of this research, it was concluded that *H. variegata* and *A. colemani* are efficient biological control agents of *M. persicae nicotianae*, both individually and in combination and it is recommended that they be conserved in tobacco fields. Thus, selective aphicides like acetamiprid should be used ahead of broad spectrum ones in tobacco production.

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Chapter 1

General Introduction

1.1 Introduction

The tobacco aphid, *Myzus persicae nicotianae* Blackman (Hemiptera: Aphididae) is one of the major insect pests attacking tobacco worldwide (Jagadish *et al.*, 2010). The pest is highly polyphagous and feeds on more than 400 plant species (Vargas *et al.*, 2005). The tobacco aphid inflicts crop damage both directly and indirectly. Direct damage resulting from sucking of plant sap by the aphids manifests as leaf deformation, stunting, poorly developed shoots, dropping of flowers as well as young fruit (Sannino *et al.*, 2000; van Emden and Harrington, 2007). Indirect damage mainly results from viral disease transmission and honeydew secretion on plant leaves. The presence of honeydew encourages black sooty mould growth resulting in poor leaf quality (van Emden and Harrington, 2007). Virus diseases transmitted by the aphid on tobacco include Potato Virus Y (PVY), bushy-top, rosette, tobacco vein mottling virus and vein banding and tobacco etch virus (Akehurst, 1981; Lampert *et al.*, 1988, 1993; Kavallieratos *et al.*, 2004; van Emden and Harrington, 2007; Masukwedza *et al.*, 2013). Recorded yield and economic losses in tobacco production due to injury caused by the tobacco aphid have been as high as 30% (Reed and Semter, 1982; Srigiriraju *et al.*, 2010).

Control of the tobacco aphid has mainly been done using chemical insecticides in most parts of the world. However, the continuous use of these chemicals has resulted in the pest developing resistance particularly to organophosphates and carbamates (Katsarou *et al.*, 2005; Vaneva-Gancheva, 2006; van Emden and Harrington, 2007; Wu *et al.*, 2012). Srigiriraju (2008) reported the red morph of *M. persicae nicotianae* to have developed resistance to more insecticides than any other insect pest species during the early 1990's. Very high resistance to imidacloprid, for

example, has been reported in many tobacco-producing parts of the world including USA, northern and southern Europe. In Zimbabwe, Masukwedza *et al.* (2013) reported possible resistance to monocrotophos and aldicarb. However, these two chemicals have since been de-registered for use in tobacco in Zimbabwe.

The extensive use of chemical insecticides, in addition to insecticide resistance, has many negative impacts on the environment. Non-target organisms can be affected by chemical drifts and accumulation of the chemical in the food chain, which has severe consequences on higher organisms (Dent, 2000; Hoddle, 2003). Pest population resurgence and replacement due to the elimination of beneficial insects can occur as a result of the use of non-selective broad spectrum insecticides (Pedigo and Rice, 2006). This implies that more preference should be given to insecticides selective to the beneficial insects (Stara *et al.*, 2010).

In view of the problems associated with the use of chemical insecticides, Katsarou *et al.* (2005) emphasized the need to harness natural enemies for control of the tobacco aphid. A number of natural enemies attack the tobacco aphid. These include parasitoids of the genus *Aphidius*, *Diaeretiella* and *Lysiphlebus*, predators such as ladybird beetles, syrphid flies, lacewings, and entomopathogenic fungi (Saljoqi, 2009). In Greece, some of the tobacco aphid natural enemies that have been recorded include the ladybird beetle predator, *Hippodamia variegata* Goeze (Coleoptera: Coccinellidae) and the parasitoid *Aphidius colemani* Viereck (Hymenoptera: Braconidae) (Kavallieratos *et al.*, 2004).

Hippodamia variegata is a widely distributed aphidophagous ladybird species common in tobacco, wheat, cotton and horticulture fields (Rebolledo *et al.*, 2009; Rahmani *et al.*, 2013). The coccinellid has been used for the control of different aphid species in cereal and oil plant

production (Rebolledo *et al.*, 2009; Hagh ghadam and Yousefpour, 2012). *Aphidius colemani* is a polyphagous, solitary aphid parasitoid most commonly used as a biological control agent of the cotton aphid, *Aphis gossypii* Glover and the Green Peach Aphid, *Myzus persicae* Sulzer (Perdikis *et al.*, 2004; Garantonakis *et al.*, 2009;). The species is most common in tropical and subtropical regions of the world and is considered an efficient biological control agent in greenhouse crops in different countries where it is produced commercially (Martinou and Wright, 2007).

Due to the status of *M. persicae nicotianae* as a major virus vector, the use of aphicides, either as planting hole systemics (e.g. imidacloprid or thiamethoxam) or foliar sprays (e.g. dimethoate, acephate or acetamiprid) in tobacco production is indispensable in Zimbabwe. Although high levels of ladybird predation and parasitism by *Aphidius* are generally noticeable on tobacco plants towards the end of the season, there are no published reports of any local research to evaluate the impact of these natural enemies. This study investigated the effects of predation by *H. variegata* and parasitism by *A. colemani* on the tobacco aphid as individual natural enemies as well as in combination and how aphicide sprays affect them. Such studies are very important as they provide information which can lead to more effective utilization of aphid natural enemies in agroecosystems where insecticides are currently viewed as the only reliable pest control strategy.

1.2 Justification

Woodend (1995) described tobacco production as very demanding in terms of its chemical requirements. About 60-65% of the total chemicals used in Zimbabwean agriculture are utilized in tobacco production (Woodend, 1995). Heavy reliance on insecticides for controlling the tobacco aphid has led to the development of insecticide resistance and negative environmental

effects. High costs of production imposed by the large requirements of chemicals for tobacco production (Hanyani-Mlambo, 2002) also reduce the profit margin from that which would otherwise be achieved if less of the chemicals were to be used. Given the importance of the tobacco crop, it is necessary to ensure that the highest possible yields are attained but at minimum cost.

Negative health and environmental impacts of chemical insecticides which are a problem of major concern worldwide will be reduced by the adoption of an effective IPM programme in which biological control is a major component. Currently, we are unsure of the extent to which many of the foliar-applied tobacco aphicides impact on the pest suppression potential of *H. variegata* and *Aphidius* spp. Thus, effective utilization of *H. variegata* and *A. colemani* in the control of *M. persicae nicotianae* will lead to an overall reduction in costs of controlling tobacco pests as well as ensure compliance with ever-stringent requirements on maximum pesticide residue limits on the cured tobacco leaf by major external markets, especially the European Union. As *H. variegata* and *Aphidius colemani* also attack *M. persicae nicotianae* on other crop hosts outside the tobacco-growing season, results from this study could also indicate how best to conserve the natural enemies on “overwintering hosts” for later re-introduction into tobacco early in the season.

1.3 Objectives

General objective

To investigate the effectiveness of *H. variegata* and *A. colemani* as biological control agents of *M. persicae nicotianae* coupled with the evaluation of the toxicity of aphicides on tobacco aphid natural enemies.

Specific objectives

- a) To determine the impact of tobacco aphid predation by *H. variegata*.
- b) To determine the impact of tobacco aphid parasitism by *A. colemani*.
- c) To determine the impact of combined *H. variegata* predation and *A. colemani* parasitism on the tobacco aphid.
- d) To assess intraguild predation of *A. colemani* by *H. variegata*.
- e) To determine the toxicity of aphicidal sprays on natural enemies.

1.4 Hypotheses

- a) *Hippodamia variegata* predation significantly reduces tobacco aphid population growth.
- b) There are no significant differences in aphid predation rates by *H. variegata* adults and larvae.
- c) *Aphidius colemani* parasitism significantly reduces tobacco aphid population growth.
- d) Combined aphid predation by *H. variegata* and *A. colemani* parasitism is not significantly different from the effects of either of the natural enemies acting alone.
- e) There is no significant intraguild predation of *A. colemani* by *H. variegata*
- f) Aphicides adversely affect ladybird predation of the tobacco aphid.

Chapter 2

Literature Review

2.1 Tobacco (*Nicotiana tabacum* L.) in Zimbabwe

Tobacco is a solanaceous crop grown in many parts of the world for many different uses which include smoking, chewing pipe, snuffing and for the manufacture of pesticides and medicines. The types of tobacco produced in different parts of the world include Virginia, Burley, Oriental, Amirelo and Local (Ministry of Agriculture, Mechanisation and Irrigation Development, 2010).

Tobacco is an economically important crop for most producing nations in the world which include Zimbabwe, Malawi, China India, and Brazil. The crop is the most widely grown non-food crop worldwide. In Zimbabwe tobacco ranks high among the largest foreign currency earners (Magadlela, 1997; Hanyani-Mlambo, 2002). Keyser (2002) reported an annual contribution of 10% to the country's GDP from tobacco exports as well as the tobacco production sector providing employment for 250000 people. An increase in the tobacco output to 123.5 million kilograms in Zimbabwe in 2010 led to a rise in the country's GDP (Makina, 2010), an indication that the crop is of major importance.

2.2 Economic importance of aphids

Tobacco is attacked by a number of important pests capable of reducing yields drastically if not controlled or if control measures fail. The major tobacco pests include the tobacco aphid (*Myzus persicae nicotianae* Blackman), whitefly (*Bemisia tabaci*), Thrips (*thrips tabaci*), tobacco wireworm (*Conoderus vespertinus*), budworm (*Helicoverpa armigera*), tobacco flea beetle (*Epitrix hirtipennis*) and cutworms (*Agrotis ipsilon*) (Hill and Waller, 1998).

The family Aphididae comprises approximately 4,700 species distributed all over the world. About 100 of these species have attained pest status in many different crops (van Emden and Harrington, 2007). Some of the most agriculturally important aphid species include the green peach aphid, *Myzus persicae* Sulzer, tobacco aphid, *M. persicae nicotianae*, cotton aphid, *Aphis gossypii* Glover., Russian wheat aphid, *Diuraphis noxia* Kurdj., cowpea aphid, *Aphis craccivora* Koch., and the black bean aphid, *Aphis fabae* Scopoli. (van Emden and Harrington, 2007). The feeding behaviour of aphids involves piercing plant surfaces and sucking sap causing mechanical injury and in most cases, virus transmission in the process (Miller and Fottit, 2009).

Myzus persicae nicotianae is one of the most damaging pests of tobacco worldwide. The pest is problematic in tobacco production both in the nursery and in the field (Sannino, 2000; Syed *et al.*, 2005; Srigriraju *et al.*, 2010). Tobacco aphids reduce crop yield and quality through direct and indirect damage. Direct damage manifests as the deformation of plant leaves, leaf chlorosis and necrosis and reduced overall plant growth (Vaneva-Gancheva, 2006). The aphids also secrete honeydew on plant leaves, which attracts infection by fungi such as *Fumago vegans* Pers and *Capnodium citri* Berk and Desm., resulting in the formation of black sooty moulds. The honeydew also attracts ants which in turn defend the aphids from some of their natural enemies (Pedigo and Rice, 2006). Exoskeletons shed by the aphids as they moult remain attached on plant leaves till the crop ripens. These effects interfere with the leaf curing process consequently reducing the market value of the crop.

The tobacco aphid damages crops indirectly by the transmission of viral diseases such as tobacco etch virus (TEV), tobacco vein mottling virus (TVMV), cucumber mosaic virus (CMV), and potato virus Y (PVY). *Myzus persicae nicotianae* is a vector of the viruses causing these diseases in tobacco. Transmission of tobacco mosaic virus (TMV) occurs through wounds the

pest causes on the plant when feeding and moving on the plant surfaces (Lojek and Orlob, 1972; Lampert *et al.*, 1988; Lampert *et al.*, 1993; Woodend, 1995; Vaneva-Gancheva, 2006; Masukwedza *et al.*, 2013). Yield losses ranging from 5 to 30% can occur in tobacco due to the tobacco aphid (Srigiriraju *et al.*, 2010). The tobacco aphid is also a problem pest in peach, pepper, potato and brassicas. Weeds such as *Chenopodium album* L. and *Datura stramonium* L. are other hosts of *M. persicae nicotianae* from which winged adult aphids usually bring the viruses to crops (Lampert *et al.*, 1988; Cabrera-Brant *et al.*, 2010).

2.3 *Myzus persicae nicotianae* taxonomy, identification and biology

2.3.1 Taxonomy

Myzus persicae nicotianae is a subspecies of the green peach aphid, *M. persicae*. The tobacco aphid is adapted and specialized to feeding on tobacco (Vargas *et al.*, 2005). Earlier taxonomic studies by Blackman (1987) concluded the classification of the tobacco aphid as a species separate from *M. persicae*. This was based on morphological features which were found to be different between the two “species”, leading to the naming of the tobacco-adapted form as *Myzus nicotianae* Blackman.

