ESTABLISHMENT OF AN EXOTIC PARASITOID C*OTESIA VESTALIS*, (HYMENOPTERA: BRACONIDAE) IN SELECTED STUDY SITES AS A BIOLOGICAL CONTROL AGENT OF DIAMONDBACK MOTH, *PLUTELLA XYLOSTELLA L.* (LEPIDOPTERA: PLUTELLIDAE) IN KENYA

JOAN MUKIRI MUKEMBU

MASTER OF SCIENCE

(Zoology)

JOMO KENYATTA UNIVERSITY

OF

AGRICULTURE AND TECHNOLOGY

2022

Establishment of an exotic parasitoid C*otesia vestalis,* (Hymenoptera: Braconidae) in selected study sites as a biological control agent of Diamondback Moth, *Plutella xylostella L.* (Lepidoptera: Plutellidae) in Kenya

Joan Mukiri Mukembu

A Thesis Submitted in Partial Fulfillment of the Requirements for the Degree of Master of Science in Zoology (Agricultural Entomology) of the Jomo Kenyatta University of Agriculture and Technology

2022

DECLARATION

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

. Signature……..Date……..

Joan Mukiri Mukembu

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

. Signature……..Date……..

Dr. Shadrack M. Muya

JKUAT, Kenya

. Signature……..Date……..

Dr. Steven Ger Nyanjom

JKUAT, Kenya

. Signature……..Date……..

Dr. Saliou Niassy

ICIPE, Kenya

. Signature……..Date……..

Dr. Johnson Nyasani

KALRO, Kenya

DEDICATION

This work is dedicated to my dear son, Abel and my loving family for their encouragement, and for supporting me tirelessly during the entire MSc journey.

Thank you very much.

ACKNOWLEDGEMENT

I am profoundly grateful to my supervisors Dr. Shadrack Muya, Dr.Ger Nyanjom, Dr.Saliou Niassy and Dr. Johnson Nyasani, for their guidance not only during my research work but when writing the dissertation. I am also grateful to Dr, Fathiya Khamis, Mr. Levi Ombura and the pathology unit for allowing me to use their facilities and for technical assistance during my research. I am thankful to the Zoology Department for giving me the opportunity to undertake this programme at the Jomo Kenyatta University of Science and Technology.

Janet Ambale and Charles Kanyi are gratefully acknowledged for their assistance during the study and Dorcas Kareithi for her guidance with statistical analysis. I also extend my gratitude to the German Federal Ministry of Economic Cooperation and Development (BMZ) who funded the Diamondback Moth Bio-control Project as well as the Capacity building Unit, *icipe* and the Board of Postgraduate studies at JKUAT for the academic and technical support provided during this study.

I also express my gratitude to Mr. Aritho for all his support and encouragement throughout the study.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABBREVIATIONS AND ACRONYMS

DBM Diamondback moth

GPS Geographic Positioning System

ICIPE International Centre for Insect Physiology and Ecology

PCR Polymerase Chain Reaction

DNA Deoxyribonucleic acid

COI Cytochrome oxidase I

ITS Internal Transcribed Spacer regions

Bt *Bacillus thuringiensis*

ABSTRACT

The diamondback moth *Plutella xylostella* (L) is a serious pest of cruciferous crops in most parts of the world. Use of chemical pesticides has been the main method adopted by farmers in a bid to control this pest. However, with the indiscriminate pesticide use, this pest has developed resistance to several insecticides. Moreover, insecticides are hazardous to the environment, consumers, sprayers as well as beneficial insects. Biological control using natural enemies has been suggested as an important component in the integrated management of the *P. xylostella*. In an effort to control the pest and minimize insecticide misuse, ICIPE was involved in a classical biological control project that involved importation and release of *Cotesia vestalis* from South Africa into the lowlands of Kenya in 2004. However, a survey carried out two years later indicated that the parasitoid had not established since only few parasitoids were recovered from the sites of release. Additional releases were done in Kwale, Kajiado, Kitui, Machakos and Makueni counties. The objectives of this study were to follow-up on the establishment and parasitism rates of the released *C. vestalis*, to characterize the samples through molecular techniques and identify factors affecting its establishment. Surveys were carried out in five Counties of Kenya: Kajiado, Kitui, Makueni, Machakos and Kwale County where sampling of ten randomly selected cabbages or kales per farm and collection of parasitoid pupa and the *P. xylostella* larvae and pupa. The numbers of *P. xylostella* and the parasitoids that emerged from the collected samples were recorded and the data was used to calculate the parasitism rate by *C. vestalis*. A well-structured questionnaire was used to collect information on cultural practices and farmers knowledge on use of natural enemies in pest management. The study showed that *C. vestalis* had established in its release sites but its parasitism rates remain very low, molecular techniques confirmed that the samples were Cotesia vestalis and cultural practices affecting establishment were irrigation, intercropping and use of pesticides. Therefore, farmers need to reduce the use of chemical insecticides and adopt more use of *Bt* products, increase farmers knowledge on biological control and adopt use of trap crops.

CHAPTER ONE

INTRODUCTION

1.1 Background to the study

Cabbages are very important in the Kenyan diet and also important for the local economy. Cabbages (*Brassica oleraceae* var *capitata*) belong to the cruciferae family which also include other economically important crops such as kales, broccoli and cauliflower (Varela *et al.*, 2003; Macharia, Löhr & De Groote, 2005). Cabbage is rich in carbohydrates, proteins, vitamin A and C, iron, riboflavin, calcium and phosphorous (Raemaekers *et al.*, 2001).

Production of cabbage is done for both subsistence use and income generation (Macharia *et al.*, 2005; Wambani *et al.*, 2007). Globally, cabbage is produced on approximately 2.5 million ha of land with China ranked as the largest grower on 980,914 ha while India was the second on 400,140 ha of land (FAOSTAT, 2014). Cabbages are also widely grown in North Korea (Grossrieder *et al.*, 2005) ,Thailand (Rowell *et al.*, 2005), Nigeria(Elizabeth & Zira, 2009), Ghana (Ezena, 2015) and Benin (Cherry, 2004). Additionally, the crops are very important smallholder subsistence crops in Ethiopia, Zimbabwe, Mozambique, Tanzania, Uganda, Malawi, and Kenya having 90% of their production on 0.1–0.5 ha plots (Ayalew *et al.*, 2002; Macharia *et al.*, 2005; Löhr *et al.*, 2007). Cabbage production is done during both the rainy and dry seasons (Badenes-Perez & Shelton, 2006; Cobblah *et al.*, 2012).

