ESTABLISHMENT OF *COTESIA VESTALIS* (HALIDAY) AND *DIADROMUS COLLARIS* (GRAV.) PARASITOIDS OF THE DIAMONDBACK MOTH, *PLUTELLA XYLOSTELLA* (L.), AND ASSESSMENT OF THE EFFECTIVENESS OF *C. VESTALIS* AS A BIOLOGICAL CONTROL AGENT IN ZAMBIA

by

PHILEMON HAKAINDA SOHATI

(BSc., UNZA, Zambia; MSc., McGill University, Canada)

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A Thesis Submitted to the University of Zambia in Fulfilment of the Requirements of the Degree of Doctor of Philosophy in Agricultural Sciences (Entomology)

LUSAKA, ZAMBIA

2012

DECLARATION

I, **Philemon Hakainda Sohati**, hereby declare that this thesis represents my own original work and that it has not been previously submitted for a degree, at this or any other University.

.....

Signature

.....

Date

APPROVAL

This thesis of **Philemon Hakainda Sohati** is approved as fulfilling the requirements for the award of the degree of Doctor of Philosophy in Agricultural Sciences (Entomology) by the University of Zambia.

Signature	**Date:**
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Internal Examiner	
.....	.....
Internal Examiner	
.....	.....
External Examiner	

DEDICATION

I dedicate this thesis to: my wife, Joyce M. Haluchiso-Sohati; my son, Lweendo; my two daughters, Luyando and Lushomo; my two grandsons, Lubomba and Luumuno; granddaughter Maimbo and my late granddaughter, Lisa.

"*And we know that all things work together for good to those who love God, to those who are called according to his purpose".* Roman 8:28 (NKJV).

ABSTRACT

The goals of the study were to confirm establishment of two exotic parasitoids, *Cotesia vestalis* (Haliday) (Hymenoptera: Braconidae) and *Diadromus collaris* (Grav.) (Hymenoptera: Ichneumonidae), released in Lusaka Province, Zambia, during the 1970s and 1980s, to control the diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), a serious pest of vegetables in Zambia, and to assess the effectiveness of one of the released parasitoids, *C. vestalis*, as a biological control agent of the pest. Confirmation of the establishment of the exotic parasitoids was through field sampling and collection of various developmental stages of the parasitoids, in and around the release sites in Lusaka Province. The assessment of the effectiveness of *C. vestalis*, as a biological control agent of the crop pest was through laboratory experiments.

The specific objectives were to: i) Sample and collect various developmental stages of the parasitoids, *C. vestalis* and *D. collaris*, in and around the 1977-1984 parasitoids release sites in Lusaka Province, Zambia; ii) determine the types of pesticides used by local farmers in the Chongwe, Kafue and Lusaka districts on common vegetable pests and their impacts on the exotic parasitoids, *C. vestalis* and *D. collaris*; iii) determine the phenology of *P. xylostella* and identify its parasitoids in the selected study area; and iv) assess the effectiveness of *C. vestalis* as a biological control agent of *P. xylostella* under laboratory conditions.

Vegetable farms, in and around the original release sites of parasitoids in Lusaka west and in the Makeni area were selected for the study. Parasitoid presence was the criterion used to confirm establishment. The sampled vegetable fields were geo-referenced using a Global Positioning System (GPS) and the information obtained was used to construct a pest/parasitoid field sampling map. The nearest plant after every 2 m in the campus direction on a 33.2 m diagonal line transect was sampled for the diamondback moth larvae and pupae.

A structured questionnaire was employed to collect information on pesticide usage by local farmers in the Chongwe, Lusaka and Kafue district on common vegetable pests in their areas, while the assessment of the impacts of pesticides on *C. vestalis* involved use of a stock culture of the parasitoid established in the insectary in the School of Agricultural Sciences at the University of Zambia. Determination of the phenology of *Plutella xylostella* involved establishment of a pesticide-free, hand-weeded, drought and disease tolerant hybrid cabbage plot (cultivar Pannar®, Star 3308) at the University of Zambia, School of Agricultural Sciences, Field Station, following normal agronomic practices. The effectiveness of *C. vestalis* as biological control agent was tested using different second larval instar densities (5, 10, 25, 50 and 100 per adult female) of *P. xylostella* under laboratory conditions. The impact of pesticides on *C. vestalis* was assessed using four insecticides namely, acephate at 10, 30, 100, 300, 500, 1000, 1500, 1600 and 1700 ppm, cypermethrin at 2, 4, 6, 8, 10 ppm, λ-cyhalothrin at 2, 4, 6, 8, 10 and 50 ppm, and dichlorvos at 4, 6, 8, 8.5, 9, 9.5 and 10 ppm. Ten unsexed parasitoids were put in individual Petri dishes containing the different bioassays treatments. Parasitoid mortality was recorded after 12, 24, 36, 48, 60 and 72 h of exposure.

The exotic parasitoids, *C. vestalis* and *D. collaris*, were confirmed to have established themselves in the Lusaka Province, Zambia, in this study. Both immature stages and adults of both species of parasitoids were found and collected from the field. *Cotesia vestalis* was collected from five farms (Liempe, Malambo, Miller, Mwenya and Uzakulweni) while *D. collaris* was recorded only at Malambo farm. The level of parasitism by *C. vestalis* ranged from 4.4% to 54.9% while *D. collaris* was 14.3%. The other parasitoid recorded was the hyper-parasitoid of *C. vestalis*, namely, *Aphanogmus* sp. (Hymenoptera: Ceraphronidae). The hyper-parasitoid, *Aphanogmus* sp. is being reported for the first time in Zambia by this study. Its parasitism rate was 15.3% under field conditions.

A survey on pesticide use in Chongwe, Kafue and Lusaka Districts of Zambia in this study revealed that the insecticides used to control vegetable pests belonged to the

pyrethroids, (43.5%), organophosphates (40.6%), carbamates (3.3%) and insect growth regulators (2.4%). Aphids were perceived by farmers (33.4%) to be the most important vegetable pests, followed by spider mites (19.8%) mainly on tomato, *P. xylostella* (13.7%) on brassicas and bollworm (12.9%) on tomato.

The larval populations of *P. xylostella* were recorded from April to December and the pupal populations were recorded from June to early December. The larval populations of *P. xylostella* reached the highest peak in mid-September while the pupal populations reached the highest peak in late September. The local parasitoid species of *P. xylostella* found was *O.sokolowskii*.

Plutella xylostella larvae were parasitized predominantly by the larval parasitoid *C.vestalis*, which peaked in late June, and the larval-pupal parasitoid, *O.sokolowskii*, which was most abundant from September to October. Other parasitoids found were the larval-pupal parasitoid *D. collaris*, which occurred in small numbers from July to November and the pupal parasitoid *D. mollipla*, recorded from June to October. An unidentified species of *Aphanogmus*, a hyper-parasitoid of *C. vestalis*, was also recorded.

Toxicity tests of four insecticides on *C. vestalis* in this study, indicated that the pyrethroid, cypermethrin and the organophosphate, dichlorvos were the most toxic, while another pyrethroid, λ-cyhalothrin was intermediate and the organophosphate, acephate was the least toxic after 12 to 72 h of exposure. At the recommended field rate for each insecticide, i.e. acephate 75% WP (3g/L), cypermethrin 20% EC (1ml/L), dichlorvos 100% EC (3ml/L) and λ-cyhalothrin 20% EC (1ml/L), there was 100% mortality of *C. vestalis* adults under laboratory conditions. *Cotesia vestalis* was found to have high potential for use as biological control agent of *P. xylostella*. Laboratory tests on its effectiveness as a biological control agent indicated a type II Holling disc functional response, that is, the proportion of the parasitized second instar larvae of *P. xylostella* over 24 h period decreased exponentially as the density was increased from 5 to 100 per Petri dish.

Failure of the established parasitoids to control the diamondback moth in Lusaka District is discussed. The following conclusions were drawn from this study: i) The exotic parasitoids, *C. vestalis* and *D. collaris*, established themselves in Lusaka district, ii) Though established, the population densities of the two parasitoids were not high enough to effectively control the diamondback moth in the district, iii) One possible contributing factor to the ineffectiveness of the parasitoids to control the diamondback moth was the use of highly toxic pesticides by farmers to control local vegetable pests such as dichlorvos, iv) Acephate was more friendly insecticide to *C. vestalis* adults than cypermethrin, dichlorvos and λ-cyhalothrin, v) *C. vestalis* was an effective biological control agent of the diamondback moth in laboratory bioassays.

The following recommendations were made regarding the future of the biological control programme of the diamondback moth in Lusaka Province: i) Assessment of the potential of *P. xylostella* parasitoids should also be done under field conditions, ii) Parasitism and functional responses of parasitoids need to be investigated under field conditions, to further the development of a biological control programme for *P. xylostella,* iii) There is need to mass rear *C. vestalis* for release in selected farms to augment the existing populations of this parasitoid, iv) The impact of all insecticides used against *P. xylostella* on its key parasitoids need to be investigated, v) Acephate should be recommended for use by vegetable farmers to control *P. xylostella* for it has been shown that it is less toxic to *C. vestalis*, and vi) Since the phenologies of *O. sokolowskii* parasitoid and the diamondback moth larva were found to be in synchrony and that of *O. sokolowskii* is also density dependent it is recommended that future studies on the diamondback moth take this parasitoid into consideration as biological control agent.

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LIST OF ABBREVIATIONS AND ACRONYMS

AVRDC	:	Asian Vegetable Research and Development Centre
Bt	:	*Bacillus thuringiensis*
CABI	:	Commonwealth Agricultural Bureaux International (UK)
CSO	:	Central Statistical Office
GTZ	:	Deutsche GesellschaftfürTechnischeZusammenarbeit GmbH (Germany Agency for Technical Co-operation)
GPS	:	Global Positioning Systems
GV	:	Granulovirus
MAWD	:	Ministry of Agriculture and Water Development
NSW	:	New South Wales
SPSS	:	Statistical Programme for Social Scientists
UNZA	:	University of Zambia

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CHAPTER 1. INTRODUCTION

1.1 Background

The diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) is one of the most destructive cosmopolitan insect pests of brassica plants in the world (Talekar and Shelton, 1993), including Zambia (Ahmed, 1977; Mingochi and Luchen, 2000). Chemical control of *P. xylostella* worldwide is estimated to cost about one billion US dollars annually (Talekar, 1992; Grzywacz *et al.*, 2010). It is believed that *P. xylostella* originated from the Mediterranean area of the world (Harcourt, 1954; Talekar *et al*., 2002).

The host range of *P. xylostella* includes common cabbage, cauliflower, Chinese cabbage, collards, broccoli, kale, radish, rape and mustard. The damage is caused by the larvae through perforation of leaves of vegetables during feeding, thereby reducing the quality and the cosmetic value of the vegetable crops. Crop damage due to *P. xylostella* is especially serious in the tropics where host plants and ideal temperatures for insect development and multiplication exist throughout the year. In Europe, *P. xylostella* causes relatively little damage and control measures are rarely required because about 40 endemic natural enemy species attack the pest's larvae and pupae, thereby keeping populations well below the levels where they cause economic damage to vegetables (Talekar *et al*., 2002). Lack of effective natural control is considered to be the major cause of *P. xylostella*'s increased pest status in many other parts of the world where it exists (Lim, 1986; Talekar and Shelton, 1993).

Like many other tropical countries, the agro-ecology of Zambia is highly suited for vegetable production. Zambia is endowed with many natural streams, rivers, dams and lakes that make it possible for smallholder farmers to grow vegetables throughout the year, especially during the cool and dry season (May-August) when family labour is more abundant for subsistence farming. In seasons preceded by drought, vegetable growing is normally taken more seriously as a mitigating strategy to the failure of staple crops such as cassava, maize and other cereals. Vegetable growing thus enhances household food security through improved incomes and supplementary foods. As a result of the often low initial investment requirement, small-scale vegetable production and marketing are important employment creators for disadvantaged groups in society, particularly women and youth (Mingochi and Luchen, 2000). It also generates income in the shortest possible time with minimal resources (Kapunda *et al.*, 2004).

Although vegetable growing provides the leverage point for most disadvantaged households to earn income, it is also usually associated with a corresponding demand for pesticides to prevent and control vegetable pests and diseases which are among the major constraints to vegetable production in Zambia (Mubita, 1996; Kapunda *et al.*, 2004; Mingochi and Luchen, 2000). Pesticide abuse by farmers, such as through fixed application regimes (‘calendar spraying’) whether there is any pest present or not, and through the use of un-recommended pesticides, results in destruction of beneficial insects and an increase in toxic pesticide residue levels on vegetables.

Overuse of pesticides classically leads to resistance and this is currently suspected in a number of pests in Zambia, especially in populations of *P. xylostella* and of red spider mites (*Tetranychus* spp.) (Mingochi and Luchen, 2000). Many pesticides, particularly organophosphates, are often highly toxic to applicators and can be very harmful to farmers' friends, the natural enemies of vegetable pests, and are environmentally unsafe. Excessive use of pesticides also results in reduction in profit and most small-scale farmers are resource-poor, hence cannot really afford the high prices of pesticides. Therefore, it is important that development of pest control strategies is aimed at developing pest control strategies that are affordable and readily available to small-scale farmers. Biological control of *P. xylostella* using local parasitoids could be one such control strategy. It would provide a more sustainable and environmentally benign control approach for use by resource-poor farmers in Zambia and other countries.

1.2 Statement of the Problem

The parasitoids, *C. vestalis* and *D. collaris*, were released during 1977-1984 period for the control of the diamondback moth, *P. xylostella* in Lusaka Province. There has been no follow up studies to determine whether or not the parasitoids established themselves in the Province and why they are not reducing populations of the diamondback moth.

1.3 Study Justification

Currently, control of *P. xylostella* in Zambia is predominantly through the use of synthetic insecticides such as monocrotophos, λ-cyhalothrin and cypermethrin and to some extent through the use of the microbial insecticide *B. thuringiensis* subsp. *kurstaki* (*Btk*). These pesticides are, however, expensive for resource-poor farmers who constitute the largest group of farmers growing vegetables in Zambia. Further, it is becoming evident that synthetic pesticides in particular are environmentally hazardous to applicators and in some cases *P. xylostella* has developed resistance to most of the pesticides. Without sufficient background information, the usual response of growers to poor control of diamondback moth is to use higher dosages and more frequent applications of insecticides, inadvertently exacerbating the problem. Perhaps the biggest worry here is the danger of such practices to human health and the environment at large.

With the rising global demand for organically grown vegetables, biological control of insect pests may provide a long-term or even permanent solution as it does not cause pollution and risk to human health and may generate increased value through the sale of organic vegetables at a premium price.

Biological control is the deliberate use of natural enemies (predators, parasites, parasitoids) to control pests and in this case, insect pests. It has three major advantages over chemical control of pests namely, safety, permanency and it is economical. In terms of safety, many natural enemies are host-specific or are restricted to a few closely related species and it is unlikely that non-target organisms

in the environment will be affected, hence natural enemies are safe to use. Once established, biological control of an insect pest is permanent and since it is self-perpertuating, requiring no additional inputs, it is economical. Its only disadvantages are, that it takes time to establish and that it may be incompatible with insecticide treatments that may have to be used in insect pest outbreak situations.

Biological control of *P. xylostella* may provide an important part of integrated pest management control strategy since it is environmentally safe, specific and once natural enemies are established, is self sustaining.

This research has been designed in the wake of the above mentioned concerns. Its results will pave the way for the use of natural enemies of *P. xylostella*, both exotic and indigenous, for the long-term environmentally friendly control of the major vegetable pest in Zambia.

1.4 Study Objectives

1.4.1 Major Objective of the Study

The major objectives of this study were to:

1. Confirm the establishment of the two exotic parasitoids, *C. vestalis* and *D. collaris*, in Lusaka Province of Zambia, and
2. Assess the effectiveness of *C. vestalis* as a biological control agent of *P. xylostella* under laboratory conditions.

1.4.2. Specific Objectives of the Study

The specific objectives of the study were to:

i. Establish the presence of the exotic parasitoids, *Cotesia vestalis* (Haliday) and *Diadromus collaris* (Grav.), in and around their 1977-1984 period release sites in Lusaka Province, Zambia, for biological control of *P. xylostella*.

ii. Determine the pesticides used by local farmers in the Chongwe, Kafue and Lusaka Districts on common vegetable pests and their impact on the exotic parasitoids, *C. vestalis* and *D. collaris,* of *P. xylostella*.

iii. Determine the phenology of *P. xylostella* and identify its parasitoids in the selected study area.

iv. Assess the effectiveness of *C. vestalis* as a biological control agent of *P. xylostella* under laboratory conditions.

1.5 Hypotheses Tested in the Study

This study tested the following five hypotheses:

i. That the exotic parasitoids of *P. xylostella*, namely *C. vestalis* and *D. collaris*, released in Lusaka Province during the 1977-1984 period in a biological control programme for the vegetable pest, had established in Zambia.

ii. That use of pesticides by vegetable farmers impacted negatively on the exotic parasitoids of *P. xylostella* resulting in low rates of parasitisms of both larvae and pupae of the crop pest.

iii. That the established exotic parasitoids of *P. xylostella* in the Lusaka Province occurred in densities that could not effectively control this vegetable pest due to the impacts of insecticides used to control *P. xylostella* by vegetable farmers on them.

iv. That there were many local parasitoids (these are parasitoids found in the field that were not reported to have been released into the environment in Zambia for biological control programme purposes) of larvae and pupae of *P. xylostella* in Zambia, some of which had the potential for use in a successful biological control programmes for the vegetable pest.

CHAPTER 2. LITERATURE REVIEW

2.1 Taxonomy of the diamondback moth, *Plutella xylostella*

The scientific classification of *P. xylostella* is as follows:

Kingdom: Animalia

Phylum: Arthropoda

Class: Insecta

Order: Lepidoptera

Family: Plutellidae

Genus: *Plutella*

Species: *Plutella xylostella* (L.)

Common names of the Vegetable Pest:

English: diamondback moth

Tonga/Nyanja: Sefa-sefa

Spanish: Plutella, Palomilla de Dorso Diamante, Rasquiña

There have been several taxonomic changes in the nomenclature of *P. xylostella* from its initial description by Linnaeus in 1758 (Moriuti, 1986; Shaw, 2003).

2.2 Origin and Geographical Distribution of *Plutella xylostella*

Plutella xylostella is a cosmopolitan pest of cruciferous crops and it is not known when it was introduced into Zambia. However, it was first reported in Zambia by the Commonwealth Institute of Entomology in 1967 (CABI, 2007). It is believed to have

originated from the Mediterranean area (Harcourt, 1954; Talekar *et al*., 2002), although Kfir (1998) speculated that *P. xylostella* might have originated from the Cape Flora of South Africa based on the large number (175) of wild plant species in the Brassicaceae recorded from South Africa (Jordaan, 1993), on which *P. xylostella* may develop. He also referred to the presence of many species of *P. xylostella* parasitoids and a bisexual form of the pupal parasitoids *D. collaris* in South Africa which may suggest that *P. xylostella* might have originated in southern Africa.

Plutella xylostella is distributed in all parts of the world where plants of the family Brassicaceae occur (Hill and Waller, 1988; Talekar and Shelton, 1993; CABI, 2007). The insect does not survive rigorous winters (Honda, 1992) but it can develop and complete its life cycle from about 10-35^{0}C (Hardy, 1938). In North America, *P. xylostella* has been collected as far north as Edmonton in Canada while Hagerty *et al*. (2008) reported the first collection of *P. xylostella* from the interior of Alaska which suggests that with the predicted continued moderation of the climate due to global warming, this species has the potential of becoming an increasingly important agricultural pest in Alaska.

2.3 Biology and Life History of *Plutella xylostella*

The biology and life history of *P. xylostella* is well known (Marsh, 1917; Harcourt, 1954, 1957; Honda, 1992; Pivnick *et al.*, 1990; Sakanoshita and Yanagita, 1972; Yamada and Koshihara, 1978; Talekar and Shelton, 1993; AVRDC, 1987). It is a holometabolous insect with four distinct developmental stages: egg, larva (four

instars), pupa and adult. There can be several to many generations per growing season (multivoltine). For example, in the tropics breeding may be continuous, with as many as 15 or more generations per annum (Hill and Waller, 1988). Under summer conditions in temperate regions, *P. xylostella* takes about 32 days to develop from egg to adult. However, the time to complete a generation may vary from 21 to 51 days depending on weather and food under field conditions (Harcourt, 1957). In Zambia, the development from oviposition to adult emergence takes approximately 14 to 18 days under field conditions; field generations usually overlap and all four-life stages may be present in the field at the same time (Ahmed, 1977).