Molecular studies on the classification of the tobacco aphid later revealed that at the species level, the tobacco aphid is similar to the green peach aphid, thus does not qualify to be a separate species (Van Emden and Harrington, 2007). Following this revelation, some scholars like Kavallieratos *et al.* (2005) and Srigiriraju *et al.* (2010) classified the tobacco aphid and the green peach aphid as one species simply named *M. persicae*. Eastop and Blackman (2005), however, renamed the tobacco-adapted form, *Myzus persicae* subspecies *nicotianae* Blackman, owing to the proven morphological differences with the green peach aphid. Vucetic *et al.* (2010) investigated the morphological differences of nine characteristics between *M. persicae*

populations from peach and those from tobacco in Serbia and Montenegro and found out that the two were morphologically distinct. In support of the work by Easton and Blackman, these results were adequate to qualify the tobacco-adapted form as a separate subspecies to be referred to as *M. persicae nicotianae*.

The full taxonomic classification of the tobacco aphid is as follows:

Order:	Hemiptera
Family:	Aphididae
Subfamily:	Aphidinae
Genus:	<i>Myzus</i>
Species:	<i>Myzus persicae</i>
Subspecies:	<i>Myzus persicae nicotianae</i>
Common name:	Tobacco aphid

2.3.2 Identification

Myzus persicae nicotianae is an oval or pear-shaped insect, 1.2-2.6 mm in body length, with red eyes (Tharp *et al.*, 2005; Pedigo and Rice, 2006). There are either green or red coloured morphs of the species (Gooden *et al.*, 2010). Adults can be winged or apterous (Plate 1), depending on the prevailing climatic conditions, population density or plant quality. Negative impacts of these factors result in the production of winged individuals which will then emigrate to colonise plants in areas where conditions are favorable.



Plate 1. *Myzus persicae nicotianae* apterous adult (left) and winged adult (right)

2.3.3 Origin and distribution

The species originated from the east and south-eastern region of Asia. From there it spread to other areas where tobacco is produced worldwide (Kephalogianni *et al.*, 2002).

2.3.4 Biology

The life cycle of the tobacco aphid varies and falls in four different categories which can either be holocyclic, anholocyclic, androcyclic or intermediate. Environmental conditions, particularly temperature and photoperiod, are the most influential in the determination of the type of life cycle as these factors are responsible for initiating the sexual cycle (Margaritopoulos *et al.*, 2000; Kephalogianni *et al.*, 2002; Poupoulidou *et al.*, 2006). The holocyclic life cycle is characterised by alternating cycles of sexual and asexual reproduction. Anholocyclic life cycles are totally parthenogenetic, that is, reproduction is permanently asexual. Mainly parthenogenetic females together with a few males occur in androcyclic aphid populations whereas in intermediate life cycles, there exist only a few males and mating females (Kephalogianni *et al.*, 2002; Poupoulidou *et al.*, 2006; Blackman *et al.*, 2007). Permanent parthenogenesis occurs in warmer climates whereas in temperate regions, sexual reproduction occurs during winter on the

primary host, peach (*Prunus persica* L.), alternating with several asexual generations on secondary hosts (Blackman and Eastop, 1984; Kephalogianni *et al.*, 2002).

Females lay eggs in the sexual cycle, followed by four nymphal instars before reaching the adult stage. Parthenogenetic females, however, are viviparous, that is, they produce live offspring. The females produce already pregnant young which will produce their offspring about a week after birth. This leads to very high rates of population increase and the species can have more than twenty generations in a year when conditions are favourable (Poupoulidou *et al.*, 2006).

2.4 Control of the *M. persicae* complex

Major pest control methods of the aphids of the *M. persicae* complex comprise chemical, cultural and biological control means.

2.4.1 Chemical control

Chemical control is the most commonly used method for controlling the tobacco aphid in tobacco. The application of aphicides becomes necessary when the aphid population in the field reaches an economic threshold of at least 50 aphids on any upper leaf of 5-50 scouted plants (Reed *et al.*, 2012). Some of the predominant aphicides currently in use include dimethoate, acephate, acetamiprid, methamidophos, thiacloprid, pirimicarb, and malathion (van Emden and Harrington, 2007; Masukwedza *et al.*, 2013).

Various problems are, however, associated with the use of chemicals for the control of insect pests. Insecticide resistance is one such a problem resulting from the continuous use of insecticides. Of the tobacco aphid morphs, the red morph appears to be associated with the most widely reported cases of insecticide resistance in many regions of the world where tobacco is grown. The pest developed resistance to aphicides in several chemical groups, e.g. carbamates,

pyrethroids, organophosphates and neonicotinoids. *Myzus persicae nicotianae* developed cross resistance against neonicotinoid aphicides in France, Japan, USA and Greece (Srigiriraju *et al.*, 2010).

Pest resurgence can also result from the application of broad spectrum insecticides. Such insecticides are non-selective. Thus natural enemies of the target pest will get killed along with the pest. When the pest reinfests the crop, its population rises to very high numbers than before insecticide application due to the absence of its natural enemies. Consequently, the pest problem becomes even worse (Norris *et al.*, 2003; Pedigo and Rice, 2006).

Insecticides can also initiate pest replacement in cases when natural enemies of a previously minor (secondary) pest are suppressed such that the pest later gains a key pest status in an agroecosystem. This happens when an insecticide is applied to control the target/ primary pest. The insecticide simultaneously eliminates natural enemies of the secondary pest while mortalities of the pest are low. Populations of the secondary pest will then suddenly rise to levels at which it replaces the primary pest as the key pest of the crop (Dent, 2000; Pedigo and Rice, 2006). A number of insecticides have been found to be harmful to non-target organisms outside the agricultural environment. The accumulation of DDT, for example, up the food chain was found to cause a reduction in populations of raptorial birds. The chemical was found to cause egg shell thinning in these bird species which caused mortalities before hatching. Insecticides in the pyrethroid group, though less harmful to most natural enemies, are poisonous to fish (Dent, 2000).

According to the World Health Organization (WHO), out of around 25 million agricultural workers poisoned by pesticides worldwide per year, 200,000 of them die, with the majority of

these cases taking place in the developing world. These chemicals have a cumulative effect on humans, resulting in symptoms of poisoning appearing later in life (Seen Environmental Learning, 2012).

2.4.2 Cultural control

Cultural control involves the manipulation of the cropping practices from planting right through the crop's growing period so as to disrupt the establishment of pests. Various strategies can be employed to reduce the tobacco aphid populations in tobacco. The planting dates of tobacco can be manipulated to avoid susceptible plant stages coinciding with periods when aphid populations are at their peak during the growing season (Reed *et al.*, 2012). Fertilizer management to reduce the amounts of nitrogen in the soil available for uptake by plants is another recommended aphid control method. High nitrogen increases the manufacture of amino acids in the plant systems which are a favorable food source for the aphids. Aphids feeding on such quality food have been shown to increase reproduction rates coupled with quick generational periods, leading to very high population growth in shorter time periods (van Emden and Harrington, 2007; Reed *et al.*, 2012).

Intercropping tobacco with crops which are not hosts of the tobacco aphid is an efficient cultural control method farmers can adopt. These plants affect the host-finding behavior of the aphids resulting in reduced aphid infestation in tobacco. Trap crops which host the tobacco aphid can be planted near the field to serve as aphid traps, thereby reducing field infestations. Conservation tillage, in which previous crop residues are not destroyed in the field either using reduced tillage or no-till methods, is an effective aphid control measure in tobacco. This avoids the destruction of diapausing stages of aphid natural enemies in previous crop residue (Reed *et al.*, 2012). Topping and sucker control in the field is an essential cultural control measure for

managing the tobacco aphid (Ministry of Agriculture, Mechanisation and Irrigation Development, 2010).

2.4.3 Biological control

Biological control in general involves the utilization of living organisms (natural enemies) to control pests with the aim of reducing pest populations to less damaging levels. More than 1,000 years ago, citrus farmers in China were already using the method for controlling caterpillar larvae using ants in their orchards (Samways, 1981; Markham, 1992; Charlet *et al.*, 2002; Pedigo and Rice, 2006).

The biological control of aphids has been successfully practiced for both field and glasshouse crops in many countries worldwide. Many predatory species of ladybird beetles, hoverfly and predatory bugs are important aphid natural enemies currently in use. *Aphidius* and *Aphelinus* species are the most common parasitoid genera being utilized with great success. Many efficient species of these natural enemies are being produced commercially for use in crop production (van Emden and Harrington, 2007).

Various studies have been conducted, and many are currently in progress to evaluate aphid natural enemy efficiency; hence select and recommend the best biological control agents to be utilized in agriculture (Bilu *et al.*, 2006; Jagadish *et al.*, 2010; Darsouei *et al.*, 2011; Jafari, 2011). Amongst the coccinellids evaluated so far to be effective aphid biocontrol agents are *Hippodamia variegata*, *Coccinella septempunctata*, *Hippodamia convergence*, and *Harmonia axyridis*. In the case of *Aphidius* parasitoids, the common species that have been studied include *A. colemani*, *A. ervi*, and *A. transpicua*, among many other species.

2.4.3.1 *Hippodamia variegata* identification and biology

Identification

Hippodamia variegata is a member of approximately 6,000 species of the family Coccinellidae in the order Coleoptera. The Old World synonym of this species is *Adonia variegata* Goeze and it is commonly named the variegated ladybird beetle. The species is originally from regions in the Palaearctic zone of the world which ranges from Asia to parts of southern Europe and northern Africa. The variegated ladybird beetle is now widespread in most parts of the world (Ellis *et al.*, 1999; Rebolledo *et al.*, 2009; Hesler and Lundgren, 2011; Rahmani *et al.*, 2013).

The adult variegated ladybird beetle has a black head and orange-coloured elytra, each with five to seven black spots. The main distinguishing morphological features between the sexes of this species are the different color patterns on the frontal part of the head and on the pronotum. The vertex of the head of the male is wholly colored yellow whereas on that of the female, black longitudinal marks almost obscure the yellow colour. The male has a black pronotum with two small spots on either side of the line arising from the yellow anterior border of the pronotum. The female pronotum has a colour pattern similar to that of the male but with smaller spots and a less developed medial line. For both sexes, the border of the pronotum is colored yellow/white. The average size of the adult ranges from 4.4 to 5.0 cm in length and the average weight is approximately 4.8 mg (Gordon and Vandenberg, 1991; Hodek *et al.*, 2012; Veesar *et al.*, 2012). The fourth instar campodeiform larva of *H. variegata* has a grayish blue body with a few segments coloured yellow on the dorsal part. Its head is brown and legs are black in color (Gordon and Vandenberg, 1991; Chapman, 1998).

Biology

Hippodamia variegata is a holometabolous insect species, that is, its life cycle undergoes complete metamorphosis (Plate 2). There is a complete change from the egg, larva, pupa to the adult stage (Gullan and Cranston, 1994; Daly *et al.*, 1998; Pedigo and Rice, 2006; Hodek *et al.*, 2012). The female lays oblong, oval and mostly orange-coloured eggs in clusters of 17-25 eggs. These eggs hatch in 2-3 days depending on the prevailing temperature conditions. Hatched larvae remain on egg shells for a period of up to 24 hours during which they feed on the egg shells and infertile eggs, an adaptation which ensures they have adequate energy before they leave in search of food. The larvae feed on available suitable prey and moult three times. The larval period spans 7-10 days after which the larva enters the prepupal stage for 1–2 days. The pupal stage lasts 4–5 days (Pedigo and Rice, 2006).

Adults feed and mate within a few hours after emergence after which the female lays her first egg batch within 0-4 days (Hodek *et al.*, 2012). Eggs are laid close to the food source preferably on the underside of leaves and the average daily fecundity rate is 21 eggs. Adult *H. variegata* mate in aggregation sites and in fields and usually the female is permanently fertile after a single mating but research has shown an increase in the number of eggs with multiple matings (Hodek *et al.*, 2012).



Plate 2. Different life stages of *H. variegata*

Hippodamia variegata is a polyphagous but predominantly aphidophagous predatory coccinellid species. The species also feeds on other insect pests such as whiteflies, lepidopteran larvae, mealybugs, thrips and psyllids (Jafari, 2011; Veesar *et al.*, 2012). The ladybird searches for these insects in a variety of cropping systems particularly in tobacco, vegetables, wheat, cotton and orchards (Rebolledo *et al.*, 2009; Rahmani, 2013). The host searching behavior of *H. variegata* is influenced by a number of factors. As aphids feed, the injured plants release volatiles which in turn attract the ladybirds (Tapia *et al.*, 2010).

The most notable natural enemies of *H. variegata* are birds and parasitoids like *Dinocampus coccinellidae* Shrank (Hymenoptera: Braconidae) which attacks adults. The parasitoid can reduce field populations of the ladybird beetle species by about 30% (Rebolledo, 2009; Hodek *et al.*, 2012). Cannibalism within this species occurs usually where food is in limited supply.

First instar larvae cannibalize eggs, whereas older instars can cannibalize younger ones as well as pupae.

2.4.3.2 *Aphidius colemani* identification and biology

Identification

Aphidius colemani is a parasitoid belonging to the family Braconidae. Within this family there are approximately 50 genera and 400 species (Darsouei *et al.*, 2011). *Aphidius colemani* is a small dark brown wasp of an average size of 2-3 mm in length. Females have slender, 15-segmented slightly pale antennae in relation to body colour. Their legs are yellowish brown to yellow in colour. Males have slightly longer, dark brown to brown antennae, as well as dark brown to brown legs. Females have a sharp pointed tip of the abdomen (ovipositor), whereas the male abdomen tip is round-shaped (Takada, 1997; Garantonakis *et al.*, 2009; Anonymous, 2010).