However, successful production of crucifers is constrained by high cost of inputs such as pesticides, insect pests, diseases and shortage of good quality irrigation water (Hines & Hutchison, 2001; Löhr *et al.*, 2007). The common diseases affecting cabbage production include; Alternaria leaf spot (*Alternaria ssp*), black rot (*Xanthomonas campestris* pv. *Campestris*), blackleg (*Leptosphaeria maculans*), fusarium yellows (*Fusarium oxysporum* f. *conglutinens)* and downy mildew (*Peronospora parasitica*) (Massomo *et al.*, 2005; Wakeham & Kennedy, 2010). On the other hand, the economic pests of cabbages include; cabbage aphids (*Brevicoryne brassicae* Linnaeus), Diamondback Moth (*Plutella xylostella* Linnaeus), cabbage whitefly (*Pieris brassicae* Linnaeus) and the cutworms (*Agrotis ipsilon* Hufnagel) (Badenes-Perez & Shelton, 2006; Munthali, 2009). The Diamondback Moth larvae are considered the most important pest in cabbage production worldwide (Sarfraz & Keddie, 2005; Badenes-Perez & Shelton, 2006). Infestations by this pest result in huge crop losses of up to 80% and 98 % in China and Kenya respectively (Shelton *et al.*, 2000; Macharia *et al.*, 2005).

In an effort to control *P. xylostella,* farmers rely heavily on insecticides and application frequencies keep rising every year (Macharia *et al.*, 2005). This pesticide usage has various shortcomings; they are expensive (Zalucki *et al.*, 2012), develop resistance (Luogen *et al.*, 2005; Sarfraz *et al.*, 2005) and contribute in elimination of natural enemies (Kfir & Thomas, 2001a; Dobson *et al.*, 2002). Exposure of *Diadegma insulare* to permethrin caused high mortality while exposure to spinosad resulted in 100% mortality (Hill & Foster, 2000).

Considering these negative impacts and reduced control efficacy of insecticides, it is evident that it is not a sustainable option for farmers. Therefore, farmers ought to adopt cheaper, more effective and environmentally friendly options by using Integrated Pest Management (IPM) with strong focus in incorporation of biological control measures (Sarfraz *et al.*, 2005).

1.2 Statement of the Problem

The major constrain in crucifer production in East Africa has been *P. xylostella*, with the pest threat in Kenya rating at 75% and 69% on kale and cabbages respectively resulting in 25% -65 % of the production cost going into pest control (Oruku & Ndung'u, 2001). Use of pesticides in their management has not been effective and the parasitism rates by indigenous parasitoids have been below 15% in East Africa; evidence that the local parasitoids have been ineffective , necessitating introduction of more effective exotic parasitoids (Nyambo & Pekke, 1995; Lohr & Gathu, 2002; Lohr & Kfir, 2004).

This prompted a classical biological control effected by ICIPE through introduction of *Diadegma semiclausum* from Taiwan in 2001 to Kenya and Uganda leading to establishment and effective management of *P. xylostella* in the highlands and parasitism rates of 60% in Kenya (Löhr *et al.*, 2007; Gichini *et al.*, 2008). Another parasitoid; *C. vestalis* from South Africa was introduced into the East African mid-altitude and they established in Uganda but despite repeated releases in Kenya, very low parasitism rates (0.5-26.9%) were recorded (Nyambo *et al.*, 2008; Kahuthia-Gathu, 2012). Although studies confirm establishment of *C. vestalis* at the release sites, most of them relied on morphological identification.

Accurate identification of natural enemies is key to the success of biological control programs and past failures in pest control have often been attributed to the introduction or release of an incorrectly identified wasp species (Huber *et al.*, 2001). The genus *Diadegma* had some taxonomic dilemma (Fitton & Walker, 1992) such as when *Diadegma insulare* from Kenya was imported and released in Hawaii, but failed to establish and it was later identified as *D. semiclausum* (Wagener *et al.*, 2004) . Moreover, in 2000, all African *Diadegma* species were classified as *Diadegma mollipla* based on common morphological characters (Azidah, Fitton & Quicke, 2000) but Wagener *et al.* (2004) separated them into seven different *Diadegma* species using PCR- RFLP. There is also evidence of distinct biological differences between some populations of *C. vestalis*, (Rincon *et al.*, 2004) . Therefore, with the existing misidentification and the pre-1950 data being considered unreliable (Furlong, Wright & Dosdall, 2013) molecular techniques can reliably identify and separate biologically distinct but morphologically identical populations (Traugott *et al.*, 2006). An additional release of *C. vestalis* in Kwale County in 2013 (Unpublished data) resulted in very low parasitism rates.

This prompted the need to: follow-up on establishment of the South African strain of *C. vestalis* in Kenya, understand the genetic identity of *C. vestalis* found in the region since integrative taxonomy of the parasitoids had not been carried out in Kenya, evaluate its parasitism rates and establish factors that could affect its establishment.

1.3 Justification of the study

The current limitations of pesticide use has led to development and implementation of biological control which has been successful such as the classical biological control in St. Helena (a small British island in the South Atlantic Ocean), which eliminated the need for pesticides in management of *P. xylostella* (Kfir & Thomas, 2001). Therefore, *C. vestalis* has been ranked as the most abundant and the only effective parasitoid in the lowlands of the tropics (Talekar & Shelton, 1993) with wide use in biological control programs worldwide (Delvare, Kirk & Bordet, 2004; Talekar, 2004). Moreover, the South African biotype of *C. vestalis* appears to be the most effective because of its abundance in both low and high altitude, its dominance despite hyperparasitism, its high parasitism rates exceeding 90% in South Africa and adaptability to a wide range of temperatures (8.14-33°C) (Liu *et al.*, 2000; Lohr & Kfir, 2004; Nofemela, 2004).

A solution to shortcomings of morphological identification is the use of molecular methods that reliably identify different species (Wagener *et al.*, 2004; Traugott *et al.*, 2006). Molecular identification can improve the success of future biological control programs due to correct identification of species (Whitfield *et al.*, 2002).

Therefore, from the above stated evidence, the study will help to understand the genetic identity of *C. vestalis* found in Kenya, its contribution in suppressing *P. xylostella* densities and factors affecting its' establishment and consequently be helpful in improving biological control for effective management of *P. xylostella.*

1.4 Hypothesis

i. The exotic parasitoid of *P. xylostella*, namely *C. vestalis* released in Kwale, Kajiado, Kitui, Machakos and Makueni County has not established in the release sites.
ii. The parasitism of *P. xylostella* by *C. vestalis* in Kwale, Kajiado, Kitui, Machakos and Makueni counties of Kenya is not high.
iii. Cultural practices limits establishment of *C. vestalis* in Kwale, Kajiado, Kitui, Machakos and Makueni County.