Egg:

The eggs of *P. xylostella* are oval and flattened, and measure 0.44 mm long and 0.26 mm wide. Eggs are yellow or pale green in colour, and are deposited singly or in small groups of two to eight eggs in depressions on the surface of foliage or occasionally on other plant parts (Harcourt, 1957; Justus *et al*., 2000). Adult females may deposit 250 to 300 eggs in their reproductive lifetime but the average is probably about 150 eggs. Development time averages 5.6 days at 30^0C. The adult, being nocturnal, lays eggs during the night; peak oviposition occurs between 1900-2000 h (AVRDC, 1987; Pivnick *et al*., 1990).

Larva:

The larva of *P. xylostella* is eruciform (i.e. caterpillar-like or caterpillar-shaped) (Fig. 2.1) and has five pairs of prolegs. There are four larval instars. The first instar larvae are leafminers (Section 2.5). The larvae are colourless in the first instar but thereafter are green. If later instars are disturbed, they wriggle violently, move backward, and spin down from the plant on a strand of silk. The total larval period varies from 14 to 28 days depending upon ambient temperatures (Hill and Waller, 1988).

Pupa:

The pupa of *P. xylostella* is obtect (i.e. with its appendages more or less glued to the body) (Fig. 2.2). Pupation occurs in a loose silk cocoon about 7-9 mm long, usually formed on the lower or outer leaves of the host plant. In cauliflower and broccoli, pupation may occur in the florets. The pupa is greenish and the pupal stage averages about 8.5 days (range 5 -15 days) in duration (Harcourt, 1957; Capinera, 2001).

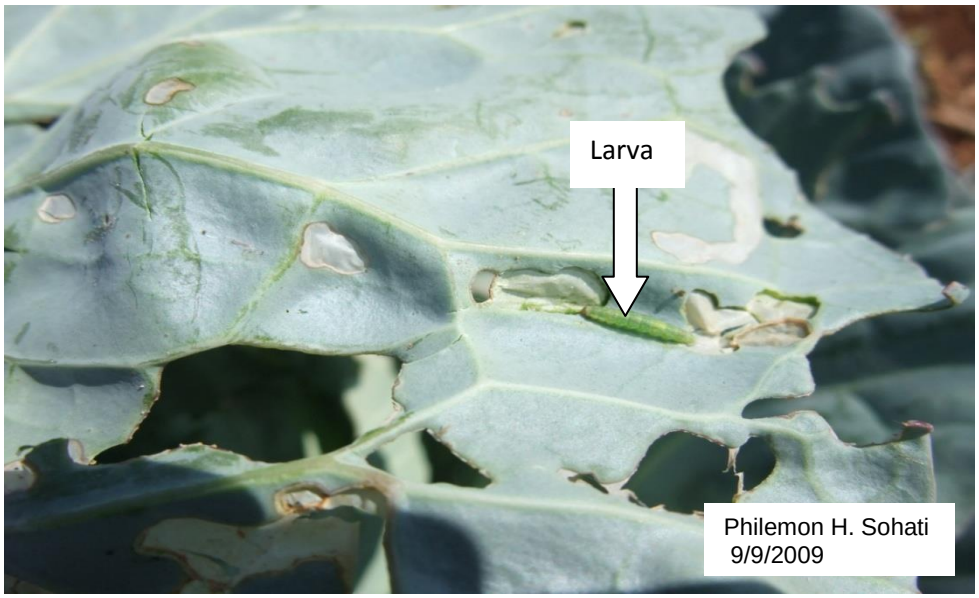

Fig. 2.1. Larva of *Plutella.xylostella* (see arrow) on *Brassica oleracea* var. *capitata.*

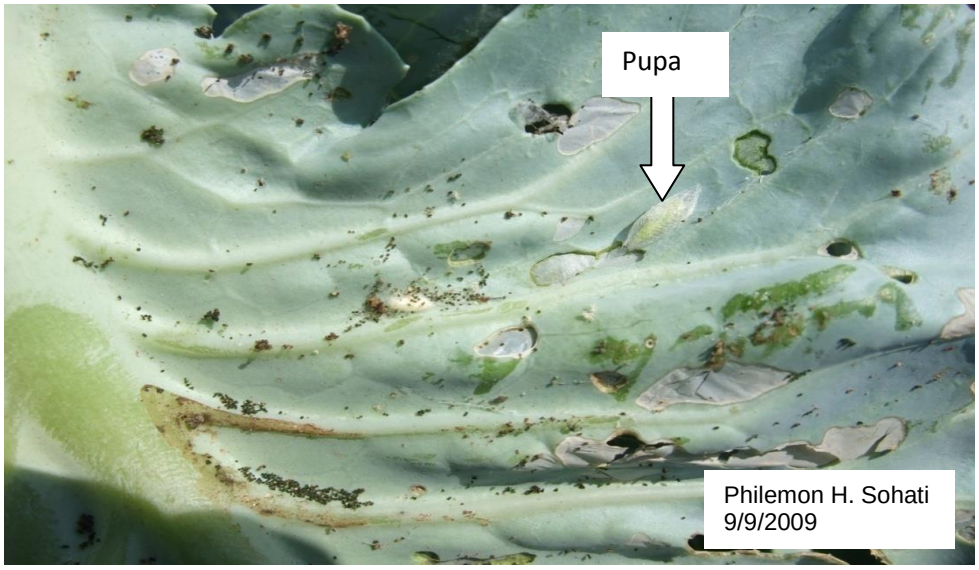

Fig. 2.2. Pupa of *Plutella xylostella* (see arrow) on *Brassica oleracea* var. *capitata.*

Adult:

The adult males and females of *P. xylostella* are small, slender, greyish-brown moths about 6 mm long. The adults have a wingspan of about 15 mm. There are three pale triangular marks along the hind margin of each forewing, and when the wings are closed these marks form a diamond pattern, which gives the moth its common name (Fig. 2.3). The hind wings have a fringe of long hairs. Adult males and females live for about 12 and 16 days, respectively; females deposit eggs for about 10 days. The moths are weak fliers, usually flying within 2 m of the ground, and not flying long distances. However, they can be carried by the wind for considerable distances (Harcourt, 1957; Talekar and Shelton, 1993; Capinera, 2001; Chapman *et al*., 2002).

Fig. 2.3. Adult *Plutella xylostella* on *Brassica oleracea* var. *capitata*.

2.4 Hosts of *Plutella xylostella*

Plutella xylostella is an oligophagous insect herbivore restricted almost exclusively to members of the Brassicaceae (Table 2.1).

2.5 Economic importance of *Plutella xylostella*

The estimated annual crop losses dueto the pest are *ca* US$ 16 million in India (Mohan and Gujar, 2003), *ca* US$ 40-70 million for cabbage andUS$ 0.4 million for broccoli in Texas (Shelton, 2004). *Plutella xylostella* outbreaks can cause as much as 90% crop loss in Southeast Asia (Verkerk and Wright, 1996), and 52% yield loss on cabbage in India (Krishnamoorthy, 2004), 80-99% damage on summer cabbage in China (Zhao *et al.*, 1996) and 12-49 t ha^{-1} of cabbage heads in Ethiopia (Ayalew, 2006) have been reported.

Plutella xylostella is considered to be one of the principal constraints to crucifer crop production worldwide (Talekar, 1992; Talekar and Shelton, 1993; Badenes-Perez and Shelton, 2006. The damage to vegetables is caused by the larvae. Soon after emergence, neonate larvae initiate feeding on foliage. The first instar mines in the spongy mesophyll tissue, whereas older larvae feed from the lower leaf surface and usually consume all tissue under high infestation except the leaf veins, thus creating a “window” in the leaf (Talekar and Shelton, 1993) (Fig. 2.4).

Fig. 2.4. Translucent "windows" caused by *Plutella xylostella* larvae damage on *Brassica oleracea* var. c*apitata*.

The larvae are particularly damaging to seedlings, and may disrupt head formation in common cabbage, broccoli and cauliflower. The mere presence of larvae in the florets can result in complete rejection of the produce by customers, even if the level of plant tissue removal is insignificant. In Zambia, most of the damage is caused during the hot dry months from July to the onset of rains, generally in October or November (Ahmed, 1977). In some areas of Zambia, *P. xylostella* populations are so high that cabbage growing has become almost economically impossible. No monetary value has been estimated for damage levels to vegetables by *P. xylostella* in Zambia but the presence of *P. xylostella* larvae on vegetables at harvest results in considerable loss of crop marketability. Mingochi and Luchen (1995) reported that *P. xylostella* was the priority number one pest of the Brassicaceae family in Zambia, followed by aphids.

Table 2.1. Summary of host plants of *Plutella xylostella* recorded from different parts of the world.

Host Plant	Country	Source
Indian mustard, *Brassica juncea* (L.) Czern	India	Singh & Rawat, 1983; Srinivasan & Krishnamoorthy, 1991, 1992
	South Africa	Charleston & Kfir, 2000
	Mauritius	Facknath, 2000
	USA	Lu *et al.*, 2004
	Guam	Muniappan *et al*., 2001
Collards, *Brassica oleracea acephala*	Guam	Muniappan *et al*., 2001
	USA	Mitchell *et al.*, 2000
		Badenes-Perez *et al*., 2004
Sugar snap peas, *Pisum sativum*	Kenya	Löhr, 2001; Löhr & Rossbach, 2001
Arabidopsis thaliana	U.K.	Barker *et al.*, 2006
Canola, *Brassica napus* L.	South Africa	Mosiane *et al.*, 2003
Collards, *B. oleracea* L. var. *acephala*, Yellow rocket, *Barbarea vulgaris* (R. Br.) var. *arcuata*	USA	Badenes-Perez *et al*., 2005a, b
Sinapis arvensis L. *Erysimum cheiranthoides L.* *Capsella bursa-pastoris (L.)*	USA	Sarfraz *et al.*, 2011
Wallflower, *Cheiranthus cheiri* Candytuft, *Iberis umbellata*, Alyssum, *Lobularia maritime* Stocks, *Matthiola longipetala* Wild mustard, *Brassica kaber*	USA (Hawaii)	Mau & Kessing, 2006
Parthenium hysterophorus	Trinidad & Tobago	Adaku Lawrence, pers. Comm., 2008
Brassica oleracea var. capitata; *Brassica pekinensis*	Zambia	Mingochi & Luchen, 1995

2.6 Control of *Plutella xylostella*

2.6.1 Chemical control of *Plutella xylostella*

Chemical control of *P. xylostella* worldwide is estimated to cost about one billion US dollars annually (Section 1.1). Sandur (2004) estimated the cost of *P. xylostella* control to India alone was 168 million US$ per annum. The conventional method of control of *P. xylostella* on cabbage has been the use of broad spectrum insecticides such as fenvalerate, cypermethrin, dichlorvos, acephate, monocrotophos, and dimethoate (Mingochi and Luchen, 2000; Mukuka *et al.*, 2002).

In Zambia, the insecticides used to control insect pests of Brassicaceae are listed in Table 2.2. Löhr *et al*. (1998) reported that application of synthetic pesticides was the preferred method of controlling pests of crucifers in East Africa, and testing of pesticides remained the major research activity. In Kenya, tests conducted in 1995/96 showed that organophosphates, carbamates and pyrethroids were no longer giving effective control of *P. xylostella* compared with new products such as insect growth regulators, the phenyl pyrazoles and *Bacillus thuringiensis-aizawai* (*Bta*)-based products (Kibata, 1997). Macharia *et al*. (2005) reported that λ-cyhalothrin (Karate™), the most commonly used insecticide, was not effective against *P. xylostella* and its continued use had negative economic returns in four separate trials in Kenya. There was also an increase in complaints from farmers and extension workers about the loss of effectiveness by the majority of commonly used

insecticides against crucifer pests, particularly *P. xylostella*, in Kenya and Tanzania (Macharia *et al*., 2005).

Table 2.2. List of common insect pests of cabbages and pesticides registered for their control in Zambia.

Insect pest	Pesticide[1]
Plutella xylostella	chloropyriphos, monocrotophos, orthene, deltamethrin, cypermethrin, α-cypermethrin, λ-cyhalothrin, fenvalerate, metamidophos, cartap hydrochloride, dichlorvos, dimethoate, carbofuran, *Bacillus thuringiensis* var *kurstaki*
Brevicoryne brassica *Lipaphis erysimi* *Myzus persicae*	dimethoate, chloropyriphos, monocrotophos, metamidophos, α-cypermethrin, deltamethrin, methomyl, pirimicarb, malathion
Bagrada hilaris	monocrotophos, λ-cyhalothrin, α-cypermtherin, dimethoate, dichlorvos
Hellula undalis	chloropyriphos, monocrotophos, Orthene, deltamethrin, cypermethrin, α-cypermethrin, λ-cyhalothrin, fenvalerate, metamidophos, cartap hydrochloride, dichlorvos, dimethoate, carbofuran. *Bacillus thuringiensis* var *kurstaki*
Agrotis spp.	carbaryl, chlorpyriphos, monocrotophos, cypermethrin, orthene, metamidophos, malathion, fenvalerate, λ-cyhalothrin
Nezara viridula	carbaryl, dimeothoate, monocrotophos, methomyl
Thripss pp. *Frankliniella* spp.	dichlorvos, disulfoton, malathion, endosulfan, formothion, malathion, parathion, sulphur
Bemisia tabaci	monocrotophos, chloropyriphos, cypermethrin, deltamethrin, dichlorvos

[1]Mingochi and Luchen, 2000; Mukuka *et al.,* 2002.

2.6.2 Cultural control of *Plutella xylostella*

Cultural control methods are amongst the oldest techniques of controlling insects and some of them have been used as an alternative control measures due to insect pest resistance to insecticides (Talekar and Shelton, 1993; Peshin and Dhawan, 2009). Some of the cultural control methods that have been used against *P. xylostella* include intercropping, use of overhead sprinkler irrigation, trap cropping, host plant resistance and pheromones (Talekar and Shelton, 1993; Walker *et al*., 2003; Grzywacz *et al*., 2010).

2.6.2.1 Use of trap crops in *Plutella xylostella* control

The use of trap crops has been employed to control various agricultural pests since time immemorial (Koul *et al*., 2004). It involves the planting of a crop to protect the main crop from damage by insects. The trap crop is usually from the same family group as the main crop but different family groups have also been used as long as they are more attractive than the main crop to the target pest. Research on the use of trap crops to control *P. xylostella* increased towards the end of the 20th century because of the increase in the resistance of *P. xylostella* to many conventional pesticides (Koul *et al*., 2004; Talekar and Shelton, 1993).

Indian mustard, *Brassica juncea* (L.) Czern, has been used as an alternative host for *P. xylostella* and the leaf webber, *Crocidolomia binotalis* Zeller (Jayarathanam, 1977; Singh and Rawat, 1983). Srinivasan and Krishnamoorthy (1992) confirmed the distinct preference for oviposition by *P. xylostella* and *C. binotalis* to Indian mustard compared with common cabbage. Their field studies showed that a planting pattern

of 15 cabbage rows followed by paired rows of Indian mustard (the first row sown 12-15 days prior to cabbage and the other sown 25 days after cabbage planting) was the most promising pest management system. Charleston and Kfir (2000) compared cabbage with four potential trap crop plants (cauliflower, Chinese cabbage, broccoli and Indian mustard) and results indicated that Indian mustard had the greatest potential as a trap crop because the mean number of *P. xylostella* eggs per leaf was significantly greater on this trap crop than the others tested while larval survival was significantly lower on Indian mustard.

Badenes-Perez *et al*. (2004) evaluated three potential trap crops (the glossy and waxy collards, *Brassica oleracea* L. variety *acephala*, Indian mustard, *B. juncea* (L.) Czern and yellow rocket, *Barbarea vulgaris* (R. Br.) variety (*arcuata*) for the control of *P. xylostella*. Their results showed that yellow rocket was the best candidate for use as a trap crop because it was highly attractive for oviposition and the larvae did not survive on it. Similar results were reported by Lu *et al*. (2004) and Badanes-Perez *et al*. (2005a, b). Lu *et al*. (2004) proposed the term 'dead-end trap cropping' referring to a plant that is highly attractive for oviposition by an insect pest such as yellow rocket, but on which offspring of the pest cannot survive. George *et al.* (2009) reported that three times as many eggs were laid on cauliflower plants that were 'unprotected' compared with those 'protected' by a trap crop of white mustard, *Sinapis alba* L.

2.6.2.2 Intercropping

Intercropping is the growing of two or more crop species on same piece of land at the same time. It reduces pest populations because plants act as physical barriers to movement of the insect pests and also may act as a reservoir for natural enemies and may have a repellent effect to the pest (Talekar *et al*., 1986; Capinera, 2001). Sithanantham (1999) in Nairobi reported that the effect of intercropping the leafy vegetable *Cleome gynandra* on *P. xylostella* at different treatment levels of cabbage to *C. gynandra* row ratios. He found that a 1:1 ratio was more effective than ratios of 2:1, 4:1 and 6:1. Similarly, Rance (2005) reported that intercropping *C. gynandra* with cabbage at 1:1 and 2:1 (*C. gynandra*: Cabbage) had a repellent effect on *P. xylostella* in Zimbabwe.

Intercropping of cabbage and tomato is reported to reduce *P. xylostella* infestation in cabbage (Facknath, 2000); while Asare-Bediako *et al*. (2010) found that intercropping cabbage with onion, tomato or pepper was as effective as spraying the cabbage with chlorpyriphos. Intercropping with sage, *Salvia offinalis*, thyme, *Thymus vulgaris* and white clover, *Trifolium repens* was found to consistently reduce damage to Brussels sprouts by *P. xylostella* (Dover, 1985, 1986). Some intercrop plants, such as garlic, onion and tomato have a repellent effect on *P. xylostella* (Endersby and Morgan, 1991; Facknath, 2000; Stöll, 2003; Silva-Aguayo, 2007).

2.6.2.3 Overhead sprinkler irrigation

Several authors (cited by Talekar and Shelton, 1993) have described the use of overhead sprinkler irrigation for the control of *P. xylostella* on cabbage. It is equally

documented that rainfall is an important mortality factor for *P. xylostella* young larvae and disrupts adult activities especially if it rains at dusk when the adults are mating (Talekar *et al*., 1986; Capinera, 2001).

2.6.2.4 Use of mosquito netting

In smallholder crops in Africa, the use of mosquito netting to cover small-scale cabbage fields has proven successful. Every day, the crop is covered with these fine-mesh nets at dusk and nets are removed again the next morning. Such labour-intensive practice is only feasible for small cabbage fields (Capinera, 2001).

2.6.2.5 Use of pheromones

Synthetic *P. xylostella* pheromones are available and mainly used for monitoring pest populations and guiding insecticide sprays in the field (Capinera, 2001). Although these traps cannot be used to predict the size of an outbreak, the trap counts can provide an early warning of an infestation. Higher concentrations of pheromones can be used for mating disruption, i.e. by confusing *P. xylostella* adults so they are less able to find mates (Talekar and Shelton, 1993; Grzywacz *et al*., 2010; Capinera, 2001).

2.6.2.6 Host plant resistance

There have been various reports of cabbage plants showing resistance to *P. xylostella*. Two types of resistance have been identified from the United States North-eastern Plant Introduction Station germplasm (Eigenbrode *et al*., 1990). A major component of resistance is the presence of leaf wax. Glossy varieties lacking the normal waxy bloom and therefore green rather than greyish green are somewhat

resistant to larval damage (Capinera, 2001). In normal bloom cabbage types, resistance is chemically based and elicits antibiosis or non-preference in the larvae (Talekar and Shelton, 1993). However, *P. xylostella*-resistant cabbage cultivars are not commercially available (Ivey and Johnson, 1997).

2.6.3 Biological control of *Plutella xylostella*

Kfir (2003) reported that although *P. xylostella* is regarded as a serious pest of crucifer crops in Africa, there is paucity of published information about the pest and its natural enemies. Most of the published information is from South Africa. Published information has increased to some extent as a result of publications by the German Agency for Technical Co-operation (GTZ) Integrated Pest Management Horticulture Project for Eastern and Southern Africa Region initiated in 1994 with its headquarters in Nairobi, Kenya (Varela *et al.*, 2003).