The species originated from regions in central Asia to the Mediterranean, particularly in the northern Indo-Pakistani area (Coping, 1998; Stary, 1999; van Lenteren, 2012). Currently *A. colemani* is now widespread in most tropical and subtropical regions of the world where the climatic conditions suit its survival (Takada, 1997; Waterhouse, 1998).

Biology and ecology

Aphidius colemani is a solitary koinobiont endoparasitoid (i.e. does not instantly kill its host) (Perdikis *et al.*, 2004; Martinou and Wright, 2007; Darsouei *et al.*, 2011). A female adult *A. colemani* lays an egg in an aphid which hatches in three days into a larva. The first instar larva feeds on aphid haemolymph until it reaches the third and last instar stage at which it feeds on the host tissue. Towards pupation, the larva makes a small hole on the aphid cuticle through which it secretes saliva to stick the already swollen aphid onto the leaf surface killing the aphid in the

process. The larva then spins a cocoon inside the aphid's body and pupates, leading to the swelling and hardening of the cuticle of the aphid. The resultant leathery, bronze-coloured structure is referred to as a 'mummy' (Plate 3).



Plate 3. Aphid 'mummies'

At the end of the pupal stage, the adult emerges through a round hole it cuts on the dorsum of the host's abdomen. The developmental time from egg to adult is temperature-dependent and spans 11-14 days (Copping, 1998; Garantonakis *et al.*, 2009). Aphids parasitized in the first and second instar stages are mummified before reaching the adult stage, whilst those parasitized in the third and fourth instar stages can reach the adult stage and produce offspring before mummification (Perdikis *et al.*, 2004; He and Wang, 2006). Adults mate within a few hours after emergence and the female mates only once whereas the male can mate multiple times during its life (Garantonakis *et al.*, 2009). A female can lay at least 100 eggs during its life time.

Aphidius colemani reproduction is arrhenotokous. Male offspring develop from unfertilized (haploid) eggs and female offspring from fertilized (diploid) eggs. The adult female controls the fertilization of its eggs as well as the type of egg it lays in an aphid. Fertilized eggs are laid in bigger hosts whereas haploid eggs are laid in smaller hosts (Jarosik *et al.*, 2003). On average an

adult can live for a period of 2-3 weeks under favorable conditions. This, however, is rarely possible as under field conditions adult longevity is only 2-3 days (van Emden and Harrington, 2007).

Aphidius colemani is a polyphagous parasitoid which parasitizes 65 aphid species. Among these species are economically important aphids including *A. gossypii*, *M. persicae*, *M. persicae nicotianae* and *D. noxia* (Sampaio *et al.*, 2008). Adults survive by feeding on plant nectar and pollen, as well as honeydew secreted by aphids (Copping, 1998).

Host searching behaviour of the parasitoid is influenced by a number of factors. Aphid sex pheromones act as kairomones which serve to attract the parasitoids. Volatiles released by plants through tissue damaged by the aphids as they feed also attract the parasitoids towards the aphids. Curled and discolored plant leaves indicate the presence of aphids and this serves to attract the parasitoids. The honeydew secreted by aphids also serves as kairomones for the parasitoids (Markham, 1992; Rehman and Powell, 2010). According to a study by Lo Pinto *et al.* (2003), *A. colemani* adult females are attracted to host plants of their prey by chemical stimuli and attraction is more to aphid-infested plants than uninfested ones. *Aphidius colemani* is attacked by hyperparasitoids especially members of the genera *Dendrocercus* and *Alloxysta* (Sullivan and Skinner, 2012).

2.4.3.3 Current use of *H. variegata* and *A. colemani* in biological control

Hippodamia variegata was found to exhibit a Type 2 functional response in its feeding on *A. gossypii* and *Acyrtosiphon pisum*, with the female adult and 4th instar larva having the most significant impact in controlling the aphids (Madadi *et al.*, 2011). An almost similar study by Dehkordi *et al.* (2012) confirmed *H. variegata* to be a successful biocontrol agent of *A. gossypii*,

with a Type 2 functional response. The Type 2 functional response has also been exhibited by many important coccinellid species utilized in biological control, like *Coccinella undecimpunctata*, *Cheilomenes sexmaxulata* and *Coccinella transversalis* (Dehkordi *et al.*, 2012). *Hippodamia variegata* was noted amongst the coccinellid species feeding on aphids in maize, beans and cowpeas intercrops in Kenya's Busia district (Nyukuri *et al.*, 2012).

Aphidius colemani is a commonly used aphid biocontrol agent and is under mass production by many different companies in European and North American countries for use in the biological control of many different aphid species (Fernandez and Netwig, 1997). Kavallieratos *et al.* (2005) found *A. colemani* amongst the spectrum of parasitoid species parasitizing aphids in tobacco in southeastern Europe. Stary (1999) also noted the importance of the parasitoid species in controlling the Russian Wheat Aphid in wheat fields. An efficiency measure of 5-65% by *A. colemani* was observed, providing desirable control of the damson-hop aphid in hop before the flowering stage (Solarska, 2004).

The behaviour of *A. colemani* is not altered by the presence of intraguild predators. The continuous presence of the parasitoid species was observed on aphid-infested plants even after encounter with ladybird larvae (Bilu *et al.*, 2006). This shows that the parasitoid species can be a useful biocontrol agent in combinations with other natural enemies of aphids.

Chapter 3

General Materials and Methods

3.1 Site

This research was conducted in the laboratory at the Biological Sciences Department (University of Zimbabwe) from October 2012 to May 2013. The average temperature and relative humidity conditions which prevailed over the period were $25 \pm 0.1^{\circ}\text{C}$ and $74 \pm 1\% \text{ RH}$.

3.2 Tobacco

Tobacco variety KRK26 from the Tobacco Research Board was used in the study. Seedlings were raised on vermiculite in asbestos trays (30 cm wide x 40 cm long) in the greenhouse. The growth media (soil and vermiculite) used for raising seedlings was sterilized in a steamer before use. The seed sowing rate chosen was to achieve the recommended rate of 3 to 6 g seed/10 m². Compound C fertilizer (6:15:12, N: P₂O₅:K₂O, 9% S) was applied proportionally to achieve the recommended rate of 1 kg/36 m². Pots were watered regularly using a perforated watering can. Germination occurred within a period of about 10 days and at two weeks after germination, seedlings were transplanted singly into soil pre-mixed with compound C fertilizer applied at 19 g per pot. Transplanting was done in 10 cm deep x 7 cm diameter plastic pots.

Plants were top-dressed with ammonium nitrate at the rate of 7.5 g per pot in split applications within a seven day period. These potted plants were used for the experiments after 2-3 weeks when they had reached an average height of 15–20 cm. As these potted plants grew, they were trained to fit in the experimental cages by cutting part of the leaves growing laterally to ensure that they grew vertically. Besides tobacco, Chinese mustard was also raised in the glasshouse using the same procedure as for tobacco. The mustard was used for aphid rearing.

Some tobacco seedlings were transplanted into 40 cm deep x 40 cm diameter asbestos pots and placed in a glasshouse. These plants were used to maintain a continuous supply of tobacco aphid populations for use in the study. The plants were also a source of leaf discs used for Petri dish aphid predation experiments.

3.3 Insects

Founder colonies of wingless (apterous) tobacco aphids, *H. variegata* and *A. colemani* (from aphid ‘mummies’) were collected from the Tobacco Research Board, Kutsaga Research Station.

Myzus persicae nicotianae

Aphid populations were maintained on older tobacco plants in the glasshouse. Additional aphids were reared in the laboratory on 20-30 cm long tobacco leaves that had the petioles dipped in water in 500 ml Consol jars. These jars were placed in insect rearing cages. Fifty to one hundred adult aphids were placed on the leaves and left to produce progeny for at most 24 hours. Newly-born nymphs were transferred to fresh leaves using a fine paint brush. The progeny could survive on the leaves for up to four days, a period during which they were mature enough to be used in the experiments.

For standardization of aphids for use in the experiments, further rearing was done in the laboratory. Nymphs were collected from tobacco plants being maintained in the greenhouse and placed on potted plants secured inside wooden-framed cages (50 cm high x 50 cm wide x 100 cm long). The cages were covered on all sides with fine plain white fabric (Plate 4). Similar but smaller cages (50 cm high x 50 cm wide 50 cm long) were used for isolation of emerged adult *H. variegata* (Plate 5).



Plate 4. Aphid rearing cage



Plate 5. Isolation cage for ladybird beetles

Hippodamia variegata

Hippodamia variegata females were placed each in an experimental Petri dish and supplied with aphids on leaf discs *ad lib* daily. Leaves were checked every 24 hours for oviposition, and eggs laid on the leaf discs were transferred to other Petri dishes. In case ladybirds laid eggs on the Petri dish rather than leaf surface, the female was transferred to another dish leaving the eggs to hatch. Hatched larvae were transferred to Consol jars and supplied with free aphids every 24 hours. This was done following the realization, in pre-trial experiments, that the first instar

larvae were unable to move on tobacco leaf discs resulting in their failure to feed and consequently starving to death. Twenty larvae were kept in each Consol jar so to reduce cases of cannibalism. When the larvae reached the late second instar stage, aphids were supplied on tobacco leaf discs as at this stage they could walk on the discs and feed provided the discs had abundant numbers of aphids. Continuous feeding was ensured daily by supplying aphid-infested leaf discs until all larvae reached the pupal stage. At this stage, the pupae and the leaves to which they were affixed were placed in isolation cages for adult emergence.

Pupae were introduced into each isolation cage for a period of up to three weeks to ensure that adults in each cage were not more than 3 weeks old. Emerging adults were supplied with aphids on 20–25 cm potted tobacco plants and leaf discs as required. Supplementary feeding was provided in the form of 50% honey solution on a soaked piece of cotton wool in a Petri dish. The honey was replaced with fresh one every 3-4 days. Each cage could host up to 200 adult ladybirds.

Aphidius colemani

Aphidius colemani populations were raised in the same type of wooden framed cages as those used for the aphids. The cages were covered with fine plain fabric on all sides to prevent parasitoids escape. A 2 mm x 2 mm leaf disc was cut out around each mummy using a sharp razor blade and 10–20 of the mummies were placed in a Petri dish for adult emergence. Food for the emerging adults was supplied as 20% honey solution on soaked pieces of cotton wool placed inside the Petri dishes. Emerging adults were sexed and isolated in pairs in the Petri dishes for 2–3 hours for mating after which females were introduced into cages containing aphid-infested tobacco leaves. The aphids used were 2-3 days old. For each mated female *Aphidius*, 20 aphids were provided for parasitization. Drops of 20% honey solution were supplied as food for the

parasitoids on the aphid infested leaves. After 2-3 days the aphids were transferred to fresh leaves until they were mummified.

3.4. Experimental materials

Experimental cages

Custom-made PVC cages (25 cm length x 12 cm diameter) with four adjacent windows (10 cm long), two above and two below in opposite sides were used in this research. The cages were covered with transparent fabric. The top and bottom parts of the cages were each covered with a 15 cm long x 15 cm wide piece of netting material which was held in place by rubber bands (Plate 6).



Plate 6. Typical experimental cage

Experimental Petri dishes

Petri dishes were used for egg laying, *H. variegata* predation experiments as well as for rearing *A. colemani*. In order to permit ventilation, a 4.5 cm diameter hole was cut out of the top lid and a circular piece of fabric stuck over it (Plate 7).

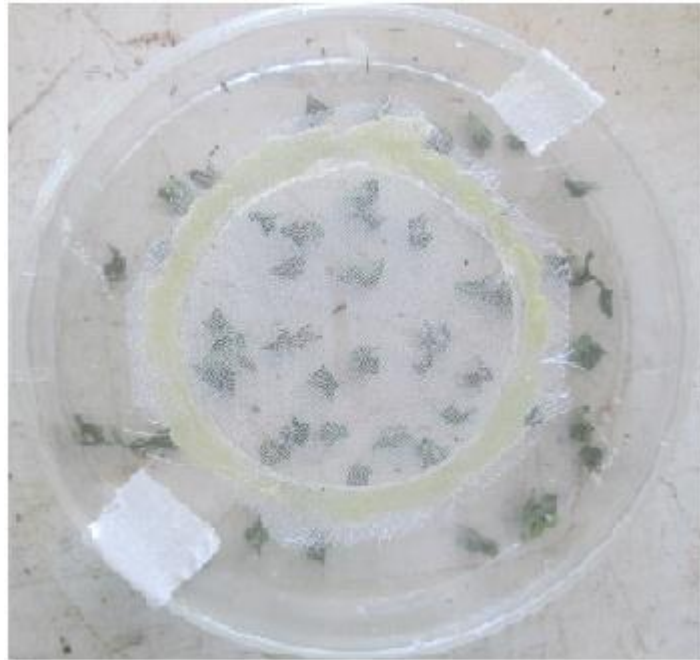


Plate 7. Experimental Petri dish

Consol jars

Glass jars (500 ml) were used for raising *H. variegata* larvae and experiments for studying the developmental time of the coccinellid. The open top of the jar was closed off using a piece of plain fabric so as to allow aeration.