1.5 General Objective

To determine the establishment of the exotic parasitoid; *C. vestalis* in and around their release sites in five counties in Kenya; Kwale, Kajiado, Kitui, Machakos and Makueni Counties for biological control of *P. xylostella*.

1.6 Specific Objectives

i. To determine the establishment and parasitism rates of *C. vestalis* on *P. xylostella* in Kwale, Kajiado, Kitui, Machakos and Makueni County.
ii. To characterize the *C. vestalis* found in Kwale, Kajiado, Kitui, Machakos and Makueni County using molecular tools.
iii. To determine cultural factors affecting the establishment of *C. vestalis* in Kwale, Kajiado, Kitui, Machakos and Makueni County.

1.7 Scope of the Study

The study was carried out to determine the establishment and parasitism rates of *P. xylostella* by *C. vestalis* in the various release sites in Kenya namely; Kwale, Kajiado, Kitui, Machakos and Makueni County, species identification of *C. vestalis* using molecular tools and cultural factors affecting its establishment. The study involved a survey in Kwale, Kitui, Makueni, Machakos and Kajiado Counties in 2015 and 2016. Questionnaires were used to collect information on cultural practices in crucifer farms that were visited. Additionally, Molecular work using PCR was done on *C. vestalis* samples collected to confirm its establishment after its release.

CHAPTER TWO

LITERATURE REVIEW

2.1 Overview of *Plutella xylostella*

The Diamondback Moth is a slender, grayish-brown moth with a light-brown colored band that form three-diamond shaped markings along the back when it fold its wings (Figure 2.1), hence its name (Capinera, 2001). Shaw, (2003) scientifically classified Diamondback Moth in the Plutellidae family, *Plutella* genus and *xylostella* (L.) species.

Figure 2.1: Image of *P. xylostella* adult (Greenlife, 2022)

2.1.1 Origin and distribution of *Plutella xylostella*

The origin of *P. xylostella* is believed to be the Mediterranean region (Talekar & Shelton, 1993). However, some believe that its origin could be in southern Africa based on presence of the highest parasitoid diversity (Kfir, 1998, 1997), long history of crucifer vegetable production (Schippers *et al.*, 2000) and presence of wide range of indigenous cruciferous species in the region (Lohr and Kfir, 2004). The distribution of *P. xylostella* is cosmopolitan (Obeng -Ofori *et al.*, 2007 ; Pratissoli *et al.*, 2008). The *P. xylostella* is the most abundant pest in Florida (Webb, 2004) and gained economic importance over time in Kenya and Ethiopia (Kibata, 1997; Ayalew *et al.*, 2002; Löhr *et al.*, 2007).

2.1.2 Biology of *Plutella xylostella*

According to Alizadeh *et al*. (2011), *P. xylostella* has four life stages: egg, larva (four instars), pupa and adult (Figure 2.2). The moth lays oval, flattened, yellowish eggs mostly on the underside of the leaves. After eggs hatch, the pale yellow first instars feed on the leaves' spongy mesophyll tissues, the pale green older ones feed and cause windowing of leaves by chewing on the lower leaf surfaces while the dark green late instars feed on all leaf tissues skeletonizing the plant. It's larvae have a characteristic violent wriggling when disturbed and typically drops from the plant while suspended on a silken thread (Sarfraz *et al.,* 2005). The last larval instar pupates on the lower or outer leaves. The pre-pupa and the pupa stages do not feed. Females lay an average of 323 eggs during their 10-day oviposition period (Webb, 2004; Alizadeh *et al.,* 2011). Development time is dependent on temperature and decrease linearly at temperatures between 10°C and 30ºC (Marchioro & Foerster, 2011).

Figure 2.2: Life cycle of *P. xylostella* (Hermansson, 2016)

2.1.3 Economic Importance of *Plutella xylostella*

The economic importance of *P. xylostella* is based on its pest status on cruciferous crops (Capinera, 2001). It's host range include crucifer vegetable such as Cabbages (*Brassica oleraceae* var *capitata*) and kales (*Brassica oleraceae* var *acephala*) as well as wild species such as *Raphanus raphanistrum, Rorippa* m*icrantha, Brassica juncea, Erucastrum arabicum* and *Rorippa nudiuscula* which act as refugia in absence of the crucifer vegetable crops (Reddy *et al*., 2004; Kahuthia-Gathu, 2007; Löhr *et al.*, 2007).

The *P. xylostella* larvae is the damaging stage which chew cabbage leaves and cabbage heads causing deformation (Figure 2.3) and forming entry points for decay pathogens (Webb,

2004). Infestations by this pest result in huge crop losses worldwide with direct losses and control costs being approximated at US$1 billion (Grzywacz *et al.*, 2010) and US$1.4 billion annual losses for cruciferous vegetables only (Zalucki *et al.*, 2012).

Figure 2.3: Image of *P. xylostella* damages on cabbage (A. M. Varela, icipe) (Infonetbiovision, n.d)

In India 70%-90% crop losses were experienced (Sandur, 2004; Webb, 2004) while 80% - 100% losses were incurred in China and Australia in absence of control measures (Shelton *et al.*, 2000). Huge annual crop losses amounting to $6 million and $8 million were incurred in California and Australia respectively (Shelton *et al.*, 2000). In India, 50% losses were incurred , management using insecticides costs up to $US34 million and a further $US20 million was used annually on spray labour (Sandur, 2004; Badenes-Perez & Shelton, 2006).Yield losses in Kenya amounted to approximately 6.8 tons/ha which added up to US$ 7.9 million annual losses and a further US$1 billion/annum in management costs (Macharia *et al.*, 2005; Zalucki *et al.*, 2012).

2.2 Management of *Plutella xylostella*

There are many ways of controlling DBM and these can be categorized into chemical, physical and bio-control.

2.2.1 Chemical management of *Plutella xylostella*

Use of conventional insecticides has been the most commonly used strategy in the management of *P. xylostella* worldwide (Kwon et al., 2006; Miranda, 2011). However, this has resulted in widespread development of resistance to virtually all classes of insecticides including carbamates, abamectins , organophosphates, and pyrethroids (Khaliq *et al.*, 2007; Oliveira *et al.*, 2011;Pu *et al.*, 2010; Talekar & Shelton, 1993) . Additionally, *P. xylostella*

was the first species reported to have developed field resistance to *Bacillus thuringiensis* (*Bt*) Cry toxins (Talekar & Shelton, 1993) and progressive increase in development of resistance was reported in Hubei, Hunan, and Guangdong provinces of china (Jiang *et al.,* 2015; Xia *et al.,* 2014).