2.6.3.1 Use of parasitoids

Many parasitoid insect species have been recorded on *P. xylostella*, attacking its egg, larval or pupal stages (Table 2.3). The larval parasitoid, *C. vestalis*, pupal parasitoid, *D. collaris* and larval-pupal parasitoid, *O. sokolowskii* were reported in Zambia (Ahmed, 1977; Yaseen, 1978). The National Biological Control Programme based at Mt. Makulu Research Station in Zambia released the two imported exotic parasitoids of *P. xylostella* namely, *C. vestalis* and *D. collaris*, in three areas of Lusaka Province between 1977 and 1984 (Albert Chalabesa, personal communication, 2008). The areas are Lusaka West before Westwood Police Post, Mwansa's Farm, Makeni and

Amorati farm, Shimabala area West of Chipongwe Post Office. The parasitoids were imported from Pakistan into Zambia for the control of *P. xylostella.*

Table 2.3. Parasitioids used in biological control of *Plutella xylostella*.

Parasitoids	Family	Country	Source
Trichogramma sp.	Trichogrammatidae	Jamaica	Alam, 1992; Shaila, 2007
		Malaysia	Lim, 1986
		Taiwan	Klemm *et al*., 1992
Trichogramma chilonis Ishii	"	Japan	Okada, 1989
	"	India	Jayarathanam, 1977
Trichogrammatoidea bactrae Nagaraja	"	Thailand	Okada, 1989
		India	Jayarathanam, 1977
Trichogrammatoidea sp.	"	Malaysia	Lim, 1986
		Taiwan	Klemm *et al*., 1992
Cotesia ippeus Nixon	Braconidae	Australia	Wilson, 1960; Goodwin, 1979; Waterhouse, 1992
Cotesia vestalis (Kurd.)	"	Australia	Wilson, 1960; Goodwin, 1979; Waterhouse, 1992
		Benin	Goudegnon *et al*., 2000
		Cape Verde Islands	Cock, 1983
		Ethiopia	Ayalew & Ogol, 2006
		India	Jayarathanam, 1977
		South Africa	Ullyett, 1947; Kfir, 2003
		Trinidad	Yaseen, 1978
		Zambia	Ahmed, 1977; Yaseen, 1978

Continued on next page

Table 2.3.continued...

Parasitoids	Family	Country	Source
Diadegma spp.	Ichneumonidae	Ethiopia	Ayalew & Ogol, 2006
Diadegma semiclausum	"	India	Jayarathanam, 1977
Diadegma insulare (Viereck)	"	Jamaica	Alam, 1992; Shaila, 2007
Diadegma eucerophaga Hortm	"	New Zealand	Muggeridge, 1939
		Australia	Muggeridge, 1939; Waterhouse, 1992
		Malaysia	Lim &Ko, 1975; Ooi & Kelderman, 1977
Diadegma mollipla	"	Kenya	Kibata, 1997; Odour *et al.*, 1997; Löhr & Kfir, 2002
Diadegma rapi (Cam)	"	Australia	Wilson, 1960; Goodwin 1979; Waterhouse, 1992
Microplitis plutellae Muesebeck	Braconidae	USA	Parker, 1971
		Cape Verde Islands	Cock, 1983
Oomyzus sokolowskii	Eulophidae	Cape Verde Islands	Cock, 1983
		Ethiopia	Ayalew & Ogol, 2006
		India	Jayarathanam, 1977
		Kenya	Kibata, 1997; Odour *et al.*, 1997; Löhr & Kfir, 2002
		South Africa	Kfir, 2003

Continued on next page

Table 2.3.continued

Parasitoids	Family	Country	Source
		Trinidad	Yaseen, 1978
		Zambia	Yaseen, 1978
Oomyzus sp.	"	Jamaica	Alam, 1992; Shaila, 2007
Diadromus collaris	Ichneumonidae	Australia	Wilson, 1960; Goodwin, 1979; Waterhouse, 1992
		India	Jayarathanam, 1977
		Malaysia	Lim & Ko, 1975; Ooi & Kelderman, 1977
		New Zealand	Muggeridge, 1939; Waterhouse, 1992
		South Africa	Kfir, 2003
		Zambia	Ahmed, 1977; Yaseen, 1978
Aphanogmus fijiensis	Ceraphronidae	Jamaica	Alam, 1992; Shaila, 2007
Horismenus sp.	Eulophidae	Jamaica	Alam, 1992; Shaila, 2007
Catoloccus sp.	Chalcididae	"	"
Pteromalus sp.	Pteromalidae	India	Jayarathanam, 1977
		South Africa	Kfir, 1996
O. sokolowskii	Eulophidae	India	Jayarathanam, 1977
Tetrastichus sp.	"	"	"
Tetrastichus sp.	Ichneumonidae	South Africa	Kfir, 1996

2.6.3.2 Taxonomy of parasitoids of *Plutella xylostella*

Plutella xylostella is attacked by several parasitoids belonging to the order Hymenoptera. The stages attacked by members of this order are the eggs, larvae and pupae. Only the Trichogrammatidae family has been reported attacking the eggs. Most of the parasitoids that have been used in biological control of *P. xylostella* attack the larvae and/or the pupae stages. It is therefore important that in order for any biological control programme to be successful it must have an accurate identification of both the natural enemy and the host. The primary hymenopteran parasitoids of *P. xylostella* belong to the following families: Braconidae, Eulophidae, Ichneumonidae and Trichogrammatidae.

The genus *Cotesia* (Hymenoptera: Braconidae) was erected by Cameron in the 19th century and *Apanteles* Foerster was listed as a synonym until the generic reclassification of Microgastrinae: Braconidae by Fitton and Walker (1992). *Cotesia* is a large group of primary parasitoids of Lepidoptera, and Rattan *et al*. (2006) recorded about 1500-2000 species worldwide. They also reported that many *Cotesia* species are important natural enemies of agricultural and forestry pests, and several have been used in temperate regions of the world. They have also become successful biological control agents of lepidopteran pests in the tropics. *Cotesia plutellae* (formerly *Apanteles plutellae*) belongs to the braconid subfamily Microgastrinae (Fitton and Walker, 1992) and was originally described in 1912 by Kurdjumov from material reared from *P. xylostella*. *Cotesia plutellae* should now be referred to as *C. vestalis* (Shaw, 2003).

Diadegma mollipla (Holmgren) belongs to the subfamily Campopleginae and Ichneumonidae family. It has been reported as a larval-pupal parasitoid of *P. xylostella* (Smith and Villet, 2001; Mosiane *et al*., 2003; Nofemela and Kfir, 2008). *Diadromus collaris* Gravenhorst (formerly *Thyreella collaris*) belongs to the subfamily Ichneumoninae and family Ichneumonidae (Fitton and Walker, 1992). It attacks the pupae of *P. xylostella* and has been widely introduced in biological control programmes in various countries (Yaseen, 1978; Talekar and Shelton, 1993; Fitton and Walker, 1992).

The eulophid, *Oomyzus sokolowskii* (formerly *Tetrastichus sokolowskii*) is a primary and facultative hyperparasitoid and it is the only chalcidoid species to have shown any real potential for biological control of *P. xylostella* (Yaseen, 1978; Fitton and Walker, 1992; Smith and Villet, 2001; Sall-Sy and Toguebaye, 2002; Ayalew and Ogol, 2006). This is a gregarious parasitoid with more than 9 small black adults emerging from a single pupa.

2.6.3.3 Measurements of Parasitoid Host Searching Efficiency (á) and Handling time (Th)

Several biological characteristics, among them searching ability, fecundity, longevity and sex ratio, have been used to assess potential efficacy of a parasitoid (Kalyebi *et al.*, 2005). Another important aspect when evaluating the efficiency of a natural enemy is the searching efficiency (attack rate) across arange of densities of the host, i.e., its functional response (Berryman, 1999). The functional response is regarded as central to understanding host-parasitoid dynamics (Hassell, 1982; Houck and Strauss, 1985; Walde and Murdoch, 1988). Searching efficiency, á and handling time, Th,

were estimated from Holling's (1959) disc equation using a non-linear least square curve–fitting programme of R (Crawley, 2007):

$$Na = N [1- \exp (á\ TP/1 + á\ ThN)]$$

Where Na = # of host larvae parasitized, N = # host larvae available, P = # of parasitoids, T = total time of experiment, Th = handling time, á = searching efficiency.

Handling time is the time spent by the parasitoid pursuing, subduing and parasitizing each larva plus time spent preparing to search for the next larva. Searching efficiency is the time at which the parasitoid finds the larva. Holling (1959) described three types of functional responses: type I is a linear, where hosts are attacked at a constant rate until a plateau is attained, beyond this there is no further attack; type II is a curvilinear increase to a plateau and there is also a decrease in searching time as the number of hosts attacked increases and type III is a sigmoid curve rising to a pleateau level which thereafter levels off under the influence of both searching efficiency and handling time.

2.6.3.4 Predators of *Plutella xylostella*

Predators have not been widely used as biological control agents of *P. xylostella* as compared with parasitoids (Lim, 1992). However, there have been reports of predators attacking *P. xylostella* under field conditions and some of them have been experimented upon under controlled laboratory conditions (Miranda *et al*., 2011). The predators of *P. xylostella* recorded in the world are shown in Table 2.4.

2.6.3.5 Use of pathogens

Bacillus thuringiensis (*Bt*) is being used with considerable success in Malaysia (Sarfraz and Keddie, 2005). However, *Bts*-resistant field populations of *P. xylostella* have been detected in several regions, such as Hawaii (Tabashnik *et al*., 1990), Central America (Perez and Shelton, 1997, and Asia (Syed, 1992; Ferre´ and van Rie, 2002). The two most widely used strains of *Bt* for the control of *P. xylostella* are *B. thuringiensis* subsp. *kurstaki* (*Btk*) and *B. thuringiensis* subsp. a*izawai* (*Bta*).

Granulovirus (GV) has also been found to be one of the key mortality factors in many populations of *P. xylostella* around the world (Abdul, 1992; Talekar and Shelton, 1993; Asayama and Osaki, 1970, Subramanian *et al*., 2010). Studies have shown that GV is infective to its larval host (Farrar *et al*., 2007; Kadir *et al*., 1999; Grzywacz *et al*., 2002, 2010). In recent studies by Dezianian *et al*. (2010), the results from pathogenicity test of GV to *P. xylostella* using the leaf disc method showed that first, second and third instars of *P. xylostella* were significantly susceptible to infection by *P. xylostella* PxGV.

In China, a nucleopolyhedrovirus isolated from *P. xylostella*, PxMNPV was three to four log cycles more potent against the pest than either AcMNPV or AfMNPV (Kariuki and McIntosh, 1999).

Table 2.4 Predators of *Plutella xylostella.*

Insect order	Family	Name	Status	Source
Coleoptera	Coccinellidae	*Hippodomia* sp	Egg	Alam, 1992
		Coleomegilla sp.	Larva	"
	Carabidae	*Chlaenius micans* (F.),	Larva	Suenaga & Hamamura, 1998
		C. posticalis	"	
	Staphylinidae	*Belonuchus*	Larva	Alam, 1992
		Gagates	Pupa	"
Diptera	Syrphidae	*Toxomerus* sp.,	Larva	"
Hemiptera	Reduvidae	*Coranus* sp.	Larva	Shaila, 2007
Pentatomidae		*Podisus nigrispinus* (Dallas)	"	Silva-Torres *et al.*, 2010
Hymenoptera	Formicidae	*Componatus sericus*,	Larva	Jayarathanam, 1977
		Pheidole spp.	"	"
		Tapinoma melanocephalum	"	"
Neuroptera	Chrysopidae	*Chrysoperla*	Egg	Shaila, 2007
		cornea Stephens	Larva	
		Ceraeochrysa sp	"	"
Araneae	Miturgidae	*Cheiracanthium*	Larva	Silva-Torres
		inclusum (Hentz		*et al*., 2010
Ciconiiformes	Ardeidae	*Bubulcus ibis*	Larva	Jayarathanam, 1977
Passeriformes	Motacillidae	*Motacilla flava*	Larva	"

Entomopathogenic fungi, including *Beauveria bassiana*, *B. brogniarti*, *Paecilomyces fumosoroseus, Verticillium lecanii* and *Metarrhizium anisopliae* have been found to

be highly pathogenic to larvae of *P. xylostella* in India (Gopalakrishnan, 1989; 2001; Kennedy *et. al*., 2002).

Entomopathogenic nematodes, families Steinernematidae and Heterorhabditidae (Nematode: Rhabditida), have been shown to be pathogenic to a wide range of agriculturally important pests and are useful alternatives to chemical insecticides for insect control (Kaya and Gaugler, 1993). *Steinernema carpocapsae* has been the most effective nematode tested against *P. xylostella* causing up to 100% mortality of larvae after 6 hours of exposure and 40% mortality of pupae (Morris, 1995; Ratnasinghe and Hague, 1997), but desiccation of the infective juveniles on foliage reduces their effectiveness (Mahar *et al*., 2004). Mason and Wright (1997) reported that the infective juveniles of two isolates of *Steinernema* spp. (SSL85), two isolates of *Steinernema* spp. (M87), *Heterorhabditis* n.sp., and *Heterorhabditis indicus* their infectivity at different temperatures differed both within and between species, with optimal infection at 20-25°C while desiccation studies revealed more marked differences between the isolate**s.** For example, at 80% relative humidity, survival of approximately 51% of infective juveniles of *Steinernema* spp. (SSL85/21) were observed, compared with 13% for *Steinernema* spp. (M87/45).

Exposure to UV-light (Gaugler and Boush, 1978; Gaugler *et al*., 1992), desiccation (Begley, 1990; Womersley, 1990) and high temperatures (Molyneux, 1985; Grewal *et al.*, 1993) tend to lower the efficacy of entomopathogenic nematodes used in a foliar application (Schroer *et al*., 2005a). Schroer *et al*. (2005b) found that the use of

a polymer formulation together with *S. carpocapsae* for the control of *P. xylostella* larvae has several advantages such as the prevention of nematodes from settling in the spray tank thus securing an even distribution and deposits nematodes on the leaf reducing loss by run-off. It also produces optimal conditions for host invasion by reducing the mobility of the insect and produces ideal conditions for infective juveniles host seeking and invasion. The results obtained by Schroer and Ehlers (2005) indicated that penetration of *S. carpocapsae* in *P. xylostella* larvae occurs within the first hour after application. Thus, the advantage of using a formulation was not to enhance nematode survival but rather to provide optimal environmental conditions that would support nematode invasion of the host on foliage.

CHAPTER 3. MATERIALS AND METHODS

3.1 The Study Area

This study was conducted in the Makeni area of Lusaka District, Zambia (Latitude, 15^0 24'S; Longitude, 28^0 17'E). Lusaka is the capital city of Zambia and also a provincial headquarters of Lusaka Province, covering some 360 $km^{2,}$ which includes Lusaka District. It is located on the central African plateau and lies at an altitude of *ca*1, 250 m above sea level. Lusaka District borders with Central Province and shares boundaries with Chibombo, Chongwe and Kafue Districts (Lusaka City Council, 2005). Fig. 3.2 is a map of study area showing release sites and sampled farm sites in Lusaka Province.

3.1.1 Geography and Soils

Lusaka District is characterized by undulating terrain of less than 10 degrees slope and is dissected by rivers and streams. The principal drainage lies to the eastern part of the District around the Chongwe river (Lusaka City Council, 2005).

The soils are mainly *Fersiallitic* type. They occur largely on parent rock materials rich in ferromagnesian minerals (dolomite, calcerous schist, etc) (Mackel, 1972). They have a moderate base status with a pH range of 5 to 7. Top soil texture ranges from clay to sandy loams, while soil thickness varies from 50 to >300 cm. The relatively wide soil variation is attributed to the proximity of parent materials. *Fersiallitic* soils are suitable for cultivation of a wide range of climatically adapted

crops. They include the most fertile Zambian soils, now widely cultivated (Mackel, 1972).

3.1.2 Climate

Lusaka District enjoys a typical Savannah climate with three distinct seasons, namely, warm and wet season (October/November to April), the cool and dry season (May to August) and the hot and dry season (September to October/November). The District as a whole receives an average annual rainfall of 650mm. The absolute maximum temperatures of 28.9^0C is experienced in October and the absolute minimum temperatures of 9.6^0C in June and July. The mean humidity rate is at 62.8% with the highest humidity in January, averaging 84% (Lusaka City Council, 2005).

3.1.3 Vegetation

The dominant vegetation type of Lusaka District is Miombo shrub, which is mixed degraded woodland consisting of *Branchystegia* spp., *Isoberlina* spp., *Julbernardia paniculata* and *Marquesia* spp. (Archer, 1972). *Acacia-Combretum-Terminalia* Savanna woodlands are the typical open, grassy woodlands on the alluvial soils of South Kabwe, Chisamba, Lusaka, Mazabuka, Choma and Chipata (Fanshawe, 1972). They form the greater part of the maize and cattle country.

3.1.4 Demography

Lusaka District had a population of *ca* 1.1 million at the last census (CSO, 2003). Its population density is just over 3,000 persons per Km^2 and is the most urbanized and

the most densely populated in the country. The population of Lusaka District is dynamic due to urban migration and due to its being the market centre for the whole country (Lusaka City Council, 2005).

3.1.5 Agriculture and Forestry

Close to 10% of the area of Lusaka District is used for cultivation and plantations. There are eight commercial farms located on the district's boundaries with Chibombo, Chongwe and Kafue Districts, while the number of emergent farms is 284, most of which are found in Lusaka East, Lusaka West and South-west of Lusaka. The majority are small-scale farms (*ca* 7000 ha) located all over the 20 agricultural camps of Lusaka District. The main agricultural crops grown include beans, cowpeas, groundnuts, maize, soybeans, wheat and assorted vegetables, while horticulture crops include citrus, flowers and other ornamentals. Cattle, sheep, goats, pigs and poultry are important livestock species kept by farmers in this district. The District has >100 fish ponds stocked with Tilapia (Cichlids) fish. It has only one gazetted forest reserve (Forest reserve number 27) which is located in Lusaka East, near Bauleni compound. The forest reserve plays an important role in protecting the Chalimbana area head waters (Lusaka City Council, 2005).

3.1.6 Vegetable Production in Lusaka District

The producers of vegetables in Zambia can be categorised into small scale farmers (with <5 ha), and medium scale emergent farmers (5-20 ha), as well as larger scale commercial farmers (>20 ha) (Nenguwo, 2004). The main production period for

vegetables is after the rainy season that is from April to October/November. Vegetable production prevails mainly in the dry season and to lesser extent in the rainy season. Garden activities in townships provide a source of vegetables for Lusaka residents. However, the supply cannot meet the demand. Micro-farming in Lusaka contributes to household food security, directly by providing the necessary food such as vegetables and indirectly by generating income (Drescher, 1997).

Lusaka District has several established council markets located almost in every township. The main council market in Lusaka city and in the country is Soweto market which is centrally located. The supply of vegetables to the markets and supermarkets is mainly from the surrounding districts such as Chibombo, Chongwe, Kafue and Mumbwa. Kabaghe *et al*. (2009) studied the three main supply districts of selected vegetables (rape, onion, and tomato) in Soweto market from January 15, 2007 to January 15, 2009 (Table 3.1).

The Makeni farmers run a co-operative society called Panjira Multi-purpose Co-operative Society Limited as an outlet for marketing their vegetables (Fig. 3.1). Some customers order vegetables directly from the individual farmers instead of buying from the Co-operative. This is common among schools and colleges, tourist lodges, hotels, motels and hospitals, which have their own transport. The farms in this area are mostly managed by women whose husbands work in Lusaka city.

Table 3.1. The three main vegetable supply districts for Soweto market (January 15, 2007-January 15, 2009 (Adapted from Kabaghe *et al*., 2009)

Tomato		Rape		Onion	
District	Share (%)	District	Share (%)	District	Share (%)
Chongwe	21.6	Chongwe	71.1	Mugabi	30.3
Lusaka	19.0	Chibombo	12.3	S. Africa	27.5
Mkushi	17.0	Mumbwa	12.0	Lusaka	21.0
Total	57.6		95.4		78.8

Tomato supplied came from a total of 17 districts, rape came from 10 districts, onion came from 19 districts.

Note: Areas outside Zambia were treated as districts such as Mugabi, S. Africa, Tanzania and Zimbabwe. Mugabi is an area on Malawi-Zambia border.

Fig. 3.1. The Panjira Multi-purpose Co-operative Society Limited, Makeni area.