Chapter 4

Biology and Effect of *H. variegata* Predation on *M. persicae nicotianae*

4.1 Introduction

Ladybird beetles have for long been the most utilized biological control agents of aphids. Many species have been evaluated and found to be effective in reducing populations of economically important aphids in a variety of crops, such as cereals, potato and orchards (van Emden and Harrington, 2007). *Hippodamia variegata* was found to be present in the spectrum of coccinellid predators in tobacco fields in central Chile (Fuentes-Contreras *et al.*, 2004). Kavallieratos *et al.* (2004) also reported the abundance of *H. variegata* in tobacco fields in central Greece. These studies serve to support the fact that the coccinellid is a notable predator of the tobacco aphid. In some states in the USA, *H. variegata* was introduced as a biological control agent of the Russian wheat aphid (Hesler *et al.*, 2011).

The efficiency of a predator, in addition to its biology and abundance, are key attributes which should be considered before it is certified an effective biological control agent (Jafari and Goldasteh, 2009). Its predation rate, fecundity rate and host preference determine its efficiency in biological control (Farhadi *et al.*, 2011). An effective biological control agent should have a desirable rate of reproduction and the ability to synchronize its life cycle to that of its prey. The species must also have the ability to adapt to different environmental conditions as well as good host-searching capacity (Veesar *et al.*, 2012).

Various factors affect the performance of coccinellids as aphid biocontrol agents. Plant morphology is one such a factor. Plants with dense trichomes generally reduce the predation of aphids by ladybird beetles as these features can impede movement on the plant surfaces, though this varies with coccinellid species. On the other hand, natural enemy activity can be reduced on

waxy plant surfaces (Markham *et al.*, 1992; Obrycki and Kring, 1998; Van Driesche *et al.*, 2008).

Research focusing on investigating the impact of *H. variegata* as a biological control agent of the tobacco aphid is currently lacking in literature though the species has been found to exist in tobacco fields. Thus it was found necessary to conduct this research using the predator as it is commonly distributed in the tobacco growing regions of the country. The objectives of this study sought to understand the biology of *H. variegata* and to determine its predation rate and effectiveness in suppressing *M. persicae nicotianae* population growth.

4.2 Materials and methods

4.2.1 *Hippodamia variegata* biology

Twenty five mated female adults (3 day-old) were individually introduced into a Petri dish and supplied with aphid-infested leaf discs. The adult female was transferred to an isolation cage once it had laid a batch of eggs within 24 hours. The number of eggs in each batch was recorded. Petri dishes were checked every 24 hours for hatched eggs. For each egg batch, the date of oviposition and hatching and the number of larvae produced were recorded.

Fifteen newly hatched larvae from the same egg batch were then introduced into a Consol jar. The larvae were supplied with free aphids to allow them to reach the late second instar stage. Thereafter, larvae were offered aphids on leaf discs *ad lib* daily until they reached the pupal stage. The number of days from hatching to adult emergence was recorded as well as the total number and sex of the emerged adults. The experiment was replicated 10 times.

4.2.2 *Hippodamia variegata* aphid predation rate

Larval predation rate

After realizing that *H. variegata* larvae had walking difficulties on tobacco leaves, it was deemed necessary to conduct this experiment separately both on tobacco and on a crop with different morphological characteristics particularly on the leaf surface. Chinese mustard was selected for the experiment. In both experiments, tobacco or mustard leaves were cut out into discs to fit into the experimental Petri dishes. All discs were cut along the leaf petiole on which a cotton piece soaked in water was tied to slow down drying. Fifty aphid nymphs (3–4 day old) were placed on a leaf disc inside a Petri dish using a fine paint brush and left to settle for about one hour. A ladybird larva (less than 24 hours old) was then transferred to each Petri dish and left to feed for 24 hours. Twenty five replicates were set up.

The number of aphids consumed in 24 hours was determined by counting the number left in the Petri dish and subtracting from the original number supplied. After counting, the aphids and leaf disc were removed and replaced with a fresh disc that had 50 new aphid nymphs. This procedure was repeated daily until pupation or death of the larva.

Adult predation rate

Newly-emerged but less than 24 hour old adults were placed in pairs (male and female) in a Petri dish and supplied with aphid-infested tobacco discs and left to mate for three days. Mated adults were then separated and placed individually in the Petri dishes. The same procedure used for the larva was followed with 25 replicates each for the male and female ladybird adults. The male was supplied with 80 aphid nymphs whereas the female was supplied with 110 nymphs every 24 hours. This was done for a period of 14 days during which time the number of eggs

laid by each female was recorded every 24 hours. Daily aphid predation rates by the male or female ladybird adult were also recorded.

4.2.3 Effect of *H. variegata* predation on *M. persicae nicotianae* population growth

A Randomized Complete Block Design (RCBD) was used for the experiment to determine the effect of *H. variegata* predation on tobacco aphid population growth. The design had five treatments replicated in time 20 times. The treatments were as follows:

- 1) 3rd instar *H. variegata* larva (x 1),
- 2) Adult female *H. variegata* (x 1),
- 3) 3rd instar *H. variegata* larva (x 1) + adult female *H. variegata* (x 1),
- 4) Adult male *H. variegata* (x 1) + adult female *H. variegata* (x 1),
- 5) Control (aphids only).

Randomization of the treatments and blocks was done using Microsoft Excel. A potted tobacco plant infested with 200 four-day old aphids was placed in an experimental cage in each treatment. Infestation was done by first introducing 35-40 adult aphids on each plant and then leaving them to reproduce for a period of 18-24 hours. By this time there were about 200-220 nymphs on each plant. The adult aphids were then removed from the plants leaving their offspring to develop for another four days. Excess aphids were then removed from the plants leaving each plant with exactly 200 aphids. This was followed by introducing the natural enemy into the experimental cages as required in each treatment. The experiment was left to run for seven days during which plants were watered as and when required using.

4.2.4 Data analysis

Data that was recorded for *H. variegata* biology determination included: (i) number of eggs/batch, (ii) egg period, (iii) number of progeny larvae/ egg batch, (iv) percent eggs hatched, (v) larval-pupal period, (vi) number of emerged adult progeny, (vii) percent adult emergence, (viii) percent female adults, (ix) percent male adults, (x) larval and adult predation rates, and (xi) adult female fecundity. These were subjected to statistical analyses to find means of the variables of concern. A one-way analysis of variance (GenStat 14.1 statistical package) was used to determine the effects of *H. variegata* predation on *M. persicae nicotianae* population growth. Where the *F*-ratio was significant, treatment means were separated using the Tukey-Kramer HSD test.

4.3 Results

4.3.1 *Hippodamia variegata* biology

Ladybird eggs took 2.6 days to hatch (range 1-4 days) (Table 1). The mean number of eggs per egg batch was 18.6 (range 10-27 eggs). The average number of hatchlings per eggs batch was 13.6 (range 6-27) giving a hatching percentage of 73.2%. The larval-pupal period, that is, from hatching to adult emergence, ranged from 12 to 17 days with a mean of 13.8 days. An average of 12.9 adults emerged (86% emergence rate) of which 52.2% were females.

4.3.2 *Hippodamia variegata* predation rate on aphids and daily female fecundity

Hippodamia variegata larva could only consume an average of 0.045 aphids on tobacco per individual. These were consumed during the first instar stage. The larvae were unable to move on tobacco leaf surfaces due to the sticky exudates that the leaf trichomes produce resulting in the death of all larvae in the 25 replicates. On mustard, the larva could survive, and each larva consumed an average of 12.1 aphids per day. The daily aphid predation rate for the larva on

mustard increased daily with age up to a maximum of 33 aphids on day 6 in the 4th instar stage after which the number drastically dropped to 0 as the larva reached the prepupal stage on day 8 (Fig 1).

Table 1. *Hippodamia variegata* biological parameters

Variable	n	Mean $\pm$ SE	Range
Number of eggs/batch	25	18.6 $\pm$ 1.0	10 – 27
Egg period (days)	466	2.6 $\pm$ 0.1	1 – 4
Number of progeny larvae/ egg batch	466	13.6 $\pm$ 1.0	6 – 27
% eggs hatched	466	73.2 $\pm$ 3.2	32 – 100
Larval-pupal period (days)	150	13.8 $\pm$ 0.1	12 – 17
Number of emerged adult progeny	150	12.9 $\pm$ 0.4	11 – 15
% adult emergence	150	86.0 $\pm$ 2.7	73 – 100
% female adults	150	52.2 $\pm$ 4.3	31 – 77

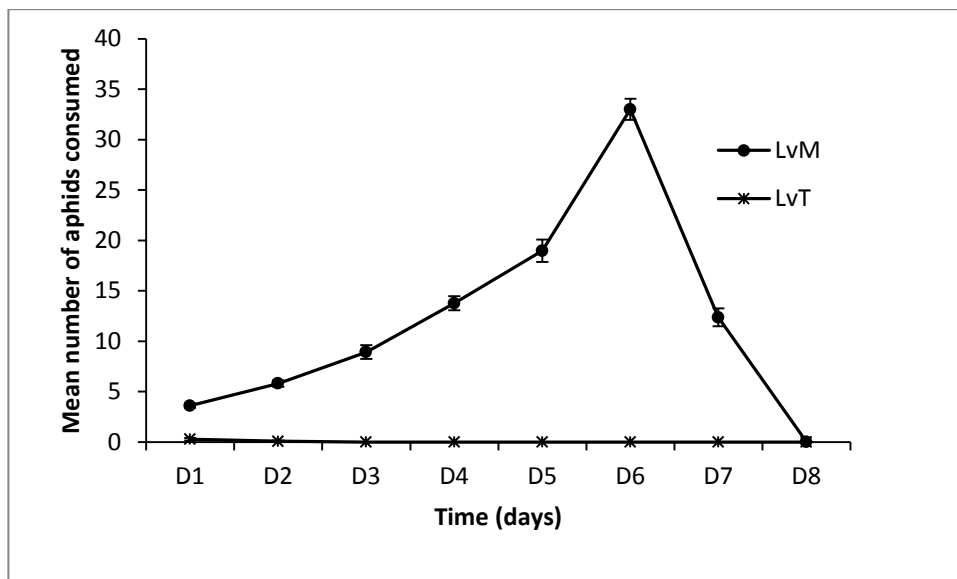


Figure 1. Tobacco aphid consumption rate by *H. variegata* larvae in tobacco (LvT) and mustard (LvM)

Adult males preyed on an average of 5.5 aphids per day per individual while the female adult daily consumption rate was 30.2 aphids per individual (Fig 2). These females produced on average 13.3 eggs per individual per day over the same period. The daily predation rate was significantly higher for female adults than males over the 14 day period. There were relatively

few significant differences in female predation rate on each of the days. The female consumed significantly higher numbers of aphids on each of the first 13 days than on the 14th day whereas on days 1 and 2, the consumption rates were significantly lower than on days 5 and 7. The daily fecundity rate was significantly low on days 1 and 2 than on the rest of the days. On day 1, the male consumed a significantly higher number of aphids than during the days which followed.

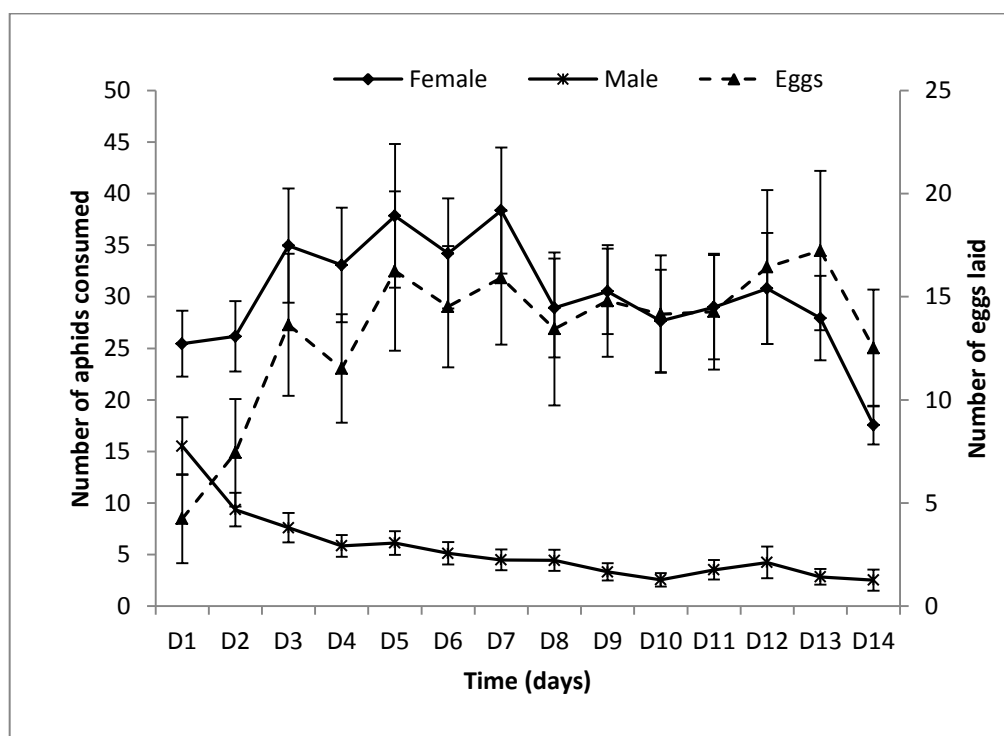


Figure 2. Daily aphid consumption rate by adult male and female *H. variegata* and the corresponding fecundity rate of the female

4.3.3 The effect of *H. variegata* predation on aphid population growth

Aphid population depression was highest in the adult male + female combination (15.4 aphids left) though this was not significantly different from that by 3rd instar larva + adult female combination (19.0 aphids left) and the adult female alone (34.8 aphids left) (Fig 3). Where the 3rd instar larva was used alone, the final aphid population was 109.8 aphids. This was significantly higher ($F_{4,19} = 101.14$, $P < 0.001$) than in the former treatments (adult male +

female and adult female alone). The control (336.2 aphids left) had significantly higher number of aphids than the rest of the treatments.