2.2.2 Cultural management of *Plutella xylostella*

Intercropping of cabbages with other crops restricts the pest insects' ability to locate and colonize the main crop by interfering with the identification of the right host plants (Talekar & Shelton, 1993) . Generally, *P. xylostella* population was lower in cabbages intercropped with onions, tomatoes and pepper compared to pure cabbage stands (Luchen, 2012; Warwick,*et al,* 2010). Tomato plants have a repelling odour which distrupts oviposition, resulting in a reduction in pest infestation (Silva-Aguayo, 2007).

Trap crops have also been used in management of *P. xylostella*. In general, the attractiveness of the trap crop as well as the proportion of trap crops the field are important factors in the success of a trap cropping system and low proportions of trap crops in a field may not be sufficient to reduce insect pest populations significantly (Badenes-Perez, Nault & Shelton, 2005; Shelton & Badenes-Perez, 2006). In situations in which trap cropping was successfully implemented, it provided sustainable and long-term management solutions to control difficult pests (Asman, 2002; Charleston & Kfir, 2000).

The use of overhead irrigation also lowers *P. xylostella* infestation. Overhead irrigation in required amounts on head cabbage was reported to have lower DBM infestation than drip or furrow-irrigated crops (Capinera, 2002; Christopher, 2020).

2.2.3 Biological Control of *Plutella xylostella*

Biological control is the use of natural enemies such as predators, entomopathogens and parasitoids in management of a pest. Biological control such as the use of parasitoids has several advantages as opposed to use of insecticide which include; no development of resistance, being environmentally friendly, self-perpetuating, self-dispersing and have a long-term effect (Van Lenteren *et al.,* 2003; Bale *et al.,* 2008). On the other hand, they have their limitations; they take time to establish, are slower in killing their host and high initial costs are involved in the process of their introduction (Bale *et al.,* 2008).

Integrated pest management of *P. xylostella* with strong biological control focus is currently suggested as the best option to overcome pesticide overuse by farmers (Hill & Foster, 2000). Integrated Pest Management (IPM) is a strategy of pest management that involves the

combination of biological, cultural and chemical control measures in an ecologically sound way to attain sustainable crop production. IPM focuses on long-term prevention or suppression of pest problems and it may include chemical control, but it seeks to minimize pesticide use so as to minimize their associated negative effects.

2.2.3.1 DBM natural enemies: Entomopathogens

DBM is attacked by a range of entomopathogens. Due to their specificity against target pests and minimal environmental impacts, microbial pest control agents (MPCAs) are welcome additions to IPM programs. The potential of various entomopathogens including bacteria, fungi, viruses, protozoa, and nematodes as biocontrol agents has been tested for DBM management.

Bacteria: Over 100 *Bt*-based pesticides have been introduced across the world(Glare, 2000). Although *P. xylostella* has shown some considerable resistance to Bt-based products(Sarfraz , 2004), *Bt* products are still effective in many crucifer producing regions and need to be used judiciously to conserve their efficacy (Braun *et al.*,2004).

Use of fungal pathogens result in death of insects in a matter of days (Glare, 2000; Inglis et al., *2001*). Several species of fungal pathogens, including *Zoophthora radicans* (Brefeld) Batko, *Beauveria bassiana* (Balsamo) Vuillemin, and *Metarhizium anisopliae* (Metsch.) have been isolated from DBM and caused 100% DBM mortality after 3 - 7 days, (Cherry, 2004; Furlong *et al.*, 2004; Kirk *et al.*, 2004) and contaminated moths effectively transmit this fungus to healthy moths and larvae foraging on plants and are highly infective (Kirk *et al.*, 2004).

A number of baculoviruses have showed promising levels of pathogenicity to *P. xylostella* including a Kenyan isolate of PxGV (Nya-01) with 82 and 90% infection rates for second and first instars, respectively (Grzywacz *et al.*, 2001). Nematodes and microsporidia have also been reported to cause infection to DBM (Idris *et al.*, 2002; Idris & Grafius, 2001) such as the *Steinernema carpocapsae* (Weiser) which gave 41% control of *P. xylostella* in Hawaii while a microsporidian, *Vairimorpha sp.*, caused 100% mortality even at a dosage of 1.5×10^3 spores per larva (Haque,Canning & Wright, 1999; Mason, Matthews & Wright, 1999).

2.2.3.2 DBM natural enemies: Predators and parasitoids

The *P. xylostella* has a wide range of parasitoids, with records of over 27 species among them being the pre-pupal, pupal and larval parasitoids in the *Cotesia, Diadegma*, *Diadromus,*

Oomyzus and the *Pteromalus* genus (Liu *et al.*, 2000; Kirk *et al.*, 2004). The, rates of parasitism of larvae and pupae were substantial and showed peaks of 60% and reached over 80% on a few occasions. Generally, the larval and pupal parasitoids such as *Cotesia vestalis, Diadegma insulare, Diadegma. semiclausum, Diadromus collaris* , and *Oomyzus sokolowskii* showed the greatest control potential in control of DBM while the egg parasitoids had very little control (Talekar & Shelton, 1993).

There are four species of parasitoids are dominant in the Eastern Cape (South Africa) namely; *Diadegma mollipla* (Holmgren), C. *vestalis* , *D. collaris* and *O. sokolowskii* with parasitism rates that varied throughout the year ranging from 10 to 80% and even 100% when the DBM moths numbers were low ((Smith & Villet, 2001;Smith & Villet 2004). In China, *C. vestalis* and *O. sokolowskii* (Kurdj.) were considered the most predominant parasitoids (Liu *et al.* 2000).

In Ethiopia, *Diadegma spp*. and *Cotesia vestalis* were responsible for over 90% of observed parasitism (Ayalew *et al.* 2004). *Diadromus* species are primary prepupal and pupal parasitoids of DBM in various regions of the world including Canada (Braun *et.al* 2004), South Africa(Kfir, 1997; Kfir, 1998;Kirk *et al.*, 2004), China (Liu *et al.* 2000), India (Chauhan & Sharma, 2004), France, Turkey, Bulgaria, Georgia and Greece (Kirk *et al.*, 2004). Biological control has been carried out with different success rates. The use of *D. collaris* resulted in parasitism rates as high as 80% and 98% in North America and South Texas respectively (Hutchison *et al.*, 2001; Smith & Villet, 2003).