3.2 Establishment of Exotic Parasitoids of *Plutella xylostella* in Lusaka Province, Zambia

The criterion used to confirm establishment of the exotic parasitoids in the Makeni area of Lusaka, was the presence of the parasitoids released in the environment. If the parasitoids were found in the environment and the length of time that had passed from the time of their release into the environment was longer than the parasitoids' longevity (i.e. length of life from hatching from eggs to natural old age death), this was taken to be indicative that the parasitoids had established themselves in the environment of release (Ross *et al*., 1982). The longevity for the female adult larval parasitoid of *P. xylostella*, *Cotesia vestalis* is 14 days (range 6 to 21 days), while that of the female adult pupal parasitoid, *Diadromus collaris* is 36 days (range 11 to 85 days) (Chua and Ooi, 1986).

Three criteria that were used in selecting sampling sites for the exotic *P. xylostella* parasitoids in this study to confirm their establishment in Lusaka Province are:

i. information available on the original release sites of the parasitoids in Lusaka Province;

ii. areas in Lusaka Province where farmers were involved in the production of vegetables especially common cabbage, Chinese

cabbage and rape which are some of the principal hosts of *P. xylostella*;

iii. the ease of accessibility to the gardens and willingness of the vegetable farmers to take part in the research involving their vegetable gardens.

Sampling for *P. xylostella* parasitoids were conducted along diagonal transects (each measuring 33.2 m long) established in and around the original release sites of the parasitoids in Lusaka West and Makeni in 1977-1984 (Fig. 3.2). Only farms that were sampled for parasitoids were geo-referenced using a Global Positioning System (GPS, model Magellan® Triton™ 400) and the way points recorded and later used to construct a map of the survey sites using Macromedia Freehand® computer software. The sampling of farms in Lusaka west, Miller and Liempe was conducted in January and February 2009 while those farms from Makeni area were sampled in September 2009. The farms were sampled once. In order to avoid bias, samples were taken at random along a diagonal transect in the cabbage field, and plants were selected at every 2 m intervals. *Plutella xylostella* larvae and pupae were sampled by examining whole cabbage plant.

The actual plant population was 3500 from the ten farms. Adjusted sample using formula (Bartlett II *et al.*, 2001):

$[(n^1 = 1/ (1/N + 1/n)$

where N is sample population, n^1 is actual sample size and n is sample size from unknown population, was 347 but only 323 plants were sampled because half of Uzakulweni farm plant population was harvested and only 13 plants were sampled instead of 24 plants.

Collected *P. xylostella* larvae were placed on moistened filter paper (Double Rings®) at the bottom of 9 cm diameter Petri dishes to which fresh leaf discs of Chinese cabbage were introduced as food for the larvae. The larvae were not put in individual vials except for the pupae. Chinese cabbage was chosen as food for the larvae because of its rapid growth and was ready to feed the larvae in 5 to 6 weeks after sowing. The larvae were then reared in the insect rearing room, at the School of Agricultural Sciences, University of Zambia where they were kept at a constant room temperature of 26^0C, 50 $\pm$ 5% relative humidity and a 16:8, L: D photoperiod until they pupated, died or a parasitoid(s) emerged from them.

Pupae were placed in individual plastic vials (15 ml) and were maintained under the same laboratory conditions as the larvae, until death, adult emergence or parasitoids emergence from them.

The parasitoids which emerged from both larvae and pupae were identified and recorded. The collected parasitoids were stored in 70 % ethanol. Parasitoid identifications from *P. xylostella* larvae and pupae were confirmed by G. L. Prinsloo of the Biosystematics Division, Agricultural Research Council, Plant Protection Research Institute, South Africa (Identification Job number 2009/222, Appendix 1).

Data analysis: Collected data on the number of *P. xylostella* larvae and pupae data was analyzed using Excel™ version 2007, Microsoft Corporation running on Windows® version 2007, Microsoft Corporation. The numbers were presented as averages. % parasitism = No. of parasitized host remains divided by No. of non parasitized host remains plus No. of parasitized host remains multiplied by 100 (Ayalew *et al*., 2004) or in case of a solitary parasitoid like *C. vestalis*, % parasitism can be defined as No. of larvae/pupae from which a parasitoid emerged divided by the total No. of larvae/pupae exposed multiplied by 100. A 95% confidence interval was calculated using the normal approximation (Steel and Torrie, 1980):

$$p \pm Z_{0.05}\,[[p(1- p)\, p]/n]^{1/2};$$

where p = observed proportion of parasitized larvae in the population, $Z_{0.05}$ = 1.96, and n = total number of observations in the sample.

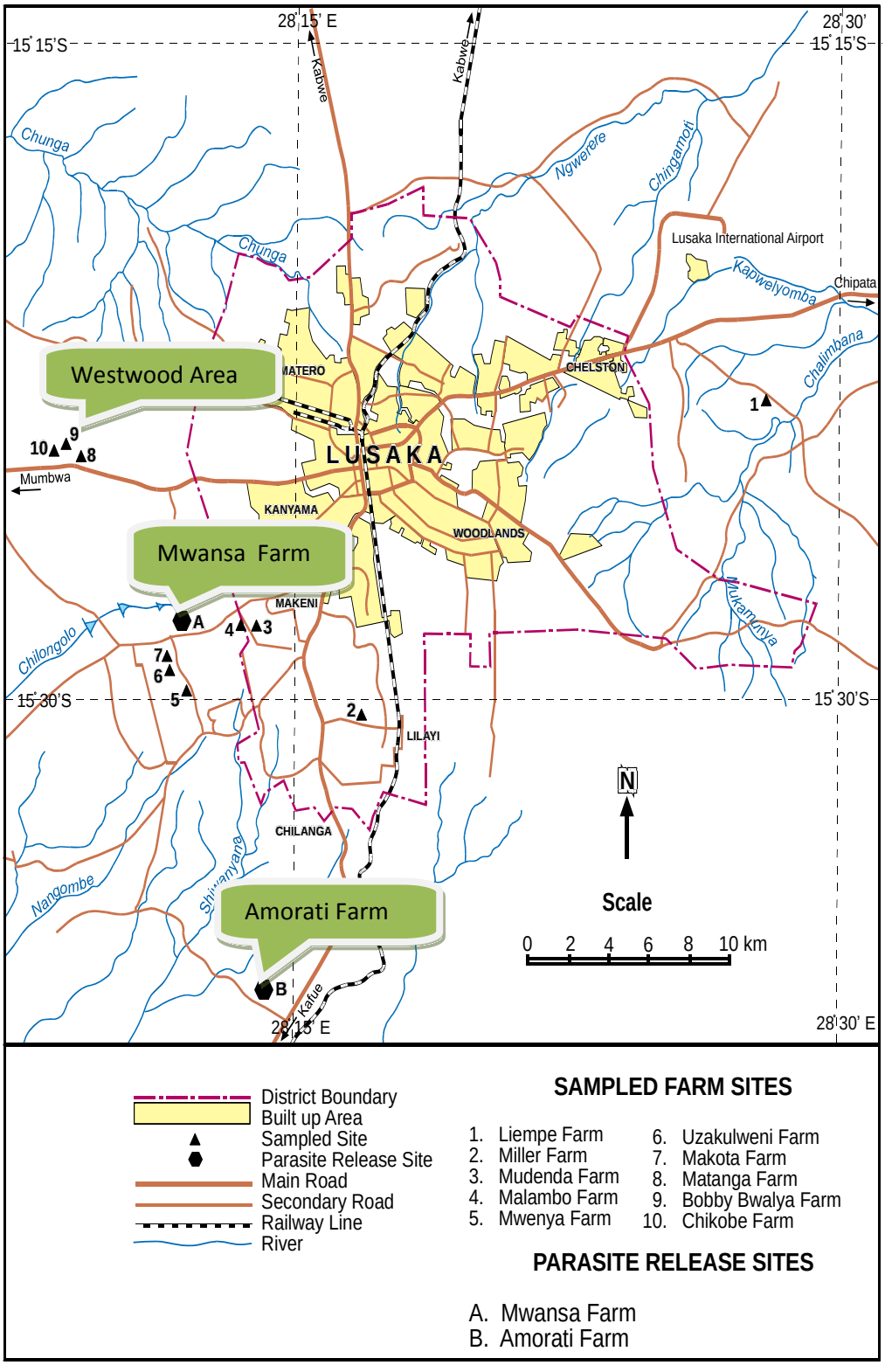

Fig. 3.2. Map of study area showing release sites and sampled Farm sites (Drawn by Joseph Chalila, UNZA, Department of Geography, 2009).

3.3 Survey of Pesticides Used by Local Farmers in Chongwe, Kafue and Lusaka Districts on Common Vegetable Pests and their Impact on the Released Exotic Parasitoids of *Plutella xylostella*

3.3.1 Pesticide survey

In order to assess the pesticides used in the environment in which the exotic parasitoids, *C. vestalis* and *D. collaris*, were released for the biological control of *P. xylostella* in Lusaka District and surrounding areas, a questionnaire-based survey of selected peri-urban vegetable farmers was conducted (Appendix 2). Assuming that the parasitoids had established themselves in the environment since their releases, some (Bobby Bwalya, Matanga, and Mwansa) but not all of the farms where the parasitoids were sampled were included in the survey. Farms from other areas of Chongwe Kafue and Lusaka districts were also included in the survey. Only households accessible by vehicle, motorbike and bicycle were included in the survey which was done during the rainy season, when most roads in the districts were impassable. The pre-selection of the farmers for the survey was based on the accessibility of their homesteads and that they were vegetable growers.

Basing on the formula and the assumption the population size was not known, the maximum sample size at 95% confidence limit was 384. However, this was adjusted to 107 basing on actual sample of 148 households by the formula:

($n^1 = 1/ (1/N + 1/n)$ where N is sample population, $n^1$is actual sample size and n is sample size from unknown population.A total of 107 questionnaires were

administered to vegetable farmers in Chongwe, Kafue and Lusaka Districts in February 2009 (Fig. 3.3). The sample size was inadequate because of the budget limitations and some of the roads were impassable as the survey was done during the rainy season. Staff from the Department of Field Services in the Ministry of Agriculture and Co-operatives of the Government of the Republic of Zambia assisted in administering the questionnaires.

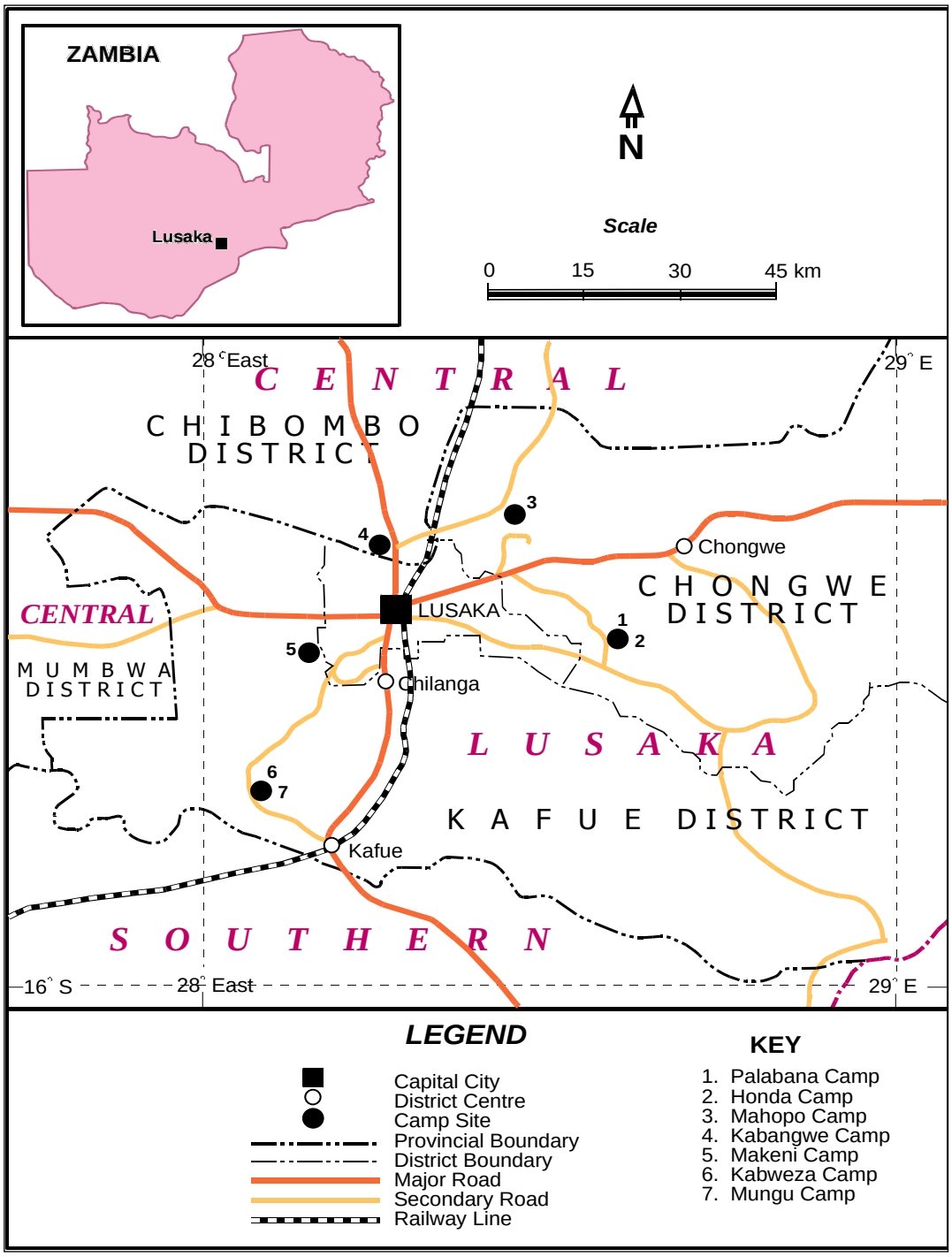

Fig.3.3. Map showing areas involved in the questionnaire survey of vegetable farmers in Chongwe, Kafue and Lusaka Districts (Drawn by Joseph Chalila, UNZA, Department of Geography, 2009).

The pesticide use survey was aimed at determining the types and impacts of the pesticides used by farmers on the exotic parasitoids of *P. xylostella* in case they established in Lusaka Province. If the exotic parasitoids did not establish in their new environment, the types of pesticides used by local vegetable farmers to control *P. xylostella* could probably have contributed to their failure to establish. If, on the other hand, the exotic parasitoids established but occurred at too low population levels to effectively control this vegetable pest, again the pesticide use environment could perhaps provide the answer.

Data Analysis: Statistical analysis of the collected data was performed using the statistical programme for social scientists (SPSS, 2007) and GenStat (2011). Data were presented as averages (absolute values or percentages, depending upon the factor investigated). The data analyzed included: Farmers' socio-economic background and general agricultural practices, pest problems and pest management practices.

3.3.2 Impact of the Pesticides on *Cotesia vestalis*

A stock culture of *C. vestalis* was established in the insectary at the School of Agricultural Sciences from the parasitoids collected from the field sampling in the parasitoid establishment study area. It was further replenished with the parasitoids from the experimental common cabbage field plot located at the School of Agricultural Sciences Field station. To assess impacts of locally used pesticides on exotic parasitoids in the laboratory *C. vestalis* were assayed on 9cm diameter Chinese cabbage leaf discs cut from plants using the base of a 9cm diameter Petri

dish to punch the leaf. Each cut leaf disc was immersed in a pesticide test solution for 10 seconds and then permitted to dry on a corrugated aluminium foil, with the underside of the leaf uppermost, for 1-2 h in order for the insecticide to completely dry up, at room temperature of 24-25^{0}C (Fig. 3.4) (Furlong, 1993 and Cordero *et al.*, 2007). Control leaf discs were immersed in distilled water. Test and control leaf discs were later placed in plastic Petri dishes (9 cm diameter) containing a single, moistened Double Rings® filter paper (9 cm diameter). Considering the time constraint in the study only the immersed disc bioassay method was used to test the impact of pesticides on *C. vestalis.* Another possible method that could have been used in the bioassay is topical applications of pesticides on the parasitoids (Gulzar, 2011).

The number of pesticides for the bio-assay was randomly selected from the 17 pesiticides that were recorded from the pesticide use survey of local farmers (section 3.3.1) and each one was replicated five times. Therefore the pesticides selected for the study were not necessarily the most used in the study areas. Four insecticides were assayed namely acephate at 10, 30, 100, 300, 500, 1000, 1500, 1600, and 1700 ppm, cypermethrin at 2, 4, 6, 8, 10 and 50 ppm, dichlorvos at 4, 6, 8, 8.5, 9, 9.5, and 10 ppm and λ-cyhalothrin at 50, 100, 200, 300, 350, 400, 450 and 500 ppm. Ten unsexed parasitoids were put in individual Petri dishes containing the different bioassay treatments (several dilution of pesticides based on Agro-chemical Company recommendation for *P. xylostella* control). The recommendations were acephate 75% WP (3g/L), cypermethrin 20% EC (1ml/L), dichlorvos 100% EC (3ml/L) and λ-

cyhalothrin 20% EC (1ml/L). A streak of honey was placed on the inside of the Petri dish as a source of food for the parasitoids (Furlong, 1993). Parasitoid mortality was recorded after 12, 24, 36, 48, 60 and 72 h exposure to the pesticide test solution. Parasitoid adults were considered dead if they did not move when gently proded with a needle.

Data analysis: Four factorial experiments in a completely randomized design were carried out to examine the effects of insecticides and time and their interaction on the mortality of the endoparasitoid, *C. vestalis* adults, of *P. xylostella* and were replicated five times. Data were transformed to Arcsin √% prior to analysis. GenStat (2011) was used for analysis of variance to determine any significant differences among treatments, regression analysis was applied to determine the relationship of the significant treatments of the transformed data.

The generalized linear model [model<-glm(y~log(conc), binomial)] of R version 2.12.1 (R Development Core Team, 2010) was used to analyze mortality data as LC_{50} values to estimate the slope and its standard error, with significance tested at the 5 % level (Crawley, 2007). Mortality data used to estimate LC_{50} values were corrected for control mortality by substracting the number of dead larvae in the control treatment from toxicant treatments. Abbott's (1925) formula was used to correct for control mortality. A function called "dose.p" from the MASS library that used logit regression analysis calculated estimated LC_{50} values and their standard errors (s.e.). Using these s.e. values, 95 % Confidence Intervals [CI; (LC_{50} ± (1.96 x s.e.))] were calculated.

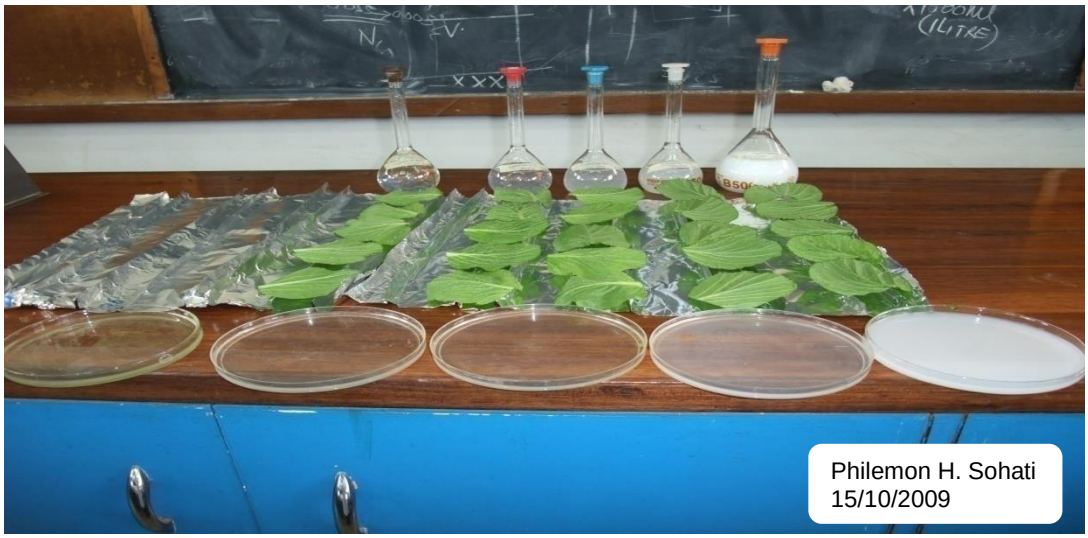

Fig. 3.4. Chinese cabbage leaf discs that have been immersed in pesticide test solutions.