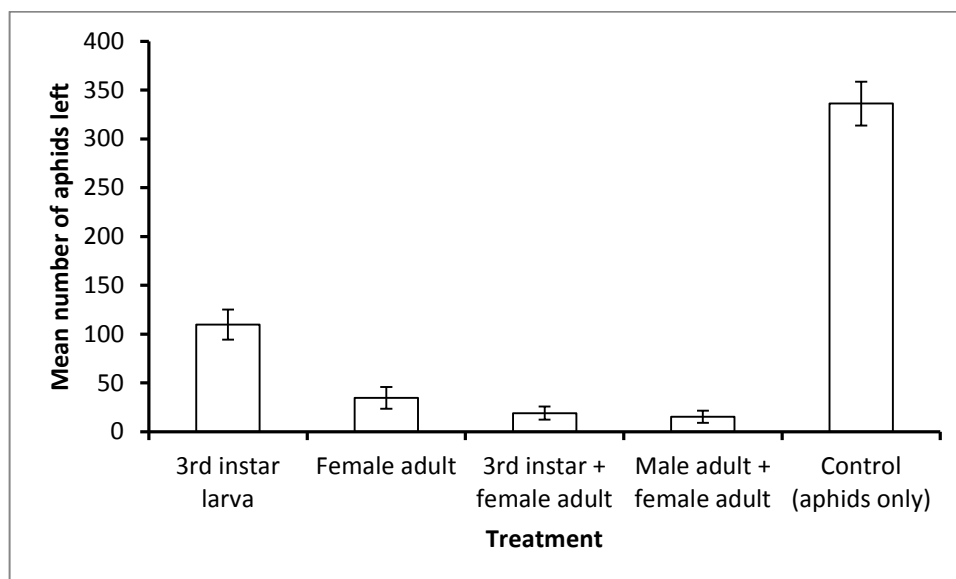


Figure 3. Impact of *H. variegata* predation on tobacco aphid population growth

4.4 Discussion

This study investigated the biology of *H. variegata*, its daily predation rate on *M. persicae nicotianae* and the ability of the predator to control aphid population growth. The *H. variegata* egg incubation period of 2.6 days (range 1-4 days) was consistent with research by Farhadi *et al.* (2011) who found out the average incubation period to be 3.03 days. The slight difference may be attributed to the environmental conditions under which the studies were conducted. Unlike the current study which was conducted at room temperature conditions, the study by Farhadi *et al.* (2011) was conducted under controlled conditions ($35\pm1^{\circ}\text{C}$ and $70\pm10\%$ RH). Temperatures are likely to have been higher at the time the current experiment was done than those used in their research. The developmental rate of coccinellids has been shown to increase with rising temperature thereby shortening the developmental periods of the insect species.

The host type is another probable contributing factor to the different egg developmental periods. Many coccinellids have different preferences for different aphid species, with a resultant difference in their biology in response to the prey type they will be consuming (Hagh ghadam and Yousefpour, 2012). Since the studies by the above mentioned researchers were done using the bean aphid (*Aphis fabae*), differences in the biology of the predator were expected as the current study used *M. persicae nicotianae* as the prey. The mean number of eggs per batch laid by a female *H. variegata* was within the range of 17 to 25 eggs recorded in many other studies conducted on the same species (e.g. Jafari, 2011). This indicates that *M. persicae nicotianae* is a preferable species for *H. variegata* and thus is a biological control agent worth conserving.

Results of the present study also demonstrate that *H. variegata* can, under natural environmental conditions, have high population growth capable of reducing tobacco aphid populations below damaging levels. The relatively shorter developmental time of 12-17 days coupled with relatively high fecundity as well as hatching and percent adult emergence show a possibility of a high rate of population increase for *H. variegata*, with more generations per season. This is indicative of an efficient biological control agent. The relatively higher percentage of females than males is also an essential attribute of an efficient biological control agent as there will be more females available to produce offspring for the population to rise faster in relation to aphid population increase.

Aphid predation by early instar *H. variegata* larvae on tobacco was shown to be nearly impossible in the current study. The inability of the first instar larvae to walk on tobacco plant leaf surfaces resulting in death of the insects due to starvation shows that the immature stage cannot be utilized as a biological control agent in tobacco especially for the older crop in the field on which plant leaf stickiness is high. However, in the nursery or seedbeds where leaves of

young plants are still smooth and 1st instar larvae are able to walk, the immature stage of *H. variegata* can be utilized with success. Though movement on older plants by late 2nd, 3rd and 4th instar larvae is possible despite some challenges, it will be a difficult and costly procedure to introduce them into the field. The adult *H. variegata*'s ability to move freely on tobacco plants implies it can be effective in controlling the tobacco aphid. The female's daily consumption rate of 30.2 aphids shows that the predator can consume a large number of prey individuals in its life time. Jafari (2011) found out that on average *H. variegata* adults can live for 55.5 days implies that a female can potentially consume at least 1,600 aphids in its life time. The ability of larvae to survive on mustard shows the possibility of utilizing brassicas for biological control of tobacco aphids with *H. variegata* in tobacco. This allows female adults to oviposit on these plants where they can survive to reach the adult stage. The crops can be intercropped with tobacco or grown next to tobacco fields.

Hippodamia variegata adults' ability to reduce aphid population growth shows that the predator is highly efficient in controlling the tobacco aphid. Though not significantly different from the female alone and female + male treatment effect, the female + male combination had relatively higher control rate for the tobacco aphid. As in nature the sexes exist together in cropping systems, this explains there is no interference to feeding caused by continuous mating but rather the combination of the sexes is additive in biological control. However, the female alone as well as the female + 3rd instar larva combination were observed to be equally effective in controlling the tobacco aphid. The 3rd instar larva also could reduce the aphid populations despite having a significantly lower rate of predation compared to the adult. The significantly lower number of aphids in the treatment than in the control supports the argument that the larva can be utilized as a biocontrol agent of the tobacco aphid.

In conclusion, results of this study on the control of the tobacco aphid by *H. variegata* show that the coccinellid is an efficient biocontrol agent of the aphid and can be deliberately harnessed for use in tobacco production. Females are generally more effective than males owing to their higher predation rates. The combination of both sexes is even more effective. The adult + larva combination can be useful in nurseries when plants are still young. Augmentative releases of *H. variegata* for short-term biological control should have more female-biased populations in order to achieve successful tobacco aphid control as the female was proven to be the most effective aphid suppression factor.

Chapter 5

The Effect of *A. colemani* Parasitism on *M. persicae nicotianae*

5.1 Introduction

Aphidius colemani has been reported to have a high preference for the green peach aphid, *M. persicae* though *A. gossypii* (cotton aphid) has been proven the most highly suitable (Perdikis *et al.*, 2004). The presence of *A. colemani* in tobacco fields shows its preference for *M. persicae nicotianae*, a subspecies of the green peach aphid, indicating the parasitoid's high preference for the aphid as a host (Kavallieratos *et al.*, 2004).

Aphidius colemani has also been shown to be a very efficient biological control agent against *M. persicae nicotianae*, and is an effective searcher, with the ability of parasitism in sparsely populated aphid populations (Copping, 1998). *Aphidius colemani* is widely used for controlling aphids in glasshouse crops. Its host instar preference for parasitism, that is, the first and second instars, is a good characteristic for biological control agents. Aphids parasitized during the early instar stages are mummified before reaching the adult stage. In contrast, those parasitized in the third and fourth instar and adult stages are able to produce offspring before mummification. Thus, as numbers of young aphids are usually higher than those of adults, these are killed before they produce any progeny making the parasitoid highly effective as a biocontrol agent (Perdikis *et al.*, 2004). *Aphidius colemani* preferred second and third instar tobacco aphids on pepper and aubergine in a study by Martinou and Wright (2007).

Aphidius colemani has also been introduced for control of aphids in field crops (Stary, 2002). Vasquez *et al.* (2006) found that *A. colemani* can effectively control *A. gossypii* in chrysanthemums producing a product with acceptable aesthetic standards in the same way chemical control does. In addition to *A. colemani*, a number of other parasitoid species exist

within the natural complex of aphids in tobacco. These include *Aphidius ervi*, *A. matricariae* and *Praon staryi* (Kavallieratos *et al.*, 2004). *Aphidius colemani* is regarded the best control agent for *M. persicae* ahead of other parasitoids like *Aphelinus abdominalis*, *Ephedrus cerasicola* and *Praon volucre* (Viridaxis, 2012). The objectives of this study were to investigate the biology, daily parasitism rate and the effect of parasitism by *A. colemani* on the population growth of *M. persicae nicotianae*.

5.2 Materials and methods

5.2.1 *Aphidius colemani* biology

A potted tobacco plant infested with fifty, 2-3 day old non-parasitized aphids was placed in an experimental cage. A mated adult *A. colemani* female (< 24 hour-old) was then introduced into the cage in order to parasitize the aphids. After 24 hours, the parasitoid was removed and the plant with aphids kept isolated in the cage. The same procedure was repeated with 15 different mated adult female parasitoids to ensure that a total of at least 240 aphid mummies would be formed. Mummies were removed by cutting a leaf disc holding the mummy and isolating a total of 30 in each experimental Petri dish. The experiment was replicated eight times, each replicate constituting an experimental Petri dish with 30 mummies. The mummies were observed for adult emergence every 24 hours. Emerged adults were removed individually using an aspirator and placed in a 2.5 cm diameter x 8 cm glass vial. The adults were then sexed under a binocular microscope. The day of emergence and sex of the adults were recorded.

To determine the daily parasitism rate and *A. colemani* adult female longevity, an adult female parasitoid, less than 24 hour old, was isolated in a 2.5 cm diameter x 8 cm glass vial with an adult male of the same age and allowed a six hour period for mating. Food for the parasitoids was provided in the form of 2–3 drops of 20% honey solution on a piece of cotton wool. The

female parasitoid was then introduced into an experimental cage in which a potted tobacco plant previously infested with fifty, 2 day-old tobacco aphids had been placed. A piece of cotton wool soaked in 20% honey solution was placed in the experimental cage to ensure an adequate supply of food for the parasitoid. After 24 hours, the plant was removed from the cage, taking great care to avoid escape by the female parasitoid. The plant was labeled and isolated in a different experimental cage. A fresh plant infested with the same number and age of aphids was placed in the cage. This procedure was repeated until the female parasitoid was dead. The experiment was replicated 25 times.

The plants were kept in isolation and watered as necessary. The number of days the female parasitoid lived was recorded. The daily parasitism rate was determined by counting the number of mummies formed on each plant after a period of 10 days from the day of aphid exposure to the parasitoid.

5.2.2 The Effect of *A. colemani* parasitism on *M. persicae nicotianae* populations

To determine the effect of *A. colemani* parasitism on the tobacco aphid populations, the experiment was laid out as an RCBD with four treatments each replicated 20 times. The treatments were as follows:

- 1) 1 x mated female *A. colemani*,
- 2) 2 x mated female *A. colemani*,
- 3) 4 x mated female *A. colemani*, and
- 4) Control (200 aphids only).

Randomization of the treatments and blocks was done using Microsoft Excel. The infestation procedure involved introducing 35–40 adult aphids on each plant, followed by isolation in an

experimental cage for 18-24 hours. After this period, the adult aphids were removed leaving their offspring, which by that time numbered on average 200-220 aphids. The aphids were left to feed undisturbed in the cages for 2-3 days. The aphids were then counted and excess aphids removed from the plants to ensure each plant was left with exactly 200 aphids

Adult parasitoids, less than 24 hour old, emerging from individually-isolated mummies in glass vials were sexed and paired male and female in vials for at least six hours to mate. About 2-3 drops of 20% honey solution on a piece of cotton wool was placed in the vial to provide food for the parasitoids. After mating, male adults were removed from the vials and the females introduced into each experimental cage in accordance with the treatment. A piece of cotton wool soaked in 20% honey solution was placed in each cage to provide food for the adult parasitoid. Plants were watered as necessary for 10 days after which the number of aphid mummies was recorded.