The solitary endoparasitoid, *Diadegma insulare* accounted for 70-90% parasitism under laboratory conditions (Monnerat *et al.,* 2002) and over 90% field parasitism rate in Texas (Legaspi *et al.,* 2000). On the other hand, the parasitoid species *C. vestalis, D. collaris* and *O. sokolowskii* had parasitism rates ranging from 10 to 60% in China (Liu *et al.*, 2000). In South Africa, *D. mollipla*, *C. vestalis*, *D. collaris* and *O. sokolowskii* recorded parasitism rates of 10-80% depending on the period of the year (Kfir & Thomas, 2001; Smith & Villet, 2003). The dominant parasitoid species in Ethiopia were *Diadegma sp* and *C. vestalis* which accounted for over 90% parasitism while *O. sokolowskii* accounted for only 1% parasitism rate (Ayalew *et al.*, 2006).

In East Africa, control of *P. xylostella* has concentrated on classical biological control using new parasitoid species and strains and conservation of natural enemies. Major initiatives by ICIPE involved introduction of *Diadegma semiclausum* from AVRDC in 2001 for highland

production and *Cotesia vestalis* from South Africa for the lowlands. Effective control was achieved by *D. semiclausum* since there was an increase in parasitism rates from 4.2% to 40.6% and a great decrease in pest density from 5.9 to 2.4 *P. xylostella* per plant three years after its release (Löhr *et al.*, 2007).

2.3 Overview of *Cotesia vestalis*

The parasitoid**,** *Cotesia vestalis* was initially named *Apanteles Foerster* by Cameron, reclassified into the *Cotesia* genus by Mason (Mason, 1981) and later renamed to *C. plutellae* by Fitton and Walker (1992). It belongs to the braconid family and the Microgastrinae subfamily (Fitton & Walker, 1992). Currently, it is known as *Cotesia vestalis* (Shaw, 2003).

The solitary parasitoid, *Cotesia vestalis* (Haliday) is the most common larval endoparasitoid of *P. xylostella* in several parts of the world including South Africa (Kawaguchi & Tanaka, 1999). Although *C. vestalis* has been reported to have a relatively wide host range, it is pre-dominantly viewed as a parasitoid of the *p. xylostella* (Shi, Liu & Li, 2002b). Cocoons production in the laboratory *by C vestalis* larvae was low (<than 10% successful parasitism) in nine host species (*Herpetogramma luctuosali, Hellula undalis, Ephestia kuehniella, Pyrausta panopealis, Helicoverpa armigera, Macdunnoughia confusa, Trichoplusia ni, Trichoplusia intermixta, and Zizeeria maha*) but no *C. vestalis* emerged from the larvae of those host species when collected in the field, revealing that they are rarely hosts of *C. vestalis* (Hiroyoshi *et al.*, 2017).

2.3.1 Origin and Distribution of *Cotesia vestalis*

The solitary parasitoid, *Cotesia vestalis* is believed to have originated from Europe and later spread throughout the world (Talekar & Shelton, 1993). It is common in western and southern Africa and the most abundant parasitoid of *P. xylostella* in South Africa (Nofemela & Kfir, 2008). It is distributed throughout China, Pakistan, Thailand, India, Indonesia (Kfir, 1997; Liu *et al.*, 2000; Rowell *et al.*, 2005) and Taiwan (Talekar, 2004).

Furthermore, *C. vestalis* was introduced into Australia, Dominica, Fiji, Thailand and United States (DeAngelis & Waterhouse, 1987; Rowell *et al.*, 2005).It's also widely distributed in Africa including South Africa, St. Helena, Ghana, Ethiopia and Kenya (Kfir & Thomas, 2001; Ayalew *et al.*, 2002; Cobblah *et al.*, 2012; Kahuthia-Gathu, 2012).

2.3.2 Biology of *Cotesia vestalis*

Life stages of the *C. vestalis* include egg, larvae, pupa and the adult stage (Figure 2.4). Molting of the first two larval instars occurs inside the host whereas the third instar molts outside the host to pupate (Yu *et al.*, 2008).

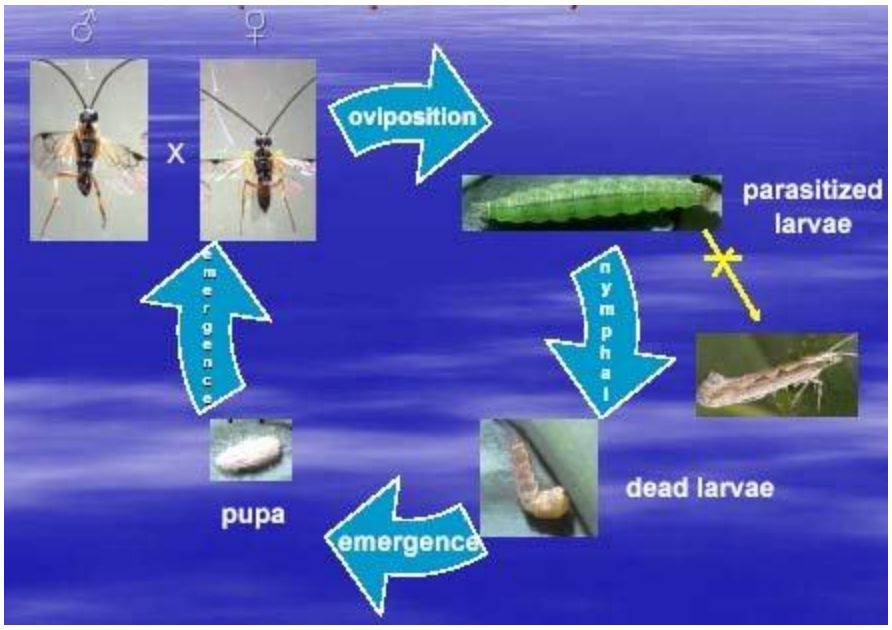


Figure 2.4: Life cycle of *Cotesia vestalis* (Soyelu, 2010)

The life cycle of *C. vestalis* is as follows; females lay spindle-shaped eggs in *P. xylostella* larvae for up to 10 days and the eggs hatch into small larvae which undergo three larval instars. The first instar stage is caudate-mandibulate, pale with a large head and no defined segmentation. The second instar is vesiculate, greenish, have anal vesicle and form an exit hole on the host before entering the third instar stage. The third instar is hymeniptoriform, yellowish-green, lack the anal vesicle and form a cocoon immediately on exiting its host. The pre-pupa is bright yellow and has 13 segments and pupate in about a day. Its pupa is oval and white, emerging in an average of 5 days. The adult is black with transparent yellow legs, a wingspan about 6mm wide. Its average lifespan is 16days (Yu *et al.*, 2008; Alizadeh *et al.*, 2011). The *C. vestalis* preferably parasitizes the second and the third instars of *P.xylostella* and its development rate increases considerably with an increase in temperature (Nofemela, 2004).