3.4 Determination of the Phenology of *Plutella xylostella* in Selected Study Area and Identification of its Local Parasitoids

Phenology is defined as periodic phenomena that are correlated with climatic conditions and in this case, study of phenology of *P. xylostella* aimed at correlating the life cycle of the pest with weather conditions and parasitoid life cycles. In order to understand establishment of parasitoid in the environment it is important to relate their life cycles to those of their hosts. To determine the phenology of *P. xylostella* in the study area a hybrid cabbage (cultivar Pannar®, Star 3308) plot was established at the University of Zambia, School of Agricultural Sciences, Field Station (Longitude, $15^{0}39^{'}463^{''}$S; Latitude, $28^{0}33'610''$E; altitude, 1274 m) in February 2009. The guidelines followed in establishing the plot were those recommended by Mingochi and Zulu (1995). The inter-row spacing was 0.60 m while the intra-row spacing was

0.45 m and the rows were 20 m long. Cabbage was first sownon a nursery at the Field Station on January 29, 2009. It was transplanted to the study plot on February 25, 2009. On March 5, 2009, basal granular fertilizer of compound D (10N:20P:10K:5S) at 800 kg per ha was applied by hand to enhance the growth of cabbage. Top dressing granular fertilizer of Urea (46%N) was applied at 200 kg per ha. The plot was pesticide-free and was hand weeded using hoes. This hybrid cabbage was chosen because it is tolerant to drought and diseases, responds well to good crop husbandry practices, and yields well in well drained, light soils with adequate irrigation facilities. In 2009, a total of three 20 m-wide staggered cabbage plots (cv. Star 3308), 46, 23 and 23 m long, were sown adjacent to each other on February 25, June 26 and September 21, 2009, respectively. Phenology data is presented as population densities of the host/pest during one year cycle.

The sample unit was the common cabbage plant. In order to determine the distribution of *P. xylostella* larvae, a preliminary sample of 20 cabbage plants was taken along a diagonal transect. From these 20 plants, the mean (average number of larvae per plant) and the variance were calculated. The mean was 10.45 larvae per plant and variance was 135.39. Since variance was larger than the mean, then the distribution was clumped. Typically for pest management sampling, the desired precision is 0.2 to 0.3. This meant that the standard error of the estimate was to be within 20-30% of the true mean. The formula used to determine sample size was

$N = [s/(m*D)]^2$,

where N = sample size, s= standard deviation (square root of variance), D = desired precision and m = mean (Southwood, 1984). The sample size was calculated as 31 cabbage plants. Since the germination was not very good which resulted in low plant population, 27 cabbage plants were sampled instead.

The sampling plan used for *P. xylostella* larvae and pupae from the cabbage plants was along the established diagonal transects. The larvae and pupae of *P. xylostella* were removed from common cabbage plants sampled. The collected larvae and pupae of *P. xylostella* were reared in the insectary for parasitoid emergence. The number of larvae per plant and number of parasitoids emerging from the reared larvae and pupae of *P. xylostella* were recorded. The selection of plants to sample for the pest in a crop row was to the nearest plant after every 0.90 m in a diagonal transect. The same plant was sampled twice per week after four days and was only sampled once during the study.

The local parasitoids of *P. xylostella* were collected as described in Section 3.2. The parasitoids were reared from larvae and pupae of *P. xylostella* collected from the common cabbage plants sown at the School of Agricultural Sciences field station (see Section 3.4). The identity of the collected parasitoids was made with the use of published literature (Polaszek, 1998; Azidah *et al*., 2000). Parasitoid identifications from *P. xylostella* larvae and pupae were confirmed by G. L. Prinsloo of Biosystematics Division, Agricultural Research Council, Plant Protection Research Institute, South Africa (Identification Job number 2009/222, Appendix 1).

3.5 Assessment of the Effectiveness of *Cotesia vestalis* as a Biological Control Agent of *Plutella xylostella* in Zambia under laboratory conditions

The Chinese cabbage (Pannar®variety: Granat) was used to rear *P. xylostella* larva. Plants were grown in a greenhouse (Fig. 3.5) under uncontrolled conditions at the School of Agricutlural Sciences, University of Zambia, Great East Road Campus, Lusaka, Zambia. The Chinese cabbage plants were grown in 13 cm diameter plastic pots placed in screened cages (L 1.5 m x W 0.72 m x H 1.4 m; Fig. 3.6) to prevent other insects interfering with the plants. Each cage contained nine trays (L0.40 m x W 0.30 m) with six pots per tray. The plants were to be grown in properly cured compost soil. The compost soil was obtained from Kasisi Agricultural Training Centre in Chongwe district. It was mixed with compost soil from School of Agricultural Sciences. The soil obtained from Kasisi Agricultural Training Centre was properly cured organic soil but was insufficient for the needs of the study, and hence had to be mixed with compost soil obtained from the School of Agricultural Sciences Field Station. The two compost soils were mixed at the ratio of 1:1. The plants were used in experiments when they were five to six weeks-old. They were placed in a rearing cage of L 0.55 m x W 0.27 m x H 0.45 m dimensions (Fig. 3.7). The top and bottom of the cage were covered with fibre glass, while the sides were covered with fine nylon netting. A stock culture of *P. xylostella* was established from a pair of adults (male and female) collected from the cabbage plot at UNZA Field station. The culture was maintained at a temperature of 26^0C ± 2^0C using a split wall mounted air conditioner (Galanz®) and a photoperiod of 16L:8D which was maintained by a Wexcel® timer. The relative humidity of 50% ± 5% was controlled

by placing buckets of water in the insectary with a Barigo® hygrometer used to measure humidity. The emerging *P. xylostella* adults were supplied with 5% honey solution poured on a 4.5 cm diameter Petri dish with cotton wool as food source upon emergence.

Fig. 3.5. The greenhouse containing cages and Chinese cabbage plants.

Fig. 3.6. Nylon screened cage for growing Chinese cabbage in greenhouse.

Fig. 3.7. Rearing cage of *P. xylostella* in the insectary.

A stock culture of *C. vestalis* was established in the insectary at the School of Agricultural Sciences from the parasitoids collected from the parasitoid sampling survey. It was further replenished with the parasitoids from the experimental common cabbage field plot located at the School of Agricultural Sciences Field station. One day-old *C. vestalis* parasitoid female and male were put in a 50 ml vial (Fig. 3.8) with 10 mm diameter hole drilled on top of the cap for provision of air. This was capped with a nylon netting to prevent the parasitoids from escaping. A streak of honey was placed on the inside of the vial as a source of food for adult parasitoids. The insects were permitted to mate for 24 h, after which one female was put in 9 cm diameter Petri dish containing different second instar larvae of *P.*

xylostella host density (5, 10, 25, 50 and 100) with a moistened filter paper (Double Rings®) and untreated fresh Chinese cabbage leaf disc.

Fig. 3.8. A 50 ml clear plastic vial used for mating *P. xylostella* parasitoids.

The second instar larvae were used because they are mostly preferred by *C. vestalis* (Kawaguchi and Tanaka, 1999). The Petri dish was securely held with a rubber band to prevent any parasitoid from escaping. A streak of honey was placed on the inside of the Petri dish as a source of food for adult parasitoids. Second instar *P. xylostella* larvae were allowed to settle and commence feeding before the introduction of the female parasitoid in the Petri dish. After 24 h the female parasitoid was removed from the Petri dish while the larvae were left in the Petri dish with Chinese cabbage disc as food. Fresh untreated Chinese cabbage leaf discs were supplied as necessary to the Petri dishes. After 11-12 days, the parasitoid cocoons had developed on the

sides of the Petri dish (Fig. 3.9). In terms of development there is one *C. vestalis* larva per diamondback moth larva (caterpillar)

Fig. 3.9. An opened 9 cm diameter Petri dish showing cocoons of *C. vestalis* and pupae of *Plutella xylostella*.

that is a solitary parasitoid. Larval mortality was recorded, that is, all the larvae that did not develop to pupal stage were considered attacked by the parasitoids after taking care of natural mortality. However, no *P. xylostella* larvae died before the end of the experiment. A total of 50 parasitoids were used in this experiment. The experiment was carried out using completely randomized design and each treatment was replicated five times.

3.6 Measurement of Parasitoid Host Searching Efficiency and Handling time

The parameters on the searching efficiency (á) and handling time (Th) were estimated using Holling's (1959) disc equation using a non-linear least square curve–fitting programme using R (Crawley, 2007).

Data Analysis: The statistical analysis was performed using R version 2.12.1 (R Development Core Team, 2010). Searching efficiency, á and handling time, Th, were estimated from Holling's (1959) disc equation using a non-linear least square curve–fitting programme of R (Crawley, 2007):

$$Na = N [1 - \exp (á\, TP/1 + á\, ThN)]$$

Where Na = # of host larvae parasitized, N = # host larvae available, P = # of parasitoids, T = total time of experiment, Th = handling time, á = searching efficiency.

CHAPTER 4. RESULTS

4.1 Establishment of Exotic Parasitoids of *Plutella xylostella*, in Lusaka Province, Zambia

A total of ten farms were each sampled once to confirm the establishment of the two exotic parasitoids of *P. xylostella* in Lusaka Province, Zambia and to determine which local parasitoids of *P. xylostella* were present in the field. The two parasitoids, *C. vestalis* and *D. collaris*, were collected from five farms and one farm, respectively, 3.5-35 Km from their original release sites. This was indicative that both parasitoids had established themselves in Lusaka Province. Over 30 years had lapsed since both parasitoids were released in Lusaka (1977-1984).

Larval parasitoid, *C. vestalis* was collected from Liempe (University of Zambia) Farm and Miller farm during January and February 2009 sampling (Table 4.1). No *P. xylostella* larvae and pupae were collected from Chikobe, Bobby Bwalya and Matanga farms in Lusaka west. The most dominant parasitoid that emerged from the larvae samples in the laboratory was *C. vestalis* and followed by a pupal parasitoid, *O. sokolowskii* and a hyper-parasitoid of *C. vestalis*, *Aphanogmus* sp. (Hymenoptera: Ceraphronidae). No *D. collaris*, emerged from *P. xylostella* pupae collected from the farms sampled during this period. Parasitism of *P. xylostella* larvae by *C. vestalis* was greater at Liempe Farm (54.9%; n=215) compared with Miller farm (18.2%; n=22). Hyper-parasitism of *C. vestalis* by *Aphanogmus* sp. was 15.3% (n=118) at Liempe farm.

Table 4. 1. Number of *P. xylostella* larvae collected and the associated parasitoids on common cabbage plants.

Sampling Date	Site	No. of larvae	No. of parasitoids	Parasitoid
25.01.09	Chikobe	0	0	0
01.02.09	Bobby Bwalya	0	0	0
01.02.09	Matanga	0	0	0
08.02.09	Liempe	215	118	*C. vestalis*
08.02.09	Miller	22	4	*C. vestalis*
09.09.09	Mudenda	36	0	0
09.09.09	Malambo	160	7	*C. vestalis*
10.09.09	Makota	28	0	0
10.09.09	Mwenya	393	27	*C. vestalis*
10.09.09	Uzakulweni	841	137	*C. vestalis*

Plutella xylostella larvae collected in September 2009 from five farms in Makeni, Lusaka showed that *C. vestalis* was the dominant parasitoid recorded with 16.3% parasitism (n=841) at Uzakulweni farm compared with 6.9% (n=393) at Mwenya's and 4.4% (n=160) Malambo's farm. There were no parasitoids recorded from larvae collected from Makota and Mudenda farms (Table 4.1). The pupal parasitoid, *D. collaris*, was only recorded at Malambo farm with 14.3% parasitism (n=28). There was a high infestation of *P. xylostella* larvae and pupae on cabbage at Uzakulweni farm and to a lesser extent at Mwenya's farm Table 4.1 and Table 4.2, respectively.

Table 4.2. Number of *P. xylostella* pupae collected and the associated parasitioids recorded on common cabbage plants

Sampling Date	Site	No. of pupae	No. of parasitoids	Parasitoid
25.01.09	Chikobe	0	0	0
01.02.09	Bobby Bwalya	0	0	0
01.02.09	Matanga	0	0	0
08.02.09	Liempe	118	18	*Aphanogmus* sp.
08.02.09	Miller	13	0	0
09.09.09	Mudenda	10	0	0
09.09.09	Malambo	28	4	*D. collaris*
10.09.09	Makota	11	0	0
10.09.09	Mwenya	51	0	0
10.09.09	Uzakulweni	139	0	0

4.2 Pesticides used by Local Farmers in Chongwe, Kafue and Lusaka Districts on Common Vegetable Pests and their Impact on Released Exotic Parasitoids of *Plutella xylostella*

4.2.1 Pesticide Use by Local Farmers

A total of 107 households were surveyed in the three study districts. The distribution of households' sampled in the study area is presented in Fig. 4.1. Ages of respondents to the questionnaire ranged from 25 to 72 years (mean = 44.6 ± 0.96 S.E.) of which 16.8% were females and 83.2% were males. The farm sizes ranged from 0.5 to 1100 ha (mean = 42.0 ± 17.9 S.E.). Mixed farms constituted 97.2% of the farms surveyed; only 1.9% of the farms were growing vegetables. The area under vegetable cultivation ranged from 0.06 to 60 ha. Manure from livestock was used as

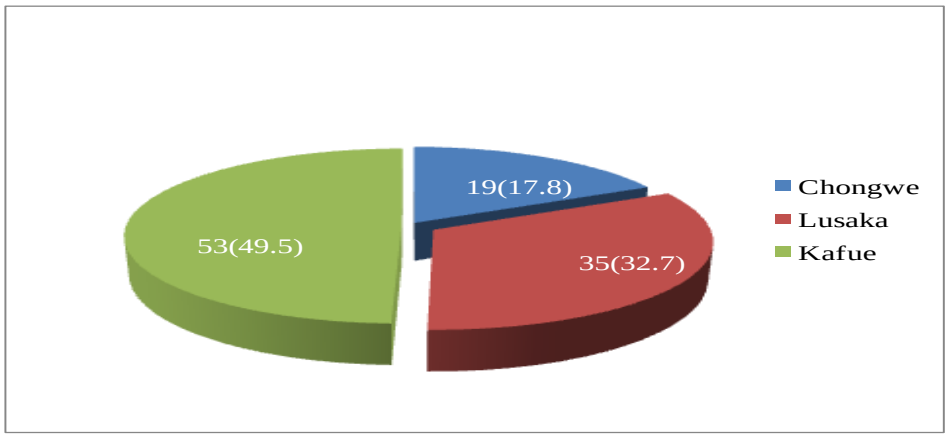

Fig. 4.1 Distribution of households' surveyed for pesticide usage in the study area, 2009. Figures in brackets represent percentage out of the total number of households visited in the three districts.

source of nutrients for crops by 57.9% of the farmers. However, some of those farmers that did not use livestock manure indicated that they did not do so because of the weed problems associated with use of improperly seasoned livestock manure.

The vegetables cultivated by the farmers surveyed were cabbage, Chinese cabbage, baby corn, okra, onion, rape, paprika, pumpkin, green pepper and tomato. Important common pests on leafy vegetables were aphids, cabbage loopers, diamondback moth and snails, while leaf miners, whiteflies, red spider mites and bollworms were reported as important pests on tomato (Table 4.3). Aphids were serious pests, causing crop damage throughout the year, while the others are occasional pests, and were more abundant during the hot-dry season (July-November).

The most important pesticides used by the farmers to control vegetable pestsare shown in Table 4.4. These were: λ-cyhalothrin (Karate®), monocrotophos (Azodrin®), α-cypermethrin (Fastac®), cypermethrin (Cymbush®), methamidophos (Methamidophos®), spear (Spear®), diazinon (Basudin®), dimethoate (Rogor®), fenvalerate (Sumicidin®), dichlorvos (Vapona®),pymetrozine (Chess®), copper oxychloride (Microsperse®), pirimicarb (Pirimor®), malathion (Malathion®), carbaryl (Sevin®), acephate (Orthene®) and abamectin(Agri-Mek®) in decreasing order of popularity.

Table 4.3. Common vegetable pests as perceived by farmers in Lusaka peri-urban area (2009).

Pest common name	Frequency	%
Aphid	88	33.4
Spider mite	52	19.8
Diamondback moth	36	13.7
Bollworm	34	12.9
Leaf miner	23	8.7
Stemborer	15	5.7
Cabbage looper	6	2.3
Snail	4	1.5
Beetle	2	0.8
Bagrada bug	2	0.8
White grub	1	0.4

The principal source of the pesticides used by most farmers was established agro-chemical outlets in the district. One farmer imported the pesticides used from South Africa. A few farmers (2.4%) sprayed tomatoes with the fungicide, copper-oxychloride, to control fungal diseases on the crop, but never used any fungicides to control fungal diseases on Brassica vegetables.

Table 4.4 Proportion of households using various agro-chemicals to control vegetable pests in Lusaka peri-urban area (2009).

Chemical name	Pesticide group	Frequency	Percent
λ-cyhalothrin	pyrethroid	44	21.3
monocrotophos	organophosphate	37	17.9
α-cypermethrin	pyrethroid	23	11.1
cypermethrin	pyrethroid	15	7.2
methamidophos	organophosphate	13	6.3
spear	nematicide	13	6.3
diazinon	organophosphate	12	5.8
dimethoate	organophosphate	8	3.9
fenvalerate	pyrethroid	8	3.9
dichlorvos	organophosphate	7	3.4
pymetrozine	antifeedant	5	2.4
copper oxychloride	fungicide	5	2.4
pirimor	carbamate	4	1.9
malathion	organophosphate	4	1.9
carbaryl	carbamate	3	1.4
acephate	organophosphate	3	1.4
abamectin	macrocyclic lactone	3	1.4

Note: compounds are insecticides unless stated otherwise.

Fifty-one % of respondents said that pests were damaging their vegetables all year round, while 48% said that there was more damage by pests during the rainy season (Nov–April) than in other seasons. The majority of respondents (96.3%) sprayed pesticides using manually operated knapsack sprayers. Eighty % of respondents waited for 5 to 7 days after spraying before harvesting their vegetables, irrespective of the pesticide used (Table 4.5). Pesticide use (frequency) was not among questions the household were asked for in the locations that were studied

Table 4.5 Pre-harvest interval of pesticides used by farmers to control vegetable pests.

Pre-harvest interval (days)	Frequency	%	[1]Valid %
1 to 4	4	3.7	3.8
5 to 7	86	80.4	81.1
8 to 10	5	4.7	4.7
>10	5	4.7	4.7
Depends on pesticide use	4	3.7	4.8
Total valid	104	97.2	100
Not sure	2	1.9	
System	1	0.9	
Total missing	3	2.8	
Total	107	100	

[1]Valid % excludes missing people because they did not answer the question and those who indicated that they did not know or were not sure.

4.2.2 Impact of the Pesticides on *Cotesia vestalis*

The LC_{50} values and 95% confidence intervals of the insecticides tested on *C. vestalis* adults are shown in Table 4.6. Cypermethrin and dichlorvos were the most toxic while acephate was the least toxic after 12 to 72 h. The four insecticides can be ranked from high-to-low toxicity to *C. vestalis* adults (based on LC_{50} values) as (cypermethrin, dichlorvos, λ-cyhalothrin and acephate). At the recommended field rate for each insecticide i.e. acephate 75% WP (3g/L), cypermethrin 20% EC (1ml/L), dichlorvos 100% EC (3ml/L) and λ-cyhalothrin 20% EC (1ml/L)) there was 100% mortality of *C. vestalis* adults after 12 to 72 h exposure.

The mortality of *C. vestalis* adults increased significantly with an increase in time of exposure and dosage among the four insecticides (Fig. 4.2-4.9). However, there were differences in the mortality of *C. vestalis* adults among the time of exposure of the same insecticideas shown by the 95% C.L. There were no significant interactions between concentration and time for cypermethrin, dichlorvos, acephate and λ-cyhalothrin ($d.f._{30,30}$, P=0.500; $d.f._{35,35}$, P=0.500; $d.f._{45,45}$, P=0.500 and $d.f._{40,40}$, P=0.500; respectively) (Appendix 3).

Table 4.6 Toxicity of four insecticides to *Cotesia vestalis* adults; n=50[1].