The effectiveness of the parasitoid was evaluated using killing power values (k -values):

$$k = -\ln(\text{survival})$$

Where survival is the proportion of hosts surviving a mortality factor, which in this case is parasitism.

$$\text{Survival} = 1 - \frac{\text{number of hosts parasitised}}{\text{initial number of hosts}}$$

5.2.4 Data analysis

The statistical analysis of data was performed using the GenStat 14.1 statistical package. Data for *A. colemani* biology, that is, the egg-adult period, number and percent adult emergence, female adult longevity and daily parasitism rates were subjected to statistical analysis to find

means of the variables of concern. A one-way analysis of variance was used to determine the effects of *A. colemani* parasitism on *M. persicae nicotianae* population growth (i.e. *k*-values). Where the *F*-ratio was significant, treatment means were separated using the Tukey-Kramer HSD test.

5.3 Results

5.3.1 *Aphidius colemani* biology

A total of 116 adult parasitoids with a male : female ratio of 1:0.9 emerged over the eight replicates. The egg-adult developmental period was 13.0 days (range 12-15 days) (Table 2). From the resultant mummies obtained over the eight replicates (240 in total), percent total adult emergence was 48.3% (range 6.7–96.7%). On average, a female adult lived for a period of 1.8 days (range 1-3 days).

Table 2. Percent adult emergence, egg-adult developmental period and female adult longevity of *Aphidius colemani*

Variable	N	Mean $\pm$ SE	Range
Male: Female sex ratio‡	116	1:0.9	-
Egg-adult period (days)‡	116	13.0 $\pm$ 0.1	12 – 15
% adult emergence	240	48.3 $\pm$ 12.1	6.7 – 96.7
Female adult longevity	25	1.8 $\pm$ 0.1	1 – 3

‡ Based on the total number of adults emerged over the 8 replicates

5.3.2 *Aphidius colemani* daily parasitism rate

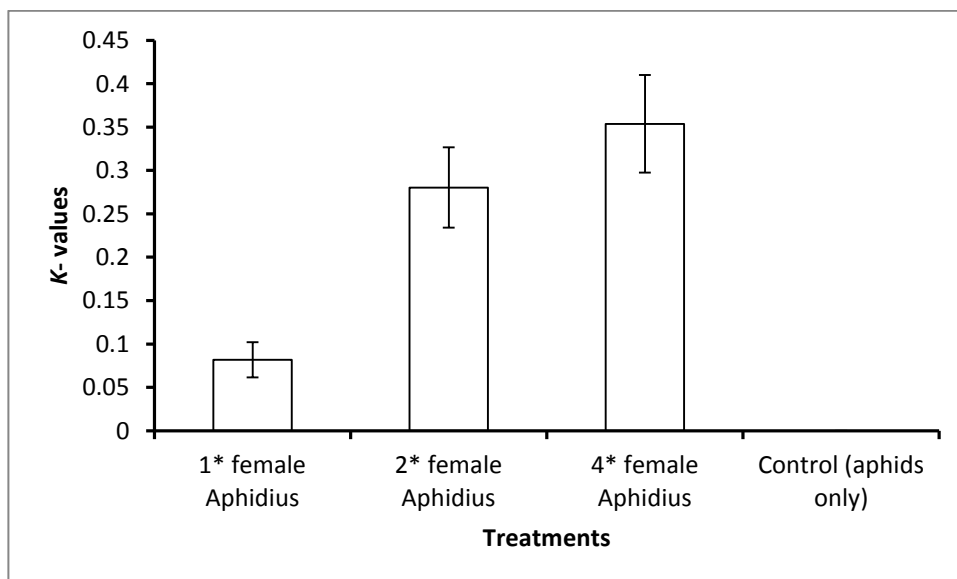
The female adult could only oviposit during the first two days of its life. The mean number of mummies arising from parasitization occurring on day 1 was 10.8 whereas for day 2 it was 5.0. On day 3, no parasitization occurred (Table 3).

Table 3. *Aphidius colemani* daily parasitism rates

Days	Number of mummies		Mean $\pm$ SE
	Minimum	Maximum	
Day 1	0	38	10.8 $\pm$ 2.6
Day 2	0	40	5.0 $\pm$ 2.3
Day 3	0	0	0.0 $\pm$ 0.0

5.3.3 The effect of *A. colemani* parasitism on *M. persicae nicotianae* populations

There were significant differences ($F_{3,19} = 20.99$, $P < 0.001$) among treatments in their effects on aphid populations (Fig 4). The use of 4 female *Aphidius* gave the highest k -value (0.4) though this was not significantly different from that where 2 females were used ($k = 0.3$). On the other hand, the effect of 1 female ($k = 0.1$) was not significantly different from that of the untreated control ($k = 0.0$). Each of the former two treatments had significantly higher k -values than the latter treatments.

Figure 4. Impact of *A. colemani* on tobacco aphid population growth

5.4 Discussion

Aphidius colemani developmental time from oviposition to the adult stage was consistent with results obtained elsewhere (e.g. Takada, 1997). Results show that more males than females emerged — an observation which, however, contradicts research findings on aphidine parasitoids. In nature, usually the proportion of female adults is slightly higher than that of males (Lanzoni *et al.*, 2004). Percentage adult emergence also appeared to be lower than normal. There is a high probability that such results were due to the quality of the aphid host, that is, tobacco. Plants used in the experiment were not exposed to natural light from the beginning of the experiment. This obviously had a negative impact on photosynthesis, leading to production of assimilates of lower quality. This led to poor quality food being available to the aphids which probably affected the parasitoids, hence their reduced fitness (in terms of lower emergence).

The low proportion of females also supports this fact in the sense that female *Aphidius* develop on bigger hosts of higher quality, thus most of the females could not get enough nutrition to reach the adult stage due to small and poor quality aphid hosts (Ode *et al.*, 2004). According to Van Driesche *et al.* (2008), the sex ratio of parasitoid progeny under laboratory conditions is usually male-biased. Most of the aphids developing in laboratory conditions are on average smaller in size, resulting in female parasitoids ovipositing haploid eggs in most of the aphids with fewer diploid eggs being laid in selected bigger hosts.

Jarosik *et al.* (2003) studied the sex ratio in *A. colemani* in relation to host size and their results indicated that higher numbers of male offspring emerged from small-sized aphids than females and vice-versa. They concluded that in smaller aphids, there is high mortality of female progeny due to food limitations. Female progeny have higher nutritional needs and reach bigger size than

males when developing in hosts of the same size. The situation should, however, be different in the field where suitable environmental conditions are available for the growth of healthy plants on which aphids develop to bigger size.

The longevity of adult female *A. colemani* (range 1-3 days) supports available literature. van Emden and Harrington (2007) explained that though *Aphidius* species can live for 2 to 3 weeks, this is rare under field conditions which usually limit the life span of the parasitoid to about 2 to 3 days. The daily parasitism rate of *A. colemani* shows that the parasitoid is most effective during the first day of its life. The rate drops by half on the second day and ceases on the third day. Sampaio *et al.* (2008) found out that the parasitism rate of *A. colemani* on *M. persicae* was 21%, a figure comparable to that obtained in the present study which reached 32% total parasitism rate if calculated from the total of the daily parasitism rates.

Results of the current study clearly indicate that with an increase in parasitoid numbers, efficiency increases as shown by the respective *k*-values of the treatments. However, the lack of significant differences in parasitism rates between 2 and 4 parasitoids indicates the existence of a possible limit in the number of parasitoids in relation to the aphid population size. High parasitoid : aphid ratios usually affect parasitoid behaviour resulting in superparasitism. Parasitoids developing this way have high mortalities which consequently results in reduced parasitism rate. On the other hand, the low rate of parasitism by a single female *A. colemani* explains the need for research to find out the most effective parasitoid : aphid ratio for augmentative parasitoid releases in tobacco production.

Results of the present study revealed that *A. colemani* significantly reduces aphid populations in tobacco, thus is an efficient control agent of the pests. However, there is need to investigate its

efficiency over several generations as this study only focused on the effectiveness of the 1st generation adults. As the parasitoid is short-lived, its actual efficacy can be more accurately determined from the impact of the next generations.

Chapter 6

Effect of Combined *H. variegata* Predation and *A. colemani* Parasitism on *M. persicae nicotianae*

6.1 Introduction

In nature, natural enemies exist as many different species together within guilds (van Emden and Harrington, 2007). When a group of different species coexist in a community and share the same food and space resources they are referred to as guilds. The species may have similar or different tactics of resource utilization. Differences or similarities among the species may also exist within the community (Polis *et al.*, 1989; Hodek *et al.*, 2012).

The consequence of sharing a similar resource is interference between or amongst the different species. The impact of natural enemies on pests within the guilds can only be significant if interference is low. In this case, their combined effect will result in better pest control than their impact as individual species (van Emden and Harrington, 2007). High interference between species within guilds manifests as intraguild predation.

Intraguild predation refers to the predation of a species by another species which is a competitor within the guild. Intraguild predation can either be symmetric or asymmetric. Symmetric intraguild predation is characterised by mutual predation between two species, that is, one species can prey on another and vice-versa. In asymmetric intraguild predation, one species is always the predator on another species whenever they interact (Polis *et al.*, 1989; Hodek *et al.*, 2012).

Intraguild predation is significant and should be considered in biological control programmes (Borer *et al.*, 2007). It is considered to affect negatively the ability of a predator to reduce a pest

population in biological control if the predator preys on another natural enemy of the same pest. The interaction between predators and parasitoids within a guild culminates in asymmetric intraguild predation in which predators are intraguild predators while parasitoids are the intraguild prey. However, ladybirds usually select non-parasitized prey ahead of parasitized ones (Bilu *et al.*, 2006, Chacon and Hempel, 2010; Hodek *et al.*, 2012). The objectives of this study was to find out whether intraguild predation exists between the combination of *H. variegata* and *A. colemani* in the biological control of *M. persicae nicotianae* and to investigate their combined effect on reducing *M. persicae nicotianae* population growth.

6.2 Materials and methods

6.2.1 Intraguild predation of parasitized aphids by *H. variegata*

Two experiments were set up to investigate intraguild predation of *A. colemani* by *H. variegata* larvae and adults. The first experiment was designed as a Completely Randomized Design with three treatments each having 20 aphids on tobacco leaf discs. The treatments were as follows:

- 1) Parasitized aphids (x 10) + non-parasitized aphids (x 10),
- 2) Parasitized aphids (x 20), and
- 3) Non-parasitized aphids (x 20)

Each treatment was replicated 10 times. The experiments were performed differently either with 1-day old parasitized aphids or 5-day old parasitized aphids for the both the 4th instar larva and the adult female *H. variegata*. To ensure homogeneity of parasitism, 2-3 day old aphids were introduced onto a leaf disc in an experimental Petri dish with a mated female *A. colemani*. Any aphid stung once was transferred to a potted tobacco plant using a fine paint brush, and the procedure repeated for up to an hour until the total number of parasitized aphids required for the experiment was reached. Each female *A. colemani* was used only once for parasitization.

Parasitized aphids were isolated on the tobacco plants in experimental cages either for a day or for five days for the two experiments.

Fourth instar *H. variegata* larvae which had just moulted and 1-3 week old female adults were first starved in isolation in experimental Petri dishes for 24 hours before the start of both experiments for each ladybird life stage. To determine intraguild predation of 1-day old parasitized aphids by the 4th instar larva, each larva was introduced into an experimental Petri dish with a tobacco leaf disc on which 20 aphids had been placed as required in each treatment. The insects were left to feed for 6 hours and then removed. The number of aphids consumed in each Petri dish was recorded. The experiment was done in a similar way for the adult ladybird. To investigate intraguild predation of 5-day old parasitized aphids, the same procedure was followed for both the 4th instar larva and the adult female *H. variegata*.

6.2.2 Choice tests for intraguild predation

Aphids were parasitized using the same procedure mentioned in 6.2.1 to obtain 1-day old parasitized and 5-day old parasitized aphids. Fourth instar ladybird larvae and female ladybird adults were starved for 24 hours before use in the experiments. For assessing intraguild predation by ladybird larvae, a total of twenty 1 day-old parasitized aphids (10 non-parasitized + 10 parasitized) were placed on a tobacco leaf disc in a Petri dish. A single ladybird larva was introduced into the Petri dish and left to feed for 6 hours. The aphids left in the Petri dish were transferred to new leaf discs and monitored daily for a period of seven days by which time all parasitized aphids would have been mummified. The numbers of parasitized and non-parasitized aphids consumed were then recorded. There were 10 replicates for each treatment. The same procedure was followed for the adult female ladybird.

Choice tests for 5-day old parasitized aphids and non-parasitized aphids were done for the 4th instar ladybird larva and adult female ladybird as was done for the 1 day-old parasitized aphids.

Prey preference was calculated using the Manly's Index of Preference:

$$\beta_1 = \log \left(\frac{e_1}{A_1} \right) / \left(\log \left(\frac{e_1}{A_1} \right) + \log \left(\frac{e_2}{A_2} \right) \right)$$

where β_1 = preference for prey type 1,
 e_i = the number of prey remaining after experimentation,
 A_i = the number of prey offered.