2.3.3 Establishment and parasitism rates of *Cotesia vestalis*

The larval parasitoid, *Cotesia vestalis* Haliday (Hymenoptera: Braconidae), has been the

subject of many classical biological control introductions, and many of them have been successful (Talekar, 2004) including South Africa where it achieved a parasitism rate of 80% (Kfir & Thomas, 2001). Moreover, introduction of *C. vestalis* in St. Helena (a small British island the South Atlantic Ocean) from South Africa whereby farmers were advised to replace chemical insecticides with *B. thuringiensis* to maximize opportunities for parasitoid establishment resulted in establishment of *C. vestalis* with high parasitism rates (27.7-80%) (Kfir & Thomas, 2001;Rami, 2005). On the other hand, an initial introduction of *C. vestalis* into some countries of the Caribbean including Dominica, St. Lucia and Jamaica did not result in effective suppression of DBM but its reintroduction in Jamaica in 1989 was successful resulting in an increase in parasitism rates from 5.4% to 88.9% (Alam, 1992; Talekar & Shelton, 1993).

The *C. vestalis* is an indigenous parasitoid in Thailand and a parasitism rates of 54% was recorded (Rowell *et al.*, 2005).In Ghana, *C. vestalis* , constituted 92% of the parasitoid species (Cobblah *et al.*, 2012) and its parasitism rates in Ethiopia ranged from 3.6% to 58.2 % (Ayalew *et al.*, 2002).In Kenya, introduction of C. vestalis in semi-arid midlands from South Africa, resulted in low parasitism rates (Löhr *et al.*, 2007;Kahuthia-Gathu, 2012)

2.3.3 *Cotesia vestalis* identification

Traditionally, insect identification was based on external morphological features, by making use of the Linnaean taxonomy which classified living things into a hierarchy, originally starting with Kingdoms.

Over time, more user-friendly and versatile matrix based keys have been developed. For instance , the INTKEY for identification of economically important species of the Cotesia genus is very useful in identification of many species of *Cotesia* even for non-*Cotesia* taxonomists (Dallwitz, Paine & Zurcher, 1999). The key uses insect features such as the cocoon colour, the body length, antennae length, hind coxa colour, forewing colour and mesosoma shape to distinguish the various *Cotesia* species (Figure 2.5).

The features that distinguish *C. vestalis* from other *Cotesia* species include; solitary cocoons which are white or yellowish-white, the body being less than 3mm long, mesososcutum being rugose posteriorly, black hind coxa, long female antennae that are almost equal to the body length and pale translucent tegula that sharply contrast its darker mesoscutum among other distinguishing features (Dallwitz *et al.*, 1999; Long & Dzung, 2014).

Figure 2.5: Pictorial identification of *Cotesia vestalis* including its forewing colour in the INT Key (Dallwitz *et al.,* 1999)

Regardless of the usefulness of morphological identification, it has its limitations such as shortage of trained taxonomist, high costs of identification and poor taxonomic keys (Stein *et al.*, 2013). Following shortcomings of morphological identification, molecular techniques have been more reliable in identification of species and studies of hymenopteran species (Behura, 2006; Greenstone, 2006).

2.4 Species identity of *Cotesia vestalis* using molecular tools

Molecular techniques have been successfully used in facilitation of ecological as well as population genetics studies among hymenoptera (Caterino *et al.*, 2000; Behura, 2006; Greenstone, 2006). Molecular markers have been used in the study of braconid parasitoids (Hufbauer *et al.*,2004) and are useful in discrimination of cryptic species (Hoy *et al.*, 2000;MacDonald & Loxdale, 2004).The molecular diagnostic approaches used include: specific PCR, DNA barcoding and microsatellite analysis which rely on PCR-based techniques that result in amplification of the specific DNA region targeted using molecular markers, to facilitate visualization of the product by gel electrophoresis (Gariepy *et al.*, 2007).

DNA (Deoxyribonucleic acid) barcoding is the amplification and sequencing of a particular DNA fragment mainly the mitochondrial cytochrome oxidase I (COI) gene (Greenstone, 2006) to identify an organism by comparing the COI sequence of an unidentified specimen to DNA sequence databases of identified and characterized species. Therefore, DNA barcoding is useful in identification and classification of specimen (Greenstone, 2006) and is widely

used due to its ability to rapidly identify both unknown and cryptic species (Hebert *et al.*, 2003).

Different gene regions are targeted for molecular studies (Greenstone, 2006; Stouthamer, 2006) but the mitochondrial DNA(mtDNA) and the nuclear ribosomal DNA (rDNA) are the most popular (Gariepy *et al.*, 2007). For the mtDNA, the most common genes used are the Cytochrome oxidase I and II, whereas for the rDNA the most common are the first and second internal transcribed spacer regions (ITS 1 and ITS 2) (Greenstone, 2006). The mtDNA is widely used in taxonomy, evolutionary and population genetic studies of hymenoptera parasitoids (Gasparich *et al.*, 1997).

The cytochrome c oxidase is a protein of electron transport chain in the mitochondria. The COI gene codes for two of seven polypeptide subunits in the cytochrome c oxidase complex and is composed of about 894 bp (Russo, Takezaki & Nei, 1996). The 5′ partition of CO1 is used for the 'Barcoding of Life' initiative (Hebert *et al.*, 2003) and its 640 nucleotide region (Vrijenhoek, 1994) is used for accurate identification of specimens (Moritz & Cicero, 2004). COI is used as the universal barcode for animals (Gariepy *et al.*, 2007). The mitochondrial CO1 is among the most commonly used markers since its transmission is strictly maternal with high mutation rate, slow changes in its amino-acid sequence (Russo *et al.*, 1996) and very robust universal primers allowing its use for most animal phyla (Vrijenhoek, 1994). Use of the CO1 region can provide a rapid and relatively inexpensive, deeper phylogenetic insights of a diverse biota since its evolution is rapid enough for discrimination of closely allied species (Cox & Hebert, 2001).