Insecticide	Time of exposure	LC_{50} (ppm) (95% C.I.)	Slope ± S.E.
cypermethrin	12	3.07 (1.56-6.04)	1.11 ± 0.42
cypermethrin	24	3.12 (2.01 - 4.84)	1.84 ± 0.59
cypermethrin	36	3.08 (2.03 - 4.68)	1.98 ± 0.62
cypermethrin	48	2.89 (1.94 - 4.31)	2.20 ± 0.67
cypermethrin	60	2.66 (1.72 - 4.10)	2.16 ± 0.69
cypermethrin	72	2.36 (1.51 - 3.70)	2.20 ± 0.75
dichlorvos	12	7.79 (7.20 - 8.44)	9.94 ± 2.76
dichlorvos	24	7.15 (6.49 - 7.88)	9.18 ± 2.26
dichlorvos	36	6.81 (6.12 - 7.59)	8.52 ± 2.08
dichlorvos	48	6.18 (5.36 - 7.12)	5.97 ± 1.44
dichlorvos	60	5.32 (4.39 - 6.72)	4.61 ± 1.18
dichlorvos	72	7.79 (7.20 - 8.44)	4.46 ± 1.19
λ-cyhalothrin	12	424 (390 - 462)	8.21 ± 2.34
λ-cyhalothrin	24	412 (315 - 540)	2.15 ± 0.70
λ-cyhalothrin	36	396 (269 - 585)	1.41 ± 0.48
λ-cyhalothrin	48	387 (265 - 567)	1.45 ± 0.49
λ-cyhalothrin	60	350 (239 - 511)	1.37 ± 0.46
λ-cyhalothrin	72	256 (182 - 358)	1.53 ± 0.44
acephate	12	1111 (852 - 1447)	2.29 ± 0.61
acephate	24	795 (550 - 1148)	1.53 ± 0.34
acephate	36	703 (483 - 1023)	1.55 ± 0.36
acephate	48	680 (489 - 945)	2.03 ± 0.47
acephate	60	497 (333 - 741)	1.63 ± 0.37
acephate	72	428 (279 - 657)	1.51 ± 0.33

[1]LC_{50} determined using the generalized linear models with binomial errors and experiments were conducted using Chinese cabbage leaf-discs.

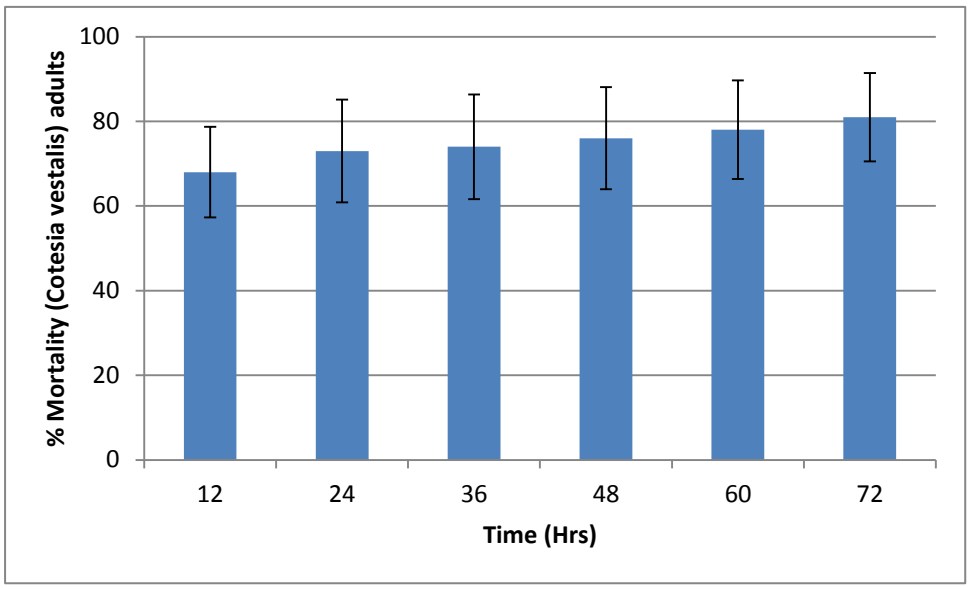

Fig.4.2. The effect of cypermethrin on mortality (% ± S.E.) of *C. vestalis* adults in relation to time.

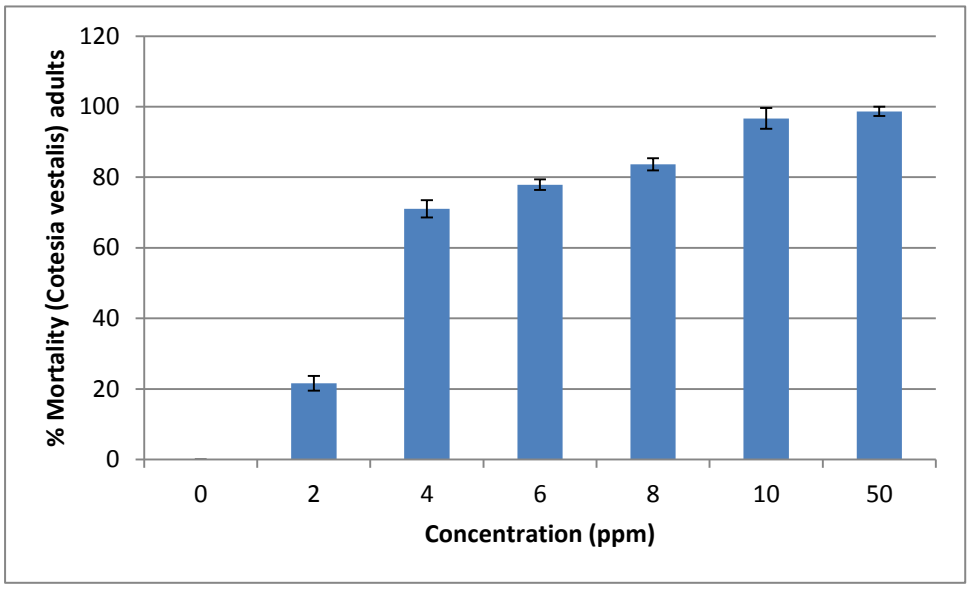

Fig. 4.3 The effect of cypermethrin on mortality (% ± S.E.) of *C. vestalis* adults in relation to dosage.

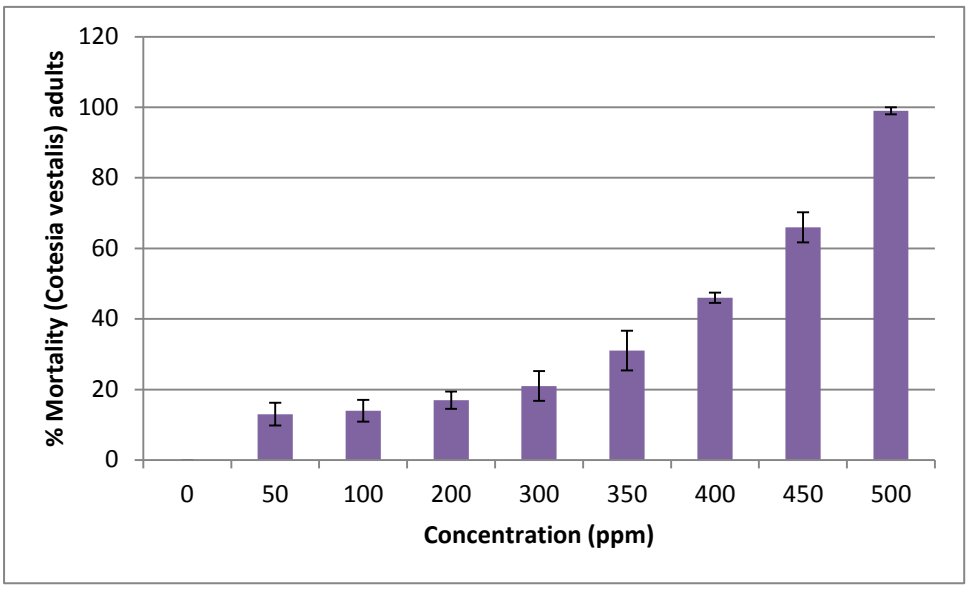

Fig. 4.4. The effect of λ-cyhalothrin on mortality (% ± S.E.) of *C. vestalis* adults in relation to dosage.

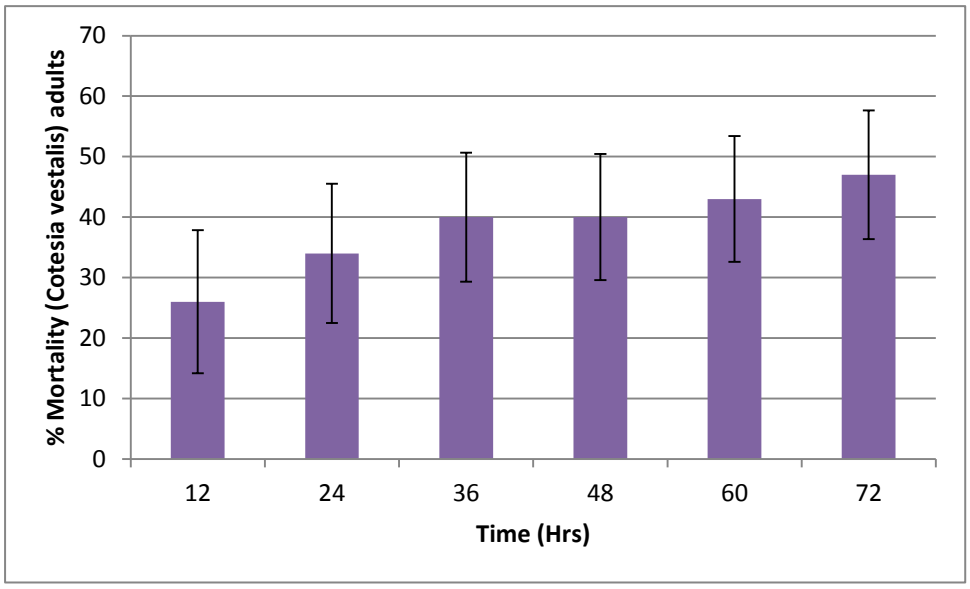

Fig. 4.5. The effect of of λ-cyhalothrin on mortality (% ± S.E.) of *C. vestalis* adults in relation to time.

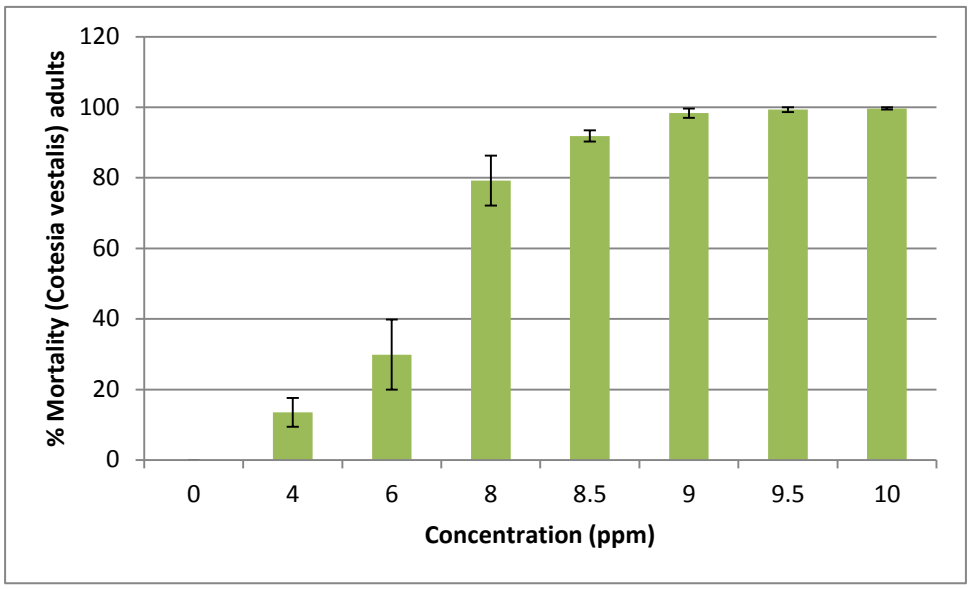

Fig 4.6. The effect of dichlorvos on mortality (% ± S.E.) of *C. vestalis* adults in relation to dosage.

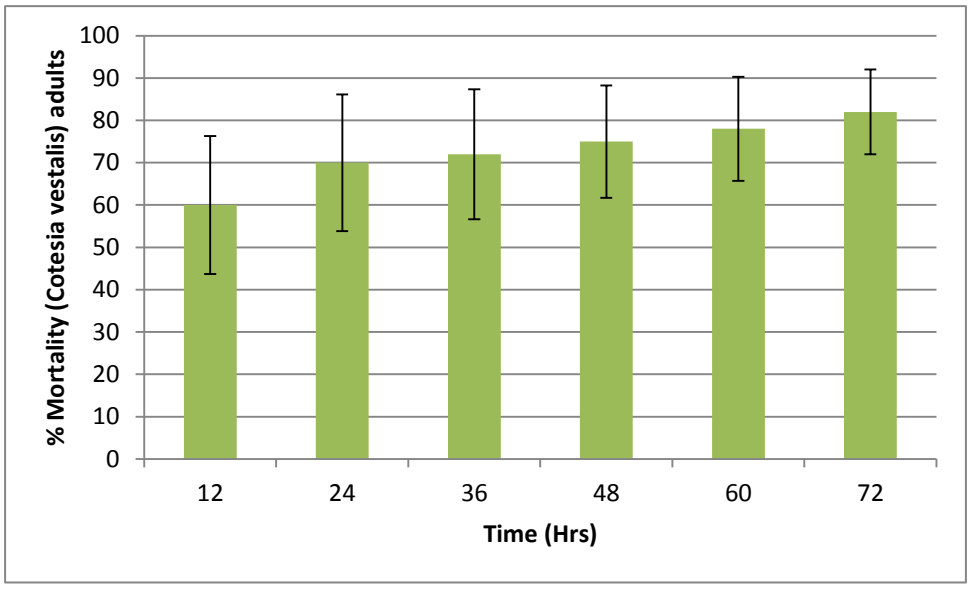

Fig. 4.7. The effect of dichlorvos on mortality (% ± S.E.) of *C. vestalis* adults in relation to time.

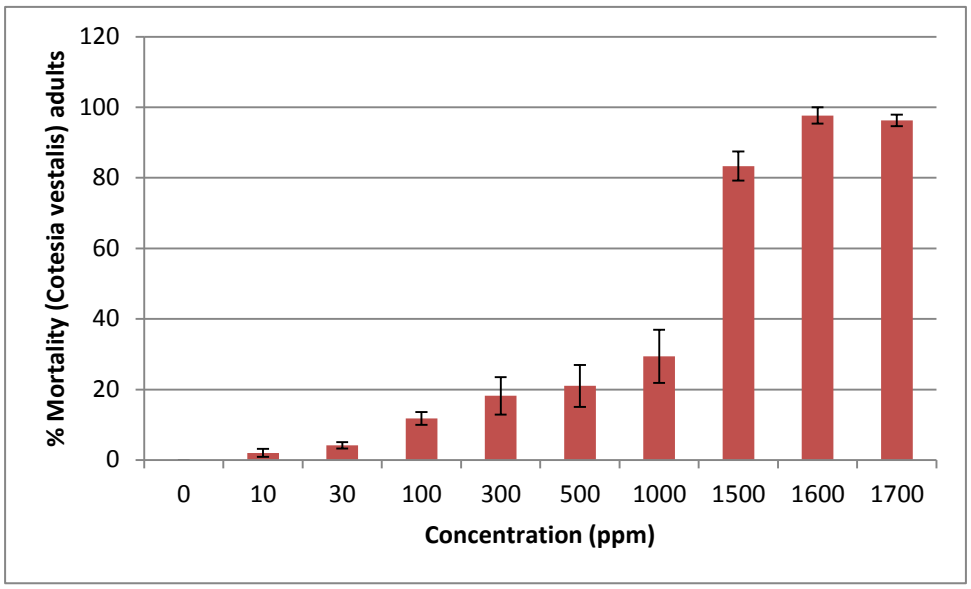

Fig. 4.8. The effect of acephate on mortality (% ± S.E.) of *C. vestalis* adults in relation to dosage.

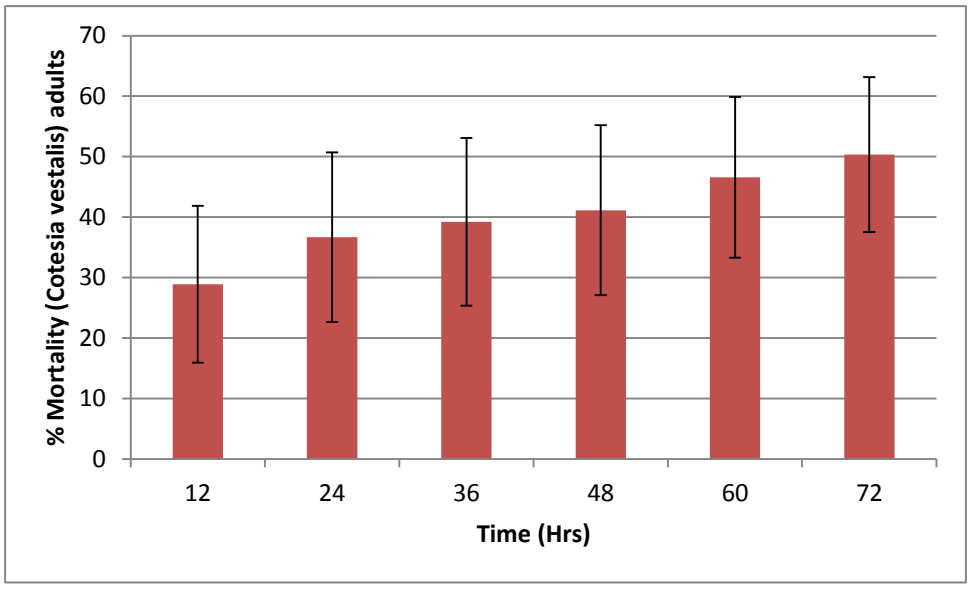

Fig.4.9. The effect of acephate on mortality(% ± S.E.) of *C. vestalis* adults in relation to time.

4.3 Phenology of *Plutella xylostella* in the Selected Study Area and Identification of its Parasitoids

The seasonal variation in the population density of *P. xylostella* larvae and pupae on cabbage is shown in Fig. 4.10. No larvae were found from March 2009 until mid-April 2009 when sampling was started. By mid-April, the larval population had increased and reached the first population peak by late June. The larval population then decreased but started increasing again in late July with a second population peak in mid-August. The highest larval peak was in mid-September. No larvae were collected from mid-December 2009 to the end of the sampling period on January 31, 2010.

Generally, the *P. xylostella* pupal population was lower than that of larvae and reached the highest peak in late September 2009 (Fig. 4.10). However, when the data was recorded on monthly basis, it vividly indicated that both the larval and pupal populations of *P. xylostella* reached their highest peak in October (Appendix 4). The larval populations of *P. xylostella* were in abundance in September and October.

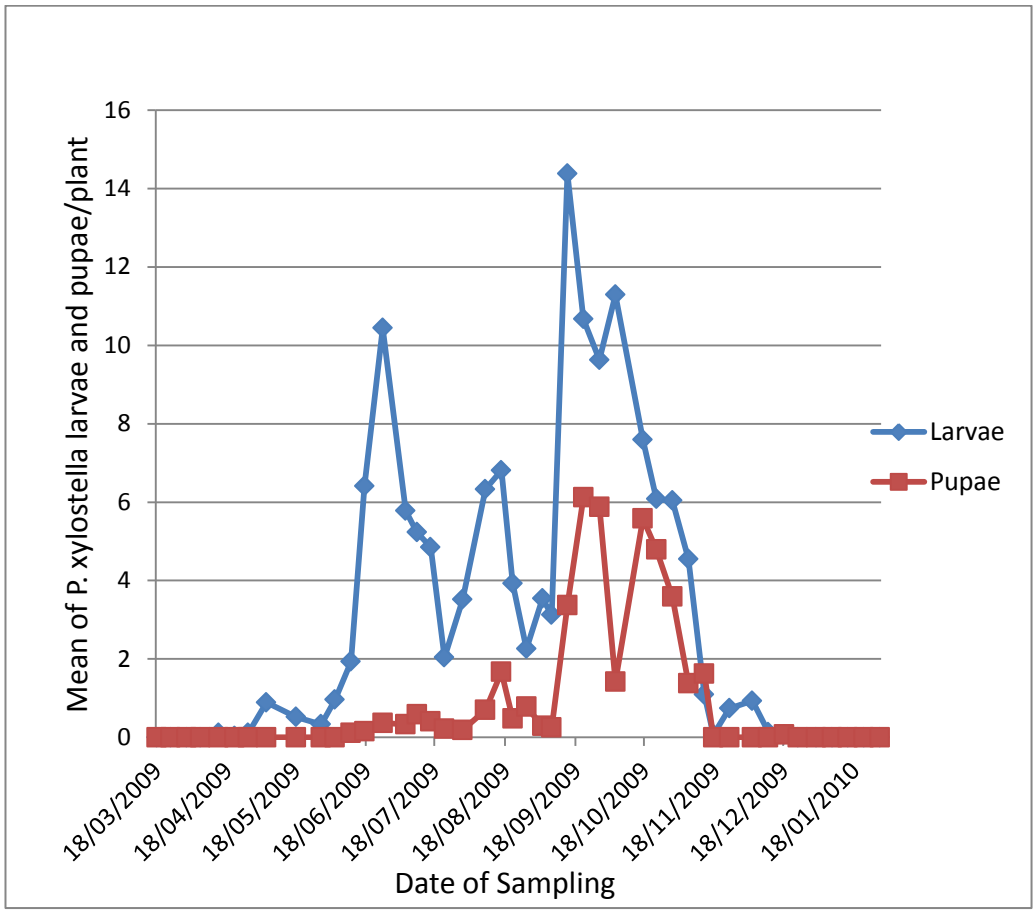

Fig.4.10. Seasonal variation of *Plutella xylostella* larvae and pupae on cabbage at the University of Zambia, School of Agricultural Sciences Field Station, 2009/2010.