An index value close to 0.5 indicates no preference for a given prey type while a value close to 1 shows preference for prey type 1. An index value close to 0 indicates predator preference for prey type 2.

6.2.3 The effect of combined *H. variegata* predation and *A. colemani* parasitism on *M. persicae nicotianae* populations

To determine the combined impact of the biological control agents on the tobacco aphid, an RCBD was used to lay out the experiment. Blocking was done in time with four treatments, each replicated 20 times. The treatments were as follows:

- 1) 3rd instar *H. variegata* larva (x 1) + mated *A. colemani* female adult (x 1),
- 2) female adult *H. variegata* (x 1) + mated *A. colemani* female adult (x 1),
- 3) mated *A. colemani* female adult (x 1), and
- 4) control (200 aphids only).

Each treatment constituted a potted tobacco plant infested with 2-3 day old tobacco aphids in an experimental cage. The randomization of blocks and treatments was done using Microsoft Excel. The natural enemies were introduced into the cages as per treatment requirement. After two days, both the predator and parasitoid were removed from the cages and the numbers of

aphids present on each plant were recorded so as to determine the number consumed by the ladybird larva and the adult. The treatments were then maintained for up to 10 days so as to enable the determination of parasitized aphids (as evidenced by mummies). Combined K -values for the predator and the parasitoid were then calculated by adding k -values for the predator and k -values for the parasitoid.

6.2.4 Data analysis

Statistical analysis for the choice test for intraguild predation experiment was done using the one sample t -test to compare the observed Manly's Index Preference value with 0.5 of no prey preference to test the null hypothesis that the predator had no choice in selecting prey type. Analysis for the intraguild predation experiment and the combined effect of *H. variegata* predation and *A. colemani* parasitism experiment was done using the one-way ANOVA to test significant differences between treatments coupled with the Tukey-Kramer HSD test where the F -ratio was significant ($P < 0.05$). Log transformation was done for the intraguild predation data (i.e. the number of parasitized and non-parasitized aphids consumed) prior to analysis. Data for the number of aphids left after 2 days and for the net number of aphids left after combined *H. variegata* predation and *A. colemani* parasitism were also log-transformed before analysis. Data for the number of mummies formed after 10 days (in three treatments, excluding the control) had a normal distribution and thus were not subjected to transformation.

6.3 Results

6.3.1 *H. variegata* predation rate on parasitized and non-parasitized aphids

There were no significant differences ($P > 0.05$) in aphid predation by 4th instar ladybird larvae 1 day after parasitization by *A. colemani* (Table 4). In the case of adult female ladybirds, there were also no significant differences among treatments in consumption rate of aphids 1 day after

parasitization. However, there were significant differences ($P < 0.001$) among treatments in 4th instar larval predation of 5-day old parasitized aphids. The number of mixed parasitized and non-parasitized aphids preyed upon by a 4th instar lady bird larva (7.2 aphids) was not significantly different from that of non-parasitized aphids (10.6 aphids). Significant differences among treatments were noted in the consumption rate of all 5-day old parasitized aphids in which the larva consumed 4.4 aphids. In the case of the adult ladybird, no significant differences were noted among treatments in the consumption of 5-day old parasitized aphids. There were also no significant differences among treatments in the consumption rate of aphids by ladybirds 5 days after parasitism.

6.3.2 *H. variegata* prey preference for non-parasitized and parasitized aphids

Prey preference was insignificant for 4th instar ladybird larva offered aphids 1-day after parasitization with a Manly's index of 0.4 (Table 5). The female adult's prey preference was also insignificant for the same type of prey. The 4th instar larva's preference on 5-day parasitized aphids was significant, with the predator's Manly index at 0.7, showing that the larva preferred non-parasitized than parasitized aphids. The female adult, however, had a Manly's index of 0.67 indicating insignificant prey preference when offered aphids parasitized 5 days previously.

Table 4. Number of *A. colemani*-parasitized aphids consumed by *H. variegata* after a 6-hour exposure

Treatment	Number of aphids	1-day old parasitized aphids		5- day old parasitized aphids	
		4 th instar larva	Female adult	4 th instar larva	Female adult
Parasitized + non-parasitized	10 + 10	4.2 ± 0.5	6.0 ± 1.0	7.2 ± 0.8 b	6.9 ± 1.1
Parasitized	20	3.4 ± 0.5	6.5 ± 1.4	4.4 ± 0.5 a	5.1 ± 1.0
Non-parasitized	20	4.6 ± 0.8	5.3 ± 1.2	10.6 ± 1.1 b	8.9 ± 1.4
d.f.		2,27	2,27	2,27	2,27
<i>F</i>		0.75	0.20	14.80	3.36
<i>P</i>		0.48	0.82	< 0.001	0.05

Means (mean ± SE) followed by the same letter within a column are not significantly different ($P < 0.05$)

Table 5. Manly's indices of preference for non-parasitized aphids by *H. variegata*

Ladybird life stage	Manly's index	<i>t</i> – value	<i>p</i> – value
(a) 1 – day old parasitized aphids			
4 th instar larva	0.42 ± 0.10	0.82	0.43
Adult female	0.67 ± 0.11	1.47	0.18
(b) 5 – day old parasitized aphids			
4 th instar larva	0.74 ± 0.03	7.59	< 0.001
Adult female	0.67 ± 0.11	1.51	0.17

6.3.3 The effect of combined *H. variegata* predation and *A. colemani* parasitism on aphid populations

There were significant treatment differences ($F_{3,19} = 27.9$, $P < 0.001$) among the treatments for the number of aphids left following *H. variegata* predation after 2 days (Fig 5). The adult female reduced the aphid population to a significantly lower value (119.1) than the other three treatments. Predation by the 3rd instar larva significantly lowered the aphid population (148.6) as compared to the *A. colemani* treatment (200.9) and the control (206.2), which were not significantly different.

The number of mummies formed after 10 days showed no significant differences ($F_{2,19} = 1.03$, $P = 0.367$) among the three treatments. The net number of aphids left after combined predation and parasitism indicated significant differences ($F_{3,19} = 26.18$, $P < 0.001$) amongst the four treatments. The female *H. variegata* adult + female *A. colemani* adult combination significantly reduced the aphid population to the lowest value (103.6) as compared to other treatments. The 3rd instar *H. variegata* larva + female *A. colemani* adult significantly lowered the aphid population (132.5) than the female *A. colemani* alone treatment (189.7) and the control (206.2). There was no significant difference noted between the former treatments.

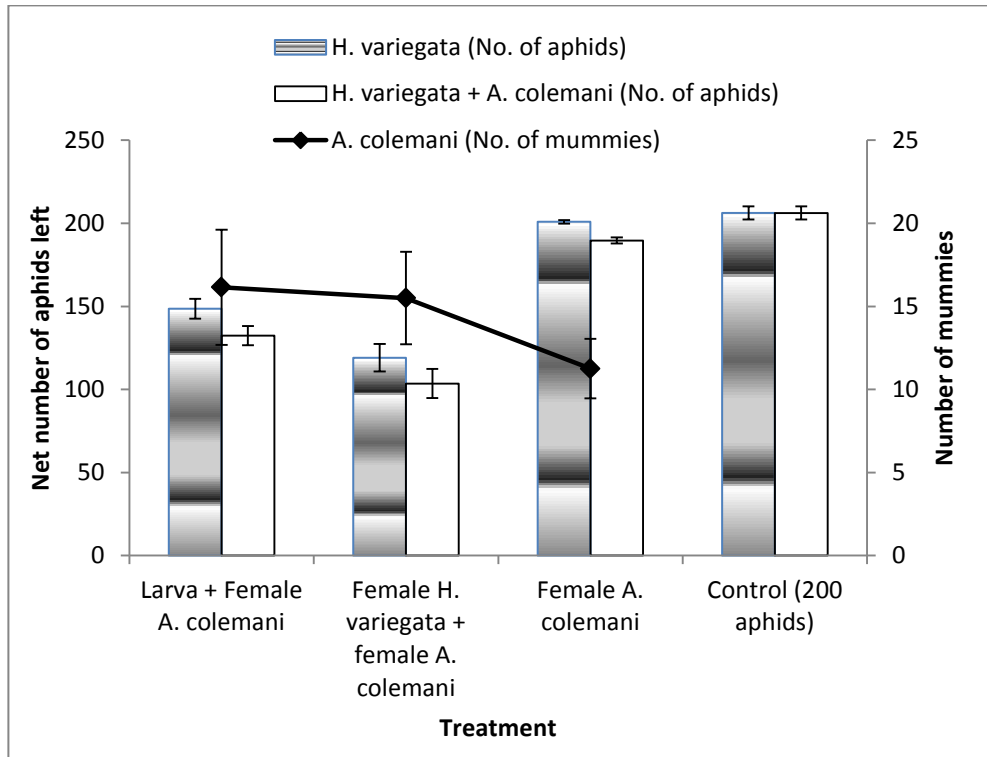


Figure 5. Net number of aphids left after predation and parasitism by *H. variegata* and *A. colemani* and the number of mummies formed after parasitism by *A. colemani*

6.4 Discussion

The present study showed that no preference for parasitized or non-parasitized tobacco aphids was shown by *H. variegata* larvae and adults on 1 day-old parasitized aphids. This reveals that at this stage of parasitoid development, the predator does not recognize the difference between parasitized and non-parasitized aphids resulting in intraguild predation of the parasitoid. The 4th instar larva could clearly select non-parasitized aphids ahead of 5-day old parasitized ones. Similarly, the female adult also showed preference for non-parasitized aphids. This shows that at this stage of parasitoid development, there is synergism in the control of the tobacco aphid by 4th instar ladybird larva and the adult and *A. colemani*. However the Manly's Index of Preference for 5-day old parasitized aphids for the adult *H. variegata* indicated lack of prey type preference. Despite not being significantly different from the no-preference index of 0.5, the

level of preference was relatively high. This shows that with an increase in the number of days after parasitism, *H. variegata* adults increase their preference for non-parasitized than parasitized aphids resulting in reduced rates of intraguild predation. The Manly's indices, however, show the existence of intraguild predation during the stages of *A. colemani* larval period before mummification. With these results, we can conclude that the larva is the better agent to be used in combination with *A. colemani* though the adult is also efficient.

Similar studies by other researchers on intraguild predation of aphid parasitoids by coccinellids are in line with the results of this study. Xue *et al.* (2012) studied intraguild predation of *Aphelinus certus* by *Coccinella septempunctata* and *Harmonia axyridis* and found out that the coccinellids could not discriminate between 3-4 day old parasitized and non-parasitized aphids. They also found out that the coccinellids preferred non-parasitized to mummified aphids and concluded that intraguild predation was not likely to disrupt biological control of the soyabean aphid.

As this study showed, *H. variegata* can be used without disrupting biological control when in combination with *A. colemani* as there was a decrease in consumption of parasitized aphids with an increase in the number of days from the first day of parasitization. *Hippodamia variegata* will be even more efficient at selecting non-parasitized aphids ahead of real mummies given the fact that it could recognize parasitized aphids before the mummy stage.

Results for the combined effect of *H. variegata* predation and *A. colemani* parasitism on the aphids after two days of exposure to both natural enemies indicate that the predator is the most effective in causing an instant population reduction of the pest. This was shown by the reduced aphid population numbers in the combination treatments than in the *A. colemani* alone treatment

and the control in which no decline in aphid numbers was noted. The results also indicated that the female adult ladybird is more effective in controlling the tobacco aphid than the larva as its impact was higher. After 10 days, the lack of significant differences in the number of mummies amongst the treatments showed that there were no differences in aphid parasitism by *A. colemani*. This translates to the fact that the parasitoid's efficiency is not altered by the presence of *H. variegata*. This shows no evidence of significant intraguild predation of the parasitoid by the predator, thus the impact of both natural enemies against the tobacco aphid is additive. Snyder *et al.* (2004) investigated the *Harmonia axyridis* (predator) + *Aphelinus asychis* (parasitoid) complimentary biological control of aphids and concluded that though the predator at times fed on mummies, there was no significant change in the number of mummies observed when the parasitoid was alone. This supports the results of this study which showed no evidence of changes in the number of mummies in the presence or in the absence of *H. variegata*, though some intraguild predation was noted in the intraguild predation experiments.

Results on the combined effect of *H. variegata* and *A. colemani* after 10 days indicate that both natural enemies can combine well to control *M. persicae nicotianae* in tobacco. The female *H. variegata* + *A. colemani* combination is the most effective. The larva + female *A. colemani* combination was also shown to be effective in controlling the aphids though at a significantly lower rate. By showing preference for non-parasitized than parasitized aphids, the efficiency of 4th instar ladybird larvae might even be higher than recorded in this study. This can be due to the fact that the walking difficulties the larvae experience on tobacco may force them to consume aphids within their vicinity regardless of whether the aphids are parasitized or not.