The most commonly used primers for braconids are the COI primers; 16SWb (5'-CACCTGTTTATCAAAAACAT-3') and 16S outer (5'-CTTATTCAACATCGAGGTC-3') (Dowton & Austin, 1994). An additional primer; CotF1 (5'-GGAACAGGTTGAACAGTTTATCCTC-3') has also been designed and combined with LepR1, targeting for Cotesia COI gene region (Malysh *et al.*, 2015). Moreover, the primer pairs, LepF 5'-ATTCAACCAATCATAAAGATATTGG-3' and LepR 5' -TAAACTTCTGGATGTCCAAAAAATCA-3' have been also used for detection of *C. vestalis*. (Hajibabaei *et al.*, 2006).

2.4 Cultural factors affecting the establishment of *C. vestalis* in Kenya

2.4.1 Use of pesticides

The indiscriminate use of pesticide common among the farmers is one of the factors that obscure establishment and conservation of parasitoid diversity, affecting contribution of natural enemies in management of pests (Devine & Furlong, 2007; Bopape *et al.*, 2014). Reports of reduced parasitism rates by *C. vestalis* and lower natural enemy densities have been recorded as a result of broad spectrum insecticides indicating their vulnerability to insecticides (Furlong *et al.*, 2008; Bommarco *et al.*, 2011). High parasitism rates by *C. vestalis* of 90% have been observed in farms untreated with pesticides as opposed to low parasitism rates of 5-10% in sprayed plots (Rami, 2005). Insecticides effect on parasitoid diversity is vivid in studies whereby treatment with dichlorvos resulted in a record of two parasitoid species, only one parasitoid species with Dipel ® treatment compared to a higher species richness of four in the control treatment without pesticides (Bopape *et al.*, 2014).

The effect of different pesticides on parasitoids varies from moderate to extremely toxic. Pesticides caused high mortality in only 24hours : fenvalerate 50% mortality, avemectin caused 100% mortality of *O. sokolowskii* and 3.3% mortality in *C. vestalis* while Chlorfluazuron caused 16.7% mortality of *C. vestalis* (Shi *et al.*, 2004). Additionally, fipronil significantly reduced the survival of *C. vestalis* adults emerging from fipronil-treated cocoons (Shi *et al.*, 2004).

Adult mortality of *C. vestalis* from pupa treated with indoxacarb and λ-cyhalothrin was as high as 100% and 88.9% respectively, 100% mortality on ingestion of spinosad and λ-cyhalothrin while lower mortality was recorded on Neemix, Ecozin, Agroneem, Xentari, Match, Crymax and Dipel (Haseeb, Liu & Jones, 2004a).

The parasitoids; *C. vestalis* are more susceptible to methamidophos, avermectin and dichlorvos than their host, *P. xylostella,* showing that the recommended dosages for management of some pests can be very toxic to parasitoids (Lin *et al.*, 2007). Treatment of leaves with spinosad, carbaryl, imidacloprid caused mortality of *D. insulare* but *B. thuringiensis* and tebufenozide caused no mortality (Hill & Foster, 2000).

2.4.2 Competition by other parasitoids

There exist competition among primary parasitoid for their host. For instance, *Hyposter horticola* outcompetes *C. melitaerum* in multiparasitized host (Tian *et al.*, 2008; Van Nouhuys & Punju, 2010). However, multiparasitism often result in high mortality rates as the parasitoids inflict physical injuries to the host as well as injection of secretions which interfere with the host's physiology (Tian *et al.*, 2008). Death of *C. melitaearum* larvae in multiparasitized host can be due to physiological mechanisms since it has suctorial mouthparts while *H. horticola* has sickle-shaped mandibles which can be utilized to harm their competitor (Tian *et al.*, 2008).

Competition in multiparasitism cases differ with order of oviposition and the parasitoid species involved. Simultaneous parasitism of *Pseudoplusia includens* by *Campoletis* sonorensis and *Microplitis demolitor* does not show any difference in survival of both parasitoids while parasitism by one parasitoid followed by parasitism by a second one 6 hours later result in higher survival rates in *C. sonorensis* (Harvey *et al.*, 2009). Multiparasitism can occur involving *Campoletis chlorideae* and *Microplitis mediator* regardless of which parasitizes its host first but M. mediator has higher parasitism rates in both simultaneous parasitization or where it parasitizes the host first (Tian *et al.*, 2008). On the other hand , some parasitoids such as *M. demolitor* was the least competitive even in cases where it parasitized its host first (Harvey *et al.*, 2009).

Interspecific competition between *O. sokolowskii* and *D. collaris* where the host is parasitized by *O. sokolowskii* six days prior to parasitization by *D. collaris*, emergence of the latter is greatly reduced (Liu *et al.*, 2001). There exists competition between *C. vestalis* and *O. sokolowskii* as well (Bai *et al.*, 2011). In instances where *D. semiclausum* parasitized its host two days prior to parasitism by *C. vestalis*, there was high mortality rates and as a result few parasitoids; few *D. semiclausum* emerge reducing the resultant efficiency of the next generation (Shi *et al.*, 2004a).

Hyperparasitoids also have a negative impact on *C. vestalis* populations. In South Africa, *C. vestalis* parasitize young host larvae limiting the availability of host for other parasitoid species which parasitize older stages of the host (Nofemela, 2004). Hyperparasitism of *C.*

vestalis by *O. sokolowskii* and *D. collaris*, leads to a decline in *C. vestalis* population and subsequently a negative impact on *P. xylostella* parasitism (Nofemela, 2013).

CHAPTER THREE
MATERIALS AND METHODS

3.1 Introduction

The study composed of a survey, data collection using a questionnaire and a molecular component. The field survey focused on five counties in Kenya; Kwale, Kajiado, Kitui, Machakos and Makueni. The selected cabbage farms were visited and ten cabbages were randomly selected, thoroughly checked for *P. xylostella* larvae and pupa as well as parasitoid cocoons on the leaves. The samples were collected, recorded and taken to the laboratory. In the laboratory, all emerging *P. xylostella* and parasitoids were recorded and the parasitism rates calculated. A questionnaire was used during the same survey to capture information on cultural practices in cruciferous vegetable production and farmer's knowledge on use of parasitoids as a biological control method in management of *P. xylostella*. The samples of *Cotesia vestalis* were used in molecular work which involved DNA extraction, Polymerase Chain Reaction (PCR), gel electrophoresis, purification, sequencing and sequence analysis.