The local parasitoid species of *P. xylostella* found was *O. sokolowskii* (Hymenoptera: Eulophidae) at the School of Agricultural Sciences Field Station (Altitude: 1274m; Latitude: $28^{0}33'610''$E; Longitude: $15^{0}39'463''$S). An unidentified species of *Aphanogmus,* a hyperparasitoid of *C. vestalis,* was also recorded from samples collected from the University of Zambia, School of Agricultural Sciences Field Station.

Plutella xylostella larvae were parasitized predominantly by the larval parasitoid *C. vestalis*, which peaked in late June, and the larval-pupal parasitoid *O. sokolowskii*, which was most abundant from September to October, the warmest months of the year in Zambia (Fig.4.11; Appendix 4). Other parasitoids found were the larval-pupal parasitoid *D. collaris*, which occurred in small numbers from early July to early November (Fig. 4.11), and the pupal parasitoid *D. mollipla*, with a total of 13 parasitoids collected during the entire sampling period from mid-June to early October (Appendix 4).

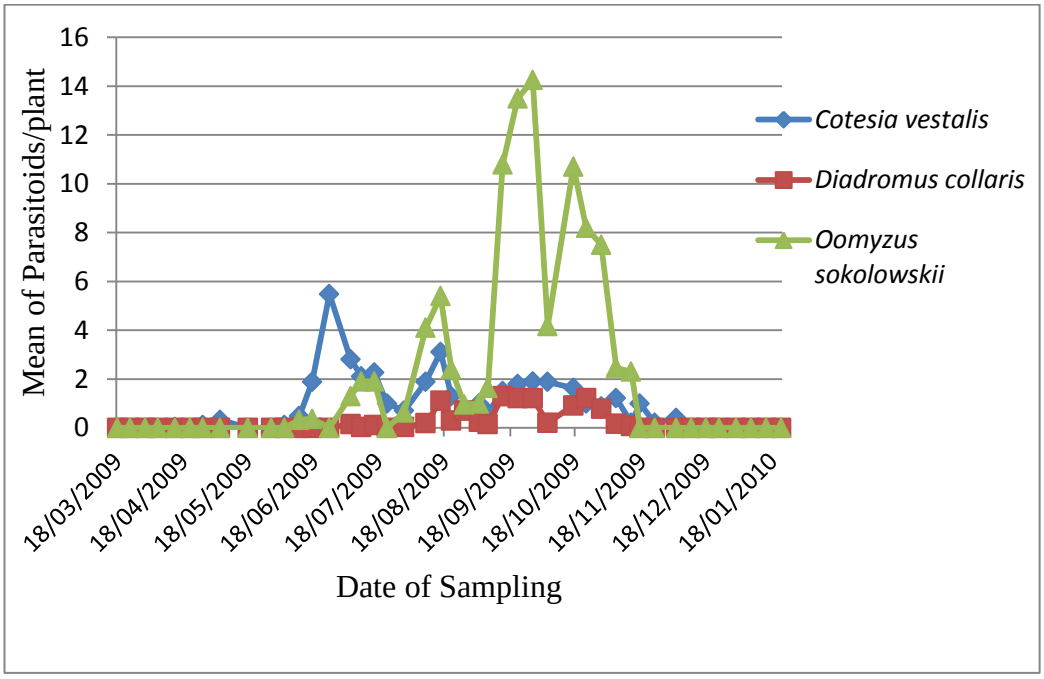

Fig.4.11. Seasonal variation of *Plutella xylostella* parasitoids on cabbage at University of Zambia, School of Agricultural Sciences Field Station, 2009/2010.

4.4 Assessment of the Effectiveness of *Cotesia vestalis* as a Biological Control Agent of *Plutella xylostella*

When mated females of *C. vestalis* were assessed for their potential to parasitize *P. xylostella* larvae, the parasitoid exhibited a type II functional response. However, the asymptote which represents parasitoid saturation was not attained in this experiment (Fig. 4.12). The searching efficiency for adult female *C. vestalis* was 0.92 h while handling time was 0.017 h. *Plutella xylostella* larval mortality due to parasitism declined with increase in its density. *Cotesia vestalis* caused more mortality of *P. xylostella* at low density than at high density. From Fig. 4.12, it appears there is a positive correlation between the number of parasitized *P. xylostella* larvae by *C. vestalis* with density. However, the mortality of the larvae is negatively correlated with larval density (Fig. 4.13). Preference was made to plot parasitized *P. xylostella* larvae against density of larvae (Fig. 4.12) than percentage of the total on density of the larvae according to Holling (1969) disc functional response equation. The functional response data is given as Appendix 5. At low larval density, a smaller proportion of *C. vestalis*'s time is spent handling the larvae, even if the parasitoid parasitizes every host available.

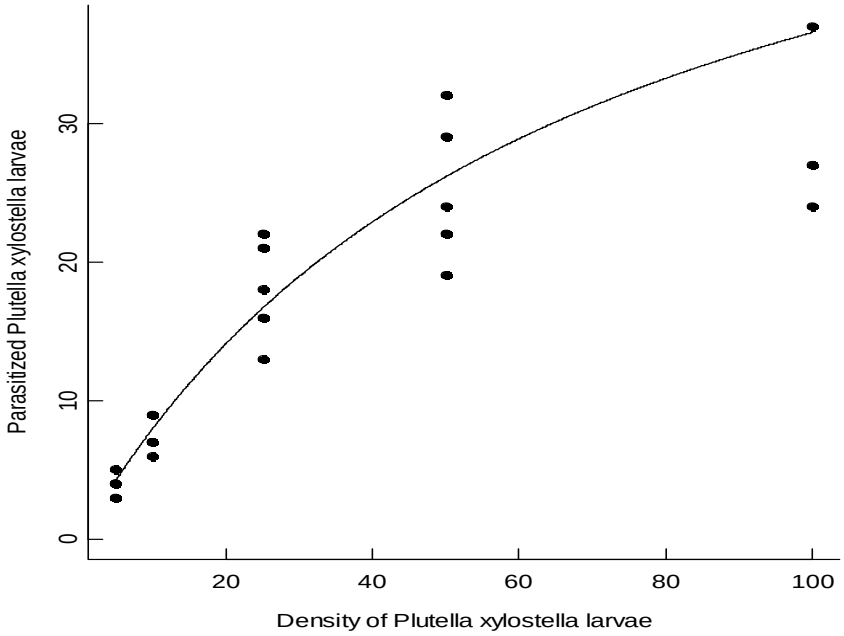

Fig. 4.12. Type II functional response of *Cotesia vestalis* to second intars of *Plutella xylostella*

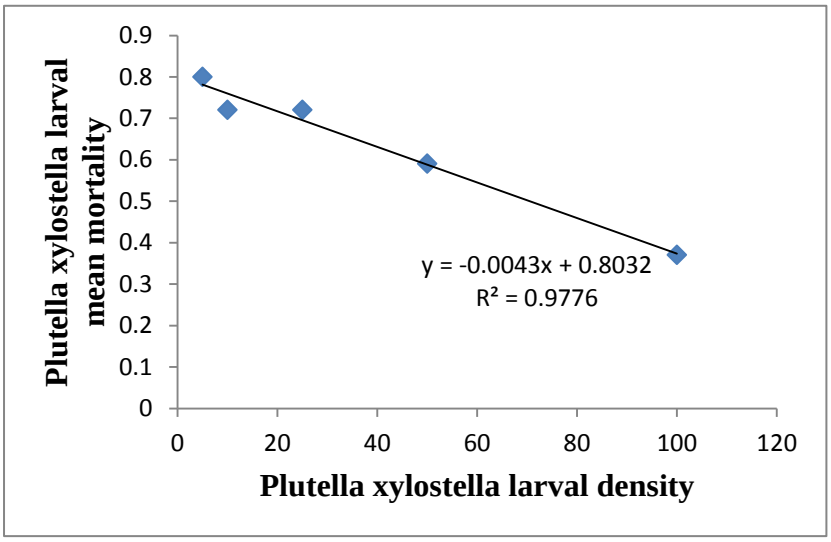

Fig. 4.13. Relationship of *Plutella xylostella* larval density to its larval mean mortality.

CHAPTER 5. DISCUSSION

5.1 Establishment of Introduced Exotic Parasitoids of *Plutella xylostella* in Lusaka Province, Zambia

The findings of this research confirm reports by Ahmed (1977) and Yaseen (1978) that the two exotic, *C. vestalis* and *D. collaris*, parasitoids of *P. xylostella* had established themselves in the Lusaka Province, Zambia. For both parasitoids to still be present in the environment in Lusaka, that is after 30 years of release, means that they have both been breeding successfully from the populations derived from the original releases in the 1970s. The presence of the larval parasitoid, *C. vestalis*, at Liempe Farm in Chongwe and the Field Station at the University of Zambia, approximately 35 Km and 10 km from the original release sites of the parasitoids respectively, could mean that the parasitoid had spread to those areas.

There was however an imbalance in the densities of the two parasitoids. The larval parasitoid, *C. vestalis*, was the most abundant while the pupal parasitoid, *D. collaris*, was the least abundant. No *P. xylostella* larvae were found in Lusaka West in January and February 2009. However, *P. xylostella* larvae and pupae were recorded from samples collected from Miller and Liempe Farms during the same period. If *P. xylostella* adults had hibernated then the insect would not have been recorded at the two farms during January and February.There is a possibility that may be the cabbage plants were just sprayed when the collection was done.

Insecticides were generally readily available to control any vegetable pests that were found damaging their vegetables. Such that if the owner failed to supply the insecticide then the farm manager was justified for not taking any control measures. So to avoid such problems the owners of the farms ensured that the insecticides were readily available on the farms. The widespread use of broad spectrum insecticides may have been responsible for the imbalance observed in parasitoid densities and also the host, *P. xylostella*, was less abundant during that time of the season. Liempe farm had the highest populations of *C. vestalis*.

The situation was different at Miller farm, which practiced both conventional and organic farming. The samples were collected from cabbage fields under conventional farming because those were the fields that had cabbage at the time of sampling. A small number of *C. vestalis* were recorded at Miller farm despite having over 5ha under cabbage production. The cabbage was sprayed with organophosphates which were directly imported from South Africa. The farmer complained that *Bt* products were no longer being sold in Zambia, as a result he was compelled to use synthetic insecticides for *P. xylostella* control.

Aphanogmus sp., a hyper-parasitoid of *C. vestalis* is being reported in Zambia for the first time by this study and its parasitism rate was 15.3% under field conditions. Jaramillo and Vega (2009) reported about 10% parasitism rate of the coffee berry borer parasitoid, *Prorops nasuta*, (Hymenopteras: Bethylidae) immature stages by *Aphanogmus* sp. in organic coffee plantation under field conditions in western

Kenya. *Aphanogmus reticulatus* (Fouts) was reported parasitizing *C. vestalis* in Benin. *Aphanogmus* has also been reared from several orders of insect orders such as Diptera, Hymenoptera, Hemiptera (Homoptera), Neuroptera, Thysanoptera and Trichoptera (Buhl *et al*., 2010; Jaramillo and Vega, 2009). This genus has also been reported in Ireland (Buhl *et al*., 2010), the UK (Polaszek and Dessart, 1996), India (Ali and Rao, 1974), Australia and New Zealand (Cumber, 1959).

Cotesia vestalis is widely distributed in Africa (Kfir, 2003). It is one of the most important biological control agents of *P. xylostella* in Southeast Asia (Lim, 1992; Talekar, 2004; Htwe *et al*., 2008, 2009). It is also the only parasitoid known to survive the hot and humid climate of the lowlands (Verkerk and Wright, 1997) in Southeast Asia.

The principal reason for *C. vestalis* abundance in the study area was not clear but this parasitoid has successfully established itself in many African countries where it was introduced for the control of *P. xylostella*. It has been reported in Benin (Goudegnon *et al*., 2000), Cape Verde Islands (Cock, 1983), Ethiopia (Ayalew and Ogol, 2006), Kenya (Akol *et al*., 2002), Togo (Karl, 1992), St. Helena Islands (Kfir and Thomas, 2001), South Africa (Waladde *et al*., 2001), Zambia (Ahmed, 1977; Yaseen, 1978) and Zimbabwe (Manyangarirwa *et al*., 2009).

Diadromus collaris is a very rare parasitoid of *P. xylostella* in Zambia and unlike *C. vestalis* it has not been widely used in *P. xylostella* control in Africa. However, *D.*

collaris has successfully established itself in South Africa (Kfir, 1997, 2003), St. Helena Island (Kfir and Thomas, 2001) and Zambia (Ahmed, 1977; Yaseen, 1978).

5.2 Pesticides used by Local Farmers in Chongwe, Kafue and Lusaka Districts on Common Vegetable Pests and their Impact on Released Exotic Parasitoids of *Plutella xylostella*

5.2.1 Pesticide Use by Local Farmers

Nearly half of the pesticides found to be used by local farmers in the study area to control common vegetable pests belonged to the organophosphate group, followed by the pyrethroids. Badenes-Perez and Shelton (2006) reported that pyrethroids and organophosphates were the two main groups of insecticides used by farmers to control *P. xylostella* in Kenya and India.

Farmers in the study area heavily depended on insecticides to control vegetable pests and similar observations were made by Ahmed (1977) and Kapunda *et al*. (2004). Most human poisonings are caused by organophosphates (Mwansa-Nyendwa, 2004). The main problem of using such pesticides on vegetable pests is the detrimental effects on non-target insect species and also on natural enemies, farmers' friends, such as the parasitoids, parasites and predators. Pesticides also have side effects on people applying them; they can result in residues on the harvested vegetables; and they can contaminate the environment. It is common to find farmers spraying highly toxic pesticides without any personal protective equipment or with equipment that

isn't suitable. The present survey also revealed that farmers rarely used fungicides to control fungal diseases in vegetables and some of the farmers interviewed had no knowledge of the diseases and insect pests of vegetables.

In the 1980s, according to Lasota and Kok (1986) worldwide control for *P. xylostella* and other vegetable pests mainly involved the prophylactic use of insecticide sprays applied on a 7-10 day schedules with little regard for pest population levels. The small scale farmers surveyed in the present study were also found to spray frequently without following recommended integrated pest management practices. These farmers did not do any scouting for insect pests in vegetables. One of the reasons why the farmers preferred to use insecticides was because insecticides give an immediate control of the insects and also resulted in higher crop yields. However, repeated application of insecticides at high dosages in order to effectively control the insects can result in pests developing resistance (e.g. Grzywacz *et al*., 2010). Mingochi and Luchen (2000) reported that *P. xylostella* had developed resistance to some synthetic pyrethroids in Zambia. There are also reports that *P. xylostella* has developed resistance to new chemistries (Talekar and Shelton, 1993; Zhao *et al*., 2001, 2006; Sarfraz and Kiddie, 2005; Sarfraz *et al*., 2006; Sayyed *et al*., 2004, 2008; Gong *et al*., 2010).

The use of synthetic insecticides for *P. xylostella* control in Africa is reportedly characterized by such practices as spraying mixtures of compounds, calendar spraying and the use of unregistered and fraudulent products of poor quality (Williamson, 2003). Surveys in a number of African countries, including Kenya

(Oruku and Ndun'gu, 2001) and Zimbabwe (Sithole, 2005), have shown that there is an overwhelming reliance on broad-spectrum insecticides (e.g. pyrethroids, organophosphates and carbamates), often applied weekly or bi-weekly (Grzywacz *et al*., 2010). The extension service in the study area, which is supposed to act as a bridge between the researchers and the farmers is weak or almost non-existence. The farmers therefore rely on other farmers for knowledge on pests and insecticides except for a few farmers who have direct links to the extension services being provided by the agro-chemical companies.

5.2.2 Impact of the Pesticides on *Cotesia vestalis* Parasitoid of *Plutella xylostella*

Several insecticides are used for the control of *P. xylostella* in Zambia (Section 2.6.1). In the present study four insecticides were tested for their toxicity to *C. vestalis*, the synthetic pyrethroids, cypermethrin and λ-cyhalothrin, and the organophosphates, dichlorvos and acephate. Dichlorvos was chosen because of being a pure (technical) grade and waiting period is one day while acephate was chosen because of being systemic and both pesticides rated highly in Kafue district. At the recommended field rate of each insecticide i.e acephate 75% WP (3g/L), cypermethrin 20% EC (1ml/L), dichlorvos 100% EC (3ml/L) and λ-cyhalothrin 20% EC (1ml/L) there was 100% mortality of *C. vestalis* adults after 12 to 72 h exposure. Kawazu *et al*. (2010) has also reported 100% mortality for *C. vestalis* adults when exposed to two other organophosphates, malathion and diazinon, and the carbamates, methomyl and alanycarb, at recommended rates for field application. It was found

that cypermethrin and dichlorvos were the most toxic to *C. vestalis* based on their low LC_{50} values, while λ-cyhalothrin was intermediate and acephate the least toxic. This agrees with the findings of Kao and Tzeng, 1992). Insecticides can belong to the same group like the two synthetic pyrethroids, cypermethrin and λ-cyhalothrin, but have different LC_{50}s. Not only that but also the same insecticide applied at different dosage may have different effects on insects (Kao and Tzeng, 1992).

Miyata *et al*. (2001) observed that fipronil, chlorfenapyr, abamectin, diafenthiuron and cypermethrin had no effect on the emergence of *C. vestalis* adults when applied on cocoons of the parasitoid. Kao and Tzeng (1992) evaluated toxicity of 17 commonly used insecticides to *C. plutellae*. Among them, seven insecticides were harmful (mortality >99%) to adults of *C. plutellae*, while the remaining 10 insecticides proved to be harmless (mortality <50%). Wang *et al*. (2008) found that dichlorvos at an LC_{50} of 15.9 mgL^{-1} was the most toxic to *Anagrus nilaparvatae* (Pang *et* Wang) (Hymenoptera: Mymaridae) among 14 pesticides tested and 100% mortality was obtained after 2 h exposure. However, the results in this study showed that dichlorvos was toxic to *C. vestalis* adults at LC_{50} lower than 15.9 mgL^{-1}. Indoxacarb, spinosad and λ-cyhalothrin caused 100% adult mortality for *Diadegma insulare* in 24 h in contact bioassays (Xu *et al*., 2004), while Hill and Foster (2000) observed 100% mortality for the same parasitoid when exposed to spinosad for 8 h.

According to Ahmed (1977) *P. xylostella* are sufficiently controlled by natural enemies in Zambia such that chemical sprays are not required from April to October each year. However, 2-4 sprays of *Btk* were needed at the end of the season if there

was a high population level of *P. xylotella* adults damaging the Brassicas. The same author reported that the rate of parasitism ranged between 75% and 90% during the peak of the moth population while in areas where broad spectrum insecticides were applied the parasitoid populations were very low and the percentage parasitism was only 21%.This study demonstrated a low rate of parasitismof *P. xylostella* larvae and pupal populations than those reported by Ahmed (1977).