Chapter 7

Effects of Aphicidal Sprays on Ladybird Predators

7.1 Introduction

The control of the tobacco aphid using chemical insecticide (aphicides) is the most dominant method in use worldwide. Aphicides in the chemical groups organophosphates, neonicotinoids, pyrethroids and carbamates are the most common (van Emden and Harrington, 2007). In Zimbabwe, some of the widely-used aphicides in tobacco production include dimethoate, acetamiprid, acephate, pirimicarb, and thiamethoxam (Masukwedza *et al.*, 2013). In this study, the impact of the aphicides acephate, dimethoate and acetamiprid on *H. variegata* were investigated.

Dimethoate is an organophosphorous systematic contact and stomach chemical for aphid control in tobacco production. The chemical also controls a wide range of pests such as lepidopteran larvae, thrips, leaf miners among many other pests in tobacco and other crops like soyabean, cotton, forestry, and horticulture. The chemical acts as an acetylcholinesterase inhibitor, that is, it disrupts the transmission of nerve impulses between two nerve cells in the insect nervous system. This results in paralysis and eventual death of the affected insects (Tomlin, 2000; van Emden and Harrington, 2007).

Acephate is a systemic organophosphate which also acts as an acetylcholine inhibitor. The chemical controls aphids and various other pests in many different crops. Acetamiprid belongs to the neonicotinoid chemical group. The mode of action of this chemical involves effecting synapses in the central nervous system by disrupting the acetylcholine receptor. The insecticide

is also used for the control of lepidopteran, thysanopteran and other hemipteran pests in many crops (Tomlin, 2000).

Many chemical pesticides are on record as causing natural enemy mortalities in crop production. Hon-Hyun and Lee (2009) reported declines in natural enemy populations in rice fields resulting from the application of broad-spectrum insecticides. Though most natural enemies are generally susceptible to pesticides, coccinellids are more tolerant than hymenopteran parasitoids. Parasitoids have also been recorded to be ever more susceptible than their hosts. This implies parasitoids should severely be affected by almost all aphicides in use against the tobacco aphid (Pedigo and Rice, 2006).

7.2 Materials and methods

The experiment was laid out as a Completely Randomized Design (CRD) with four treatments each replicated five times. The treatments were as follows:

- 1) Dimethoate – 375 ml/100 litres water,
- 2) Acephate – 90 g/ 100 litres water,
- 3) Acetamiprid – 15 g/100 litres water, and
- 4) Control (unsprayed).

A potted tobacco plant infested with at least 200 aphids was placed in an experimental cage. Ten 4th instar larvae or adult ladybirds were introduced into the cage and allowed to settle for one hour. The plants were then removed from the cages without disturbing the ladybirds and placed on the floor in a glasshouse at an in-row spacing of 1 m in each treatment. Small sprayers (1 litre) were calibrated to give a spray output of 100 litres per hectare and used for the experiment. The aphicides were then sprayed on the plants. In each treatment, spraying was done over a

walking distance of 15 m with the plants placed in the middle 5 m length. This was done to ensure a uniform spray rate was applied.

After the aphicide application, the ladybird larvae or adults in each of the treatment replicates were transferred to the laboratory and placed on caged potted tobacco plants infested with at least 200 aphids. Additional supplies of aphids on leaf discs were added as and when required. The ladybirds were monitored and mortalities recorded twice after every 48 hours. Cumulative percentage mortalities were then calculated.

The data for percent mortalities were arcsine-transformed before analysis by one-way ANOVA. Where the F -ratio was significant, treatment means were separated using the Tukey-Kramer HSD test. All analyses were performed using the GenStat 14.1 statistical package. There was no correction of mortalities as none were recorded in the untreated controls.

7.3 Results

7.3.1 Effects of aphicidal sprays on fourth instar *H. variegata* larvae

There were significant differences ($F_{3,16} = 312$, $P < 0.001$) in ladybird mortality among treatments (Table 6). Cumulative 2-day post-treatment larval mortality was highest (98%) on dimethoate-treated plants while that due to acephate was very low (18%). After four days, the cumulative mortality was 100% for dimethoate, 22% for acephate, and zero for acetamiprid and the control ($F_{3,16} = 644.1$, $P < 0.001$).

Table 6. Cummulative % mortality of *H. variegata* on aphicide-sprayed plants

Insect stage	Treatment	After 2 days	After 4 days
4 th instar larva	Dimethoate	98.0 ± 2.0 c	100 ± 0.0 c
	Acetamiprid	0.0 ± 0.0 a	0.0 ± 0.0 a
	Acephate	18.0 ± 4.9 b	22.0 ± 3.7 b
Adult	Dimethoate	98.0 ± 2.0 c	100 ± 0.0 c
	Acetamiprid	0.0 ± 0.0 a	0.0 ± 0.0 a
	Acephate	36.0 ± 8.1 b	52.0 ± 3.7 b

For each life stage, means within a column followed by different letters are significantly different ($P < 0.05$).

7.3.2 Effects of aphicidal sprays on adult *H. variegata*

At 2 days post-treatment, there were significant differences ($F_{3,16} = 122.1$, $P < 0.001$) in the effect of aphicides on adult adult ladybirds (Table 6). Dimethoate caused the highest mortalities (98%) followed by acephate (36%). After 4 days, significant differences ($F_{3,16} = 659.8$; $P < 0.001$) were again noted among treatments. Cumulative ladybird mortalities were 100 and 52% in dimethoate and acephate treatments, respectively, while no deaths were recorded in the acetamiprid treatment.

7.4 Discussion

Results in this study revealed that some of the commonly-used chemicals used for the control of the tobacco aphid are harmful to ladybird predators. The high mortalities of both *H. variegata* larvae and adults in dimethoate treatments imply that the insecticide is totally not compatible with the coccinellid for controlling the tobacco aphid. These results support the work by Croft and Brown (1975) who found *H. variegata* adults to be susceptible to direct contact with dimethoate sprays. This shows that the chemical should not be used where arthropod biocontrol agents are utilized for controlling *M. persicae nicotianae*. Santos *et al.* (2010) studied the response of coccinellids to dimethoate application in olive production in Portugal and found out

that where the aphicide was applied, coccinellid populations were drastically reduced. This supports the results of this study that dimethoate is harmful against coccinellid natural enemies of the tobacco aphid.

Acephate also caused some mortalities in the predator but the 4th instar larva exhibited some resistance to the chemical more than the adults. The aphicide caused mortalities to just more than half of the population of adults indicating that it is harmful to the adult stage of *H. variegata*. Therefore as only the adult had been found to be effective in controlling *M. persicae nicotianae* in tobacco (Chapter 4), the chemical is less compatible with *H. variegata* for tobacco aphid control. However, use of the chemical can be manipulated for combination with the coccinellid by reducing the rate of application to a level which is effective against aphids but harmless to the predator. Acetamiprid was shown in this study to be compatible with *H. variegata* in the integrated pest management of the tobacco aphid as the aphicide virtually did not cause any mortalities on the coccinellid.

In conclusion, the two organophosphate chemicals (dimethoate and acephate) are not compatible for use in combination with *H. variegata* for tobacco aphid control. Foliar sprays of neonicotinoids, represented here by acetamiprid, can be effectively used in combination with *H. variegata* for controlling the tobacco aphid. However, further research needs to be conducted to evaluate all the effects (e.g. reproductive fitness) of these chemicals on the predators surviving after coming in contact with these chemicals so as to come up with a clear picture on the effects of aphicide sprays. Additionally, the residual effects of the foliar sprays needs to be investigated as the current results may only be valid when aphicidal sprays are still fresh.

Chapter 8

General Discussion, Conclusions and Recommendations

8.1 General Discussion

Hippodamia variegata and *A. colemani* are among the most important natural enemies of *M. persicae nicotianae*. Adoption of these biological control agents for use in tobacco production will provide an alternative to the costly and environmentally unfriendly chemicals currently in use for control of the tobacco aphid in Zimbabwe and other parts of the world. The biology and effectiveness of *H. variegata* and *A. colemani* are outlined in chapters 4 and 5, respectively. The combined impact of these tobacco aphid natural enemies is discussed in chapter 6 whereas the effect of aphicides on the natural enemies is detailed in chapter 7.

The present study showed that *H. variegata* and *A. colemani* are effective biological control agents of the tobacco aphid. The larval stage of *H. variegata* was, however, found to be inefficient in tobacco as the 1st instars stick onto tobacco leaves and resultantly are unable to forage for food leading to death by starvation. During the predation experiments on tobacco, it was also observed that though 3rd and 4th instars were able to walk on leaf surfaces, their movements were impeded by hairiness of the leaves. The larva usually preferred to be on Petri dish walls than on tobacco leaves. As the tobacco aphid is polyphagous, the pest does attack other crops during the tobacco off-season. The aphid will also be feeding on weeds from which it transmits viruses to tobacco. The larva will in this case be useful as a biological control agent.

Hippodamia variegata adults were shown to exhibit high effectiveness as biological control agents of the tobacco aphid. Efficiency was observed to be high when both sexes exist as well as when the female was present alone and when both adult and larvae were present. The adult-

larva combination may, however, only be functional in other crops like brassicas with suitable plant surface morphology on which larval movement is least impeded.

Aphidius colemani was also proven to be capable of controlling the tobacco aphid in this study. The parasitoid was observed to increase efficiency with an increase in parasitoid number in relation to the aphid population. The low percent adult emergence as well as the slightly lower female : male sex ratio was an indication that the aphid hosts could not sustain the development of the endoparasitic larvae up to the adult stage possibly due to reduced photosynthates in plants following days of lack of exposure to adequate natural sunlight. It was noted in the study that some of the initial aphids introduced in the treatments died before any signs of mummification could be seen. As this study only focused on measuring the rate of parasitism through observation of mummies, there is a high probability that some aphids were parasitized but died before reaching the mummy stage. This implies the rate of parasitism could even be higher than that recorded in the current study.

Despite some intraguild predation of *A. colemani* by *H. variegata* observed in this study, the combined impact of these natural enemies on the tobacco aphid showed a positive potential for effective suppression of the pest. *Hippodamia variegata* was shown to have higher preference for non-parasitized aphids with an increase in age of the parasitized aphid. This was further supported by the lack of evidence of significant intraguild predation.

It was shown that the effects of aphicides on *H. variegata* depend on the type of chemical used. Results of the study indicate that, in general, aphicides have an adverse effect on the ladybird beetle. Acetamiprid was found to be the best aphicide to use in an Integrated Pest Management (IPM) of the tobacco aphid as the insecticide did not cause mortalities in both larvae and adults

of the ladybird beetle. The insects were able to feed and in addition adults could still mate after they were sprayed with the aphicide. Acephate toxicity showed that the chemical could possibly be used at lower than recommended rates in tobacco production as the aphicide could spare just less than 50% of the ladybird adults. However, lowering the application rate may result in poor aphid control. Dimethoate was shown to be totally not compatible with *H. variegata* in controlling the tobacco aphid due to the high mortality rates the aphicide caused on the ladybird beetles when treatments were still fresh.

The adverse impacts of the organophosphate aphicides on *H. variegata* showed that aphicides such as dimethoate and acephate should not be recommended for use in the IPM of the tobacco aphid. On the other hand, if aphid control has to be achieved through foliar sprays, farmers should be encouraged to use neonicotinoids like acetamiprid, which, in addition to their low mammalian toxicity are less toxic to *H. variegata*. Better still, tobacco aphid control should be anchored on the use of planting hole neonicotinoid systemics such as imidacloprid and thiamethoxam.

8.2 Conclusions

The results of this study show that *H. variegata* is an effective biocontrol agent which can be successfully utilized for the control of the tobacco aphid. The female adult is the most effective compared to the male and the larva. However, the early-instar ladybird larvae may not be able to provide effective aphid control as they have difficulties in walking on the hairy leaves. *Aphidius colemani* also showed high potential for controlling the tobacco aphid, thus the parasitoid needs to be conserved in tobacco fields. The use of both natural enemies in combination produces an even more effective control measure, as both were proven to have an additive impact against the tobacco aphid. It was further determined that aphicides which are

harmless to the natural enemies of the tobacco aphid should be used in the management of the tobacco aphid through foliar sprays. These selective aphicides, particularly neonocotinoids, should be preferred ahead of broad spectrum ones such as organophosphates.

8.3 Recommendations

The following recommendations are made:

- Field studies on the impact of *H. variegata* and *A. colemani* on *M. persicae nicotianae* need to be conducted to validate the findings of this study.
- The general distribution and population dynamics of both natural enemies and the tobacco aphid should be investigated in all the tobacco-producing regions of the country.
- Interactions with other natural enemies of the tobacco aphid also require extensive research so as to come up with the best available natural enemy combinations and conservation strategies.
- Insect-plant interactions will also need to be investigated to find out suitable crops to intercrop with tobacco. These are crops which are attractive to the natural enemies and serve to retain them within the field.
- Extending the study to all tobacco aphicides currently registered in Zimbabwe as well as other insecticides which are also used in tobacco against other pests (for example *Helicoverpa armigera*). This will aid in the selection of insecticides which are least harmful to all the natural enemies of the different tobacco pests.
- Agricultural extension work to educate farmers on the importance of biological control of pests and the implementation of conservation strategies for natural enemies to enhance their effectiveness in regulating tobacco aphid populations.

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