This study has shown that the greatest rate of parasitism by *C. vestalis* on *P. xylostella* larvae was 54.9% at Liempe Farm. Current control of *P. xylostella* in Zambia depends entirely on conventional insecticides. One of the reasons why farmers continue using insecticides like dichlorvos is because of its extremely short pre-harvest interval of one day. As a result, a farmer can still apply dichlorvos to vegetables that are ready for market. Synthetic pyrethroids are the most common insecticides used by farmers because of the 'cotton packs' of insecticides, which are also used to control *P. xylostella* and other vegetable pests. The current use of broad spectrum insecticides such as synthetic pyrethroids and organophosphates disrupts any potential biological control of insect pests of vegetables by parasites. Most of the farmers do not even know about *P. xylostella* and are not aware of its natural enemies.

In many countries, the destruction of the natural enemies by the widespread use of broad-spectrum insecticides such as organophosphates is responsible for the imbalance in the natural enemies observed and high pest status (Talekar and Shelton, 1993). Poletti *et al*. (2007) indicated that the sub-lethal effects of insecticides on

natural enemies may ultimately cause beneficial insects to become less effective as biological control agents in the field due to their low performance in parasitizing and preying on hosts. Jusoh *et al.* (1992) reported that the widespread and intensive use of insecticides in South East Asia led to problems such as insecticide resistance, unacceptable residues in vegetables, poisoning of farmers and labourers, reduction of natural enemies in vegetable agro-ecosystems and rising costs of production. *Plutella xylostella* has developed resistance to almost every class of insecticides used to control it (Talekar and Shelton, 1993; see also Section 2.6). The levels and types of insecticide resistance in *P. xylostella* populations in Zambia are currently not known, though Mingochi and Luchen (2000) reported that it was resistant to λ-cyhalothrin.

5.3 Phenology of *Plutella xylostella* and its Parasitoids in the Selected Study Area

The seasonal variation of *P. xylostella* larvae in cabbage plots at the School of Agricultural Sciences Field Station indicated that there were possibly five distinct generations from March to January although the peaks observed in the phenology of the pest could have been due to sampling intervals. According to Uijtewaal (2006) in tropical climates, where host plants are available all year round, more than 20 generations of *P. xylostella* may be produced. For example, Ahmed (1977) reported that the development from oviposition to adult took approximately 14-18 days under field conditions in Zambia. The larval populations were higher in the dry-hot season between September and November with a density of >14 larvae per cabbage plant.

The population dynamics reported in the present study were similar to the findings of Ahmed (1977) in Zambia and Manyangarirwa *et al.* (2009) in Zimbabwe.

The mortality observed in *P. xylostella* larval, pupal and adult populations may have been caused by two factors, overhead sprinkler irrigation and natural enemies. The natural enemies included the parasitoids *C. vestalis, D. collaris* and *O. sokolowskii* (Section 4.4) while the predators were unidentified spiders, red and black ants, lizards, coccinellids and brown lace wings. Overhead sprinkler irrigation was sometimes used instead of flood irrigation and the former has been documented to be responsible for mortality of larvae and also to disrupt the mating of the adults (Section 2.6.2.3). This is one of the reasons why *P. xylostella* is not a serious problem during the hot-dry season in Zambia when overhead sprinkler irrigation is applied at dusk. This was observed on Farms such as Makota and Mudenda where they were using overhead sprinkler irrigation. In contrast, farms that were using drip irrigation (Mwenya and Uzakulweni) had the highest *P. xylostella* larval and pupal populations.

The parasitoids recorded from *P. xylostella* larvae and pupae were *C. vestalis, D. collaris, D. mollipla, O. sokolowskii* and a hyper-parasite of *C. vestalis*, *Aphanogmus* sp. *Oomyzus sokolowskii* was the most abundant parasitoid in the hot-dry season between September and November 2009. This parasitoid seems to be density dependent that is as the host increased so was *O. sokolowskii*. Ayalew and Ogol (2006*)* reported that *O. sokolowskii* was mainly distributed in relatively heavy insecticide use areas of the Rift valley and northern Ethiopia while *C. vestalis* and

Diadegma spp. were largely confined to the south western part of Ethiopia, where insecticide use was minimal. Yaseen (1978) reported the establishment of *O. sokolowskii* in Trinidad and as being endemic to Zambia. Ahmed (1977) did not report about *O. sokolowskii* as being present in Zambia.

Cotesia vestalis and *D. collaris* were imported from Pakistan Commonwealth Institute of Biological Control into Zambia for the control of *P. xylostella* during 1977-1984 (Ministry of Agriculture and Rural Development, 1974-75, 1984). *Diadegma mollipla* was imported into Zambia at the same time for the control of the potato tuber moth, *Phthorimaea operculella* (Zeller) (Lepidoptera: Gelechiidae). The present study was the first report of *D. mollipla* attacking *P. xylostella* larvae in Zambia, although it has previously been recorded to do so in Kenya (Oduor *et al*., 1997), South Africa (Kfir, 1997, 2003; Nomefela and Kfir, 2008), and Zimbabwe (Manyangarirwa *et al*., 2009) as a larval parasitoid.

In Trinidad, both *C. vestalis* and *T. sokolowskii* were introduced and are now well established but had not given adequate control of *P. xylostella* (Yaseen, 1978). Waterhouse (1992) reported that *D. collaris, D. semiclausum* and *C. vestalis* were among the major parasitoids of *P. xylostella* established in Oceania, Australia and New Zealand. Biological control of *P. xylostella* in Cape Verde Islands has involved three parasitoids: *C. vestalis, O. sokolowskii* and *Microplitis plutellae* imported from Pakistan and released in 1981 and 1982. According to Cock (1983), the first two species were successfully established and *P. xylostella* was reported to be extremely scarce in areas where it was previously very common. Among the parasitoids

released in Malaysia in the 1970s were *D. eucerophaga* and *D. collaris*, which were reported as successfully established in the Cameron Highlands (Lim, 1986; Lim and Ko, 1975; Ooi and Kelderman, 1977).

In Cotonou, Benin, only one larval parasitoid, *C. vestalis*, has been recorded on *P. xylostella* (Goudegnon *et al.*, 2000). In surveys conducted in Kenya, Tanzania and Uganda in 2000/2001, parasitism rates of *P. xylostella* were shown to be below 15% (Löhr and Kfir, 2002). The results on the rate of parasitism of *P. xylostella* by *C. vestalis* in this study were also below 15% except for those recorded at Liempe farm which were 54.5%. Kibata (1997) and Oduor *et al.* (1997) recorded several parasitoids attacking *P. xylostella* in Kenya but with relatively low rates of parasitism. The most frequent parasitoids found in Kenya were *D. mollipla* (Holmgren) and *O. sokolowskii*. In the district of Pretoria, South Africa, Ullyett (1947) recorded nineteen parasitoids and hyperparasitoids, several predators, two bacteria and an entomopathogenic fungus associated with *P. xylostella*. Kfir (2003) compiled a total of thirty three different parasitoids and hyperparasitoids associated with *P. xylostella* in South Africa. By far the most abundant species was *C. vestalis* while other abundant parasitoids included *O. sokolowskii*, *D. collaris*, and two hyperparasitoids *Mesochorus* sp. (Hymenoptera: Ichneunomidae) and *Pteromalus* sp. (Hymenoptera: Pteromalidae).

Aphanogmus sp. was reported for the first time in Zambia in the present study (Section 5.1). The impact of this hyper-parasitoid on *C. vestalis* has not been assessed.

5.4 The Effectiveness of *Cotesia vestalis* as a Biological Control Agent of *Plutella xylostella* in Zambia

In the present study, *C. vestalis* exhibited a type II functional response whereby there was a curvilinear response, that is, the searching efficiency decreased with increased density of *P. xylostella* second instar larvae. These findings were similar to those reported by Furlong (1993). The number of parasitized *P. xylostella* larvae increased over time but did not reach the asymptote because of the decrease in searching time of the parasitoid as the number of hosts parasitized increases. The rate of parasitism eventually levels off at a plateau at which it remains constant regardless of increases in host density.

Laboratory-measured functional responses can provide useful information for understanding the host-parasitoid (or predator-prey) population dynamics (Chen *et al*., 2008). Pak and van Lenteren (1988) mentioned that *Trichogramma* strains that showed a high potential in the laboratory also had the ability to perform well in the field, while Silva *et al*. (2000) and Thomson and Hoffmann (2002) found no relationship between laboratory and field performance.The functional response describes the rate at which a biological control agent parasitizes its host at different host densities and can thus determine the efficiency of a parasitoid in regulating host populations. It has been reported that most parasitoids exhibit a type II response (Walde and Murdoch, 1988; Fernández-Arhex and Corley, 2003), including *C. vestalis* (Shi and Liu, 1999; Mitsunaga *et al*., 2004).

It has been proposed that natural enemies with type III functional responses have the greatest potential in biological control programmes (Murdoch and Briggs, 1996; Briggs *et al*., 1999). Most natural enemies show type II or III functional response (Dastjerdi *et al.*, 2009).

Between the two introduced exotic parasitoids into Zambia for the control of *P. xylostella, C. vestalis* has established widely such that it has been collected 35 Km (Liempe Farm) away from the release site. It also has other attributes which can make it a good biological control agentcandidate, such as its ease of rearing, host specificity and tolerance to insecticides. *Cotesia vestalis* has beenused in biological control programmes of *P. xylostella* in several countries because of its tolerance to insecticides (Waterhouse and Norris, 1987; Fitton and Walker, 1992). There is therefore a need to augment the field populations of *C. vestalis* in Zambia in order for it to effectively and efficiently control *P. xylostella*.

CHAPTER 6.CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

6.1.1 Establishment of Exotic Parasitoids of *Plutella xylostella* Released in Lusaka Province, Zambia

- The study confirmed the establishment of two exotic parasitoids of *P. xylostella*, namely *C. vestalis* and *D. collaris*, in Lusaka Province.

6.1.2 Pesticides used by Local Farmers in Chongwe, Kafue and Lusaka Districts on Common Vegetable Pests and their Impact on Released Exotic Parasitoids of *Plutella xylostella*.

- The study provided information on pesticides used by farmers on vegetables, which included synthetic pyrethroids, organophosphates, carbamates, insect growth regulators and a macrocyclic-lactone.
- The impact of four insecticides against *C. vestalis* was determined based on their LC_{50} values and was ranked from high-to-low toxicity as cypermethrin > dichlorvos > λ-cyhalothrin and acephate.
- Acephate was more friendly insecticide to *C. vestalis* adults under laboratory conditions.
- However, hypothesis ii of this study which stated that, "use of pesticides by vegetable farmers impacted negatively on the exotic parasitoids of *P. xylostella* resulting in low rates of parasitisms of both larvae and pupae of the crop pest", was not fully tested. This required sampling parasitoids in areas where insecticides are applied and not applied. More work is required to clarify impact of insecticides on parasitoids.

- Similarly, hypothesis iii of this study was also not fully tested. The hypothesis stated that, "the established exotic parasitoids of *P. xylostella* in the Lusaka Province occurred in densities that could not effectively control this vegetable pest due to the impacts of insecticides used to control *P. xylostella* by vegetable farmers on them". In order to fully test the parasitoid densities that are effective against the DBM required to be known in order to compare with parasitoid density figures collected from the field. Further future work is required to ascertain this.

6.1.3 Phenology of *Plutella xylostella* in Lusaka District and Identification of its Parasitoids

- Phenological data revealed that the *P. xylostella* larval population reached its highest level at the end of August, while the pupal population was in mid-September.
- The parasitoids of *P. xylostella* recorded were *C. vestalis, D. collaris, D. mollipla, O. sokolowskii* and a hyper-parasitoid of *C. vestalis*, *Aphanogmus* sp.
- *Aphanogmus* sp. is being reported for the first time in Zambia.

6.1.4 The Effectiveness of *Cotesia vestalis* as a Biological control Agent of *Plutella xylostella* in Zambia

- The effectiveness of *C. vestalis* as a biological control agent of *P. xylostella* was illustrated by its exhibiting a type II functional response with a searching efficiency of 0.91 h (54.6 minutes) and handling time of 0.017 h (1.02 minutes).

6.2 Recommendations.

The following recommendations are made:

- Assessment of the potential of *P. xylostella* parasitoids should be done under field conditions.
- Acephate should be recommended for *P. xylostella* control in vegetables for it has shown to be less toxic insecticide to *C. vestalis*.
- Parasitism and functional response of parasitoids need to be investigated under field conditions, to further the development of a biological control programme for *P. xylostella.*
- There is need to mass rear *C. vestalis* for release in selected farms to augment the existing populations of this parasitoid.
- The impact of all insecticides used against *P. xylostella* on its key parasitoids need to be further investigated.
- Since the phenologies of *O. sokolowskii* parasitoid and the diamondback moth larva were found to be in synchrony and that of *O. sokolowskii* is also density dependent, it is recommended that future studies on the diamondback moth take this parasitoid into consideration as biological control agent.

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Appendix 1. Identification Job Sheet Number 2009/222

AGRICULTURAL RESEARCH COUNCIL
LANDBOUNAVORSINGSRAAD

PLANT PROTECTION RESEARCH INSTITUTE
NA VORSINGSINSTITUUT VM PLANTBESKERMING

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REPl'BUC OF SOl'TH AFRICA / REPl'BLIEK VAN SUD- AFRIKA

BIOSYSTEMATICS DIVISION

IDENTIFICATION JOB SHEET

Material received from:

Mr Philemon H Sohati
Det of Crop Sciences
School of Agricultural Sciences
University of Zambia
PO Box 32379
Lusaka 10101
Zambia

Date: *2009/11/09*

Identification Job Number: *2009/222*

Date received: *2009/10*

Receipt acknowledged: No

Sender's Accession Number	Our Accession Number	Family Name	Species Name	Determiner & Dat
U18M1, U16F1, U15M1, U18M2, U31F1, U24M4, U5M1, U18F1, U116F2 U14F1, U29(11)		BRACONIDAE	*Cotesia vestalis* (Haliday) (previously known as C. *p/utel/ae)*	GL Prinsloo, 2009
U11(10), U25(7), U11 (4)		CERAPHRONIDAE	*Aphanogmus sp.*	GL Prinsloo, 2009

Remarks:

- C1 undetermined genus of Aphidiinae (Braconidae). Note. This is an aphid parasitoid.

Note:

- In all correspondence concerning this consignment, please refer to the job number mentioned above.
- Please enclose a completed copy of the attached form with subsequent consignments.

Yours sincerely

Vivienne Uys

V.M. Uys
Manager: Identification Services

Copy sent to RS Nofemela

Appendix 2: Farm Survey Questionnaire

FarmLocation: Gender

Date: …….

Brief Description of Farm:

Is it a mixed farm? YES/NO

If Yes, what are the crops grown and livestock kept?.....

How is livestock manure used?.....

Farm size: ...……...

Neighbouring land use:…….....

Vegetable types grown: ...…….....

What is the use of vegetables?.....

Rotation (e.g. past three years):…….....

Which are the common cabbage pests?:.....

What time of the year is the pest mentioned above most important?:.....

How do you control the pest(s)?.....

Any natural enemies observed? (e.g. spiders, wasps, parasites, ladybird beetles)
...…….....

Source of pesticides:...…….....

Which pesticides applied: …….....

…….....

How often? …….....

Mixtures applied? YES/NO

What combination:…….....

Appendix 3. Summary of the analysis of variance for *C. vestalis* mortality in leaf-dip bioassay insecticides experiment. Data transformed using arcsin √%.

Analysis of Variance: Cypermethrin

SOURCE	D.F.	S.S.	M.S.	V.R.	F. Pr.
Concentration	6	10.728300	1.788050	382.84	<0.001
Time	5	0.15047	0.030409	6.51	<0.001
Residual	30	0.140114	0.004670		
Concentration*Time	30	0.140114	0.004670	1.00	0.500
Total	41	11.020461	0.268792		

Analysis of Variance: λ-cyhalothrin

SOURCE	D.F.	S.S.	M.S.	V.R.	F. Pr.
Concentration	8	9.082479	1.135310	214.970	<0.001
Time	5	0.491200	0.098240	18.60	<0.001
Residual	40	0.211248	0.005281		
Concentration*Time	40	0.211248	0.005281	1.00	0.500
Total	53	9.784927	0.184621		

Analysis of Variance: Dichlorvos

SOURCE	D.F.	S.S.	M.S.	V.R.	F. Pr.
Concentration	7	15.33930	2.19133	199.32	<0.001
Time	5	0.47997	0.09599	8.73	<0.001
Residual	35	0.38479	0.01099		
Concentration*Time	35	0.38479	0.01099	1.00	0.500
Total	47	16.20406	0.34477		

Analysis of Variance: Acephate

SOURCE	D.F.	S.S.	M.S.	V.R.	F. Pr.
Concentration	9	15.928334	1.76915	213.40	<0.001
Time	5	0.574518	0.114904	13.85	<0.001
Residual	45	0.373205	0.008293		
Concentration*Time	45	0.373205	0.008293	1.00	0.500
Total	59	16.876057	0.286035		

Appendix 4. Percentage parasitism of *Plutella xylostella* larvae and pupae collected from cabbage at School of Agricultural Sciences Field Station, 2009/2010.

Month	Parasitoid species	No. of *P. xylostella* Larvae	Pupae	No. of Parasitoids emerged[2]	% Parasitism Larva	Pupae
		0	0			
Mar 2009	*C. vestalis*			0		
	D. collaris			0		
	O. sokolowskii			0		
	D. mollipla			0		
	Aphanogmus sp[1].			0		
		7	0			
Apr 2009	*C. vestalis*			4	57.1	
	D. collaris			0		0
	O. sokolowskii			0		0
	D. mollipla			0		0
	Aphanogmus sp.			0		
		47	0			
May 2009	*C. vestalis*			9	19.1	
	D. collaris			0		
	O. sokolowskii			0		
	D. mollipla			0		
	Aphanogmus sp.			0		
		533	17			
Jun 2009	*C. vestalis*			215	40.3	
	D. collaris			0		0
	O. sokolowskii			3(22)		17.6
	D. mollipla			1		5.9
	Aphanogmus sp.			3		1.4
		579	47			
Jul 2009	*C. vestalis*			240	41.5	
	D. collaris			9	19.1	

Continued on next page

Appendix 4 Continued

Month	Parasitoid species	No. of *P. xylostella* Larvae	Pupae	No. of Parasitoids emerged[2]	% Parasitism Larva	Pupae
	O. sokolowskii			19(154)		40.4
	D. mollipla			2		4.3
	Aphanogmus sp.			2		0.8
		522	98			
Aug 2009	*C. vestalis*			289	55.4	
	D. collaris			62	63.3	
	O. sokolowskii			39(347)		39.8
	D. mollipla			5		5.1
	Aphanogmus sp.			0		
		866	364			
Sep 2009	*C. vestalis*			148	17.1	
	D. collaris			96	26.4	
	O. sokolowskii			109(808)		29.9
	D. mollipla			4		1.1
	Aphanogmus sp.			0		
		870	387			
Oct 2009	*C. vestalis*			151	17.4	
	D. collaris			76	19.6	
	O. sokolowskii			110(814)		28.4
	D. mollipla			1		0.3
	Aphanogmus sp.			0		
		153	72			
Nov 2009	*C. vestalis*			41	26.8	
	D. collaris			6		8.3
	O. sokolowskii			20(114)		27.8
	D. mollipla			0		
	Aphanogmus sp.			0		

Continued on next page

Appendix 4 Continued

Month	Parasitoid species	No. of *P. xylostella* Larvae	No. of *P. xylostella* Pupae	No. of Parasitoids emerged[2]	% Parasitism Larva	% Parasitism Pupae
		11	1			
Dec 2009	*C. vestalis*			4	36.4	
	D. collaris			0		
	O. sokolowskii			1(4)		100
	D. mollipla			0		
	Aphanogmus sp.			0		
		0	0			
Jan 2010	*C. vestalis*			0		
	D. collaris			0		
	O. sokolowskii			0		
	D. mollipla			0		
	Aphanogmus sp.			0		

[1]Hyper-parasitoid of *C. vestalis*.

[2]Number in bracket=No. of *O. sokolowskii* emerged from *P. xylostella* pupae

Appendix 5. Functional response of *Cotesia vestalis* to 2nd instar larvae of *Plutella xylostella* after 24 hours.

Host Density	R1	R2	R3	R4	R5
5	5	4	4	4	3
10	7	9	6	7	7
25	22	16	21	18	13
50	22	19	32	24	29
100	46	27	24	49	37