3.2 Study site and study population

Surveys were carried out in 2015(Jun, Aug and Nov) and 2016 (Jan and Mar) in five counties of Kenya namely: Kajiado, Kitui, Machakos , Makueni and Kwale and the specific farms are indicated in the map which was constructed in QGIS version 6.2 (Figure 3.1). Kwale county regions were further divided into Diani, Kinondo, Lungalunga, Matuga, and Msambweni. The Kajiado, Makueni, Kitui and Machakos counties represented *C. vestalis* post-release sites for management of *P. xylostella* in the arid regions, mid-altitude (882-1918 m asl) while Kwale County represented the humid lowlands (3-416 m asl). The survey periods were further categorized as rainy (Jun 2015 and Nov 2015) and dry seasons (Aug 2015, Jan 2016 and Mar 2016). The GPS coordinates were as follows: Kajiado county (-2.5425833 to -1.8082778; 36.731 to 36.848), Kitui county (-1.374 to -3.382; 37.876 to38.334), Kwale county(; -4.129 to -4.450; 39.128 to 39.6130) , Machakos county (-1.563 to -1.453; 37.230 to 37.265), Makueni(-1.761 to -1.789; 37.587 to 37.670).

,

Kwale County has bi-modal rainfall distribution with the long rains being experienced in March to June and the short rains from October to December. The soils are fertile and mostly sandy loam while some parts are clayey. Kitui, Kajiado, Makueni and Machakos counties are hot and dry regions. The rainfall distribution in these regions is bimodal; long rains are experienced from March to May while the short rains are experienced from October to December. The soils are mainly sandy and the annual rainfall ranges between 500 mm and 1300 mm.

Most vegetable production in the survey regions is rain-fed. Cabbage and kales are the main cruciferous vegetables produced for subsistence use and commercial production to a small extent. The sample processing, rearing and molecular work was conducted at the International Centre of Insect Physiology and Ecology (*ICIPE*) campus at Kasarani, Nairobi.

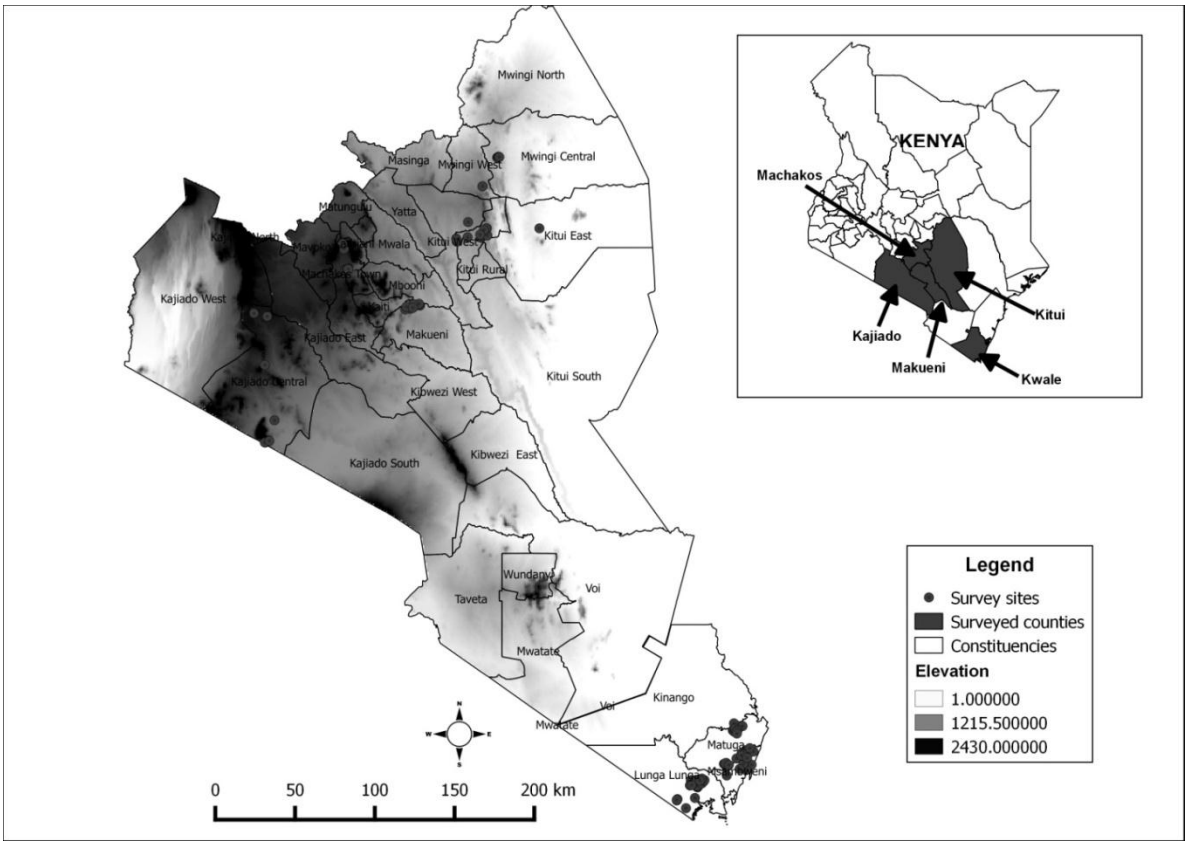


Figure 3.1: Locations of the survey sites in Kajiado, Makueni, Kitui and Machakos counties of Kenya for recovery of *Cotesia vestalis.*

3.3 Research Design

This study was descriptive in nature. Surveys were carried out in Kajiado, Makueni, Kitui and Machakos counties. During the surveys, a questionnaire was used to collect information on cultural practices used by crucifer farmers and their knowledge on the use of natural enemies in management of *P. xylostella*. The sampling of ten cabbages for each farm selected was done randomly.

The molecular work involved random selection of ten females and ten males of *C. vestalis* from each region namely: Kajiado, Makueni, Kitui and Machakos. This was followed by their DNA extraction, amplification and gel electrophoresis. Only ten samples from each group were purified, sequenced and their sequences analyzed.

3.4 Sampling Design and Sample Size

3.4.1 Sampling design

Farms on cabbage production were selected from Kitui, Kajiado, Kwale, Makueni and Machakos counties where the *C. vestalis* had been released. The farms selected were less than 0.5 ha in size and at least 1km apart.

Probability sampling was used in this study whereby every unit in the population had a chance of selection. The farms to be surveyed in Kitui, Kajiado, Kwale, Makueni and Machakos counties were selected randomly using the simple random sampling method.

Selection of ten males and ten females of *C. vestalis* from each region was done randomly. The ten samples selected for sequencing from each region was also randomly selected from the samples that had successfully amplified PCR products using the table of random numbers (Table 3.1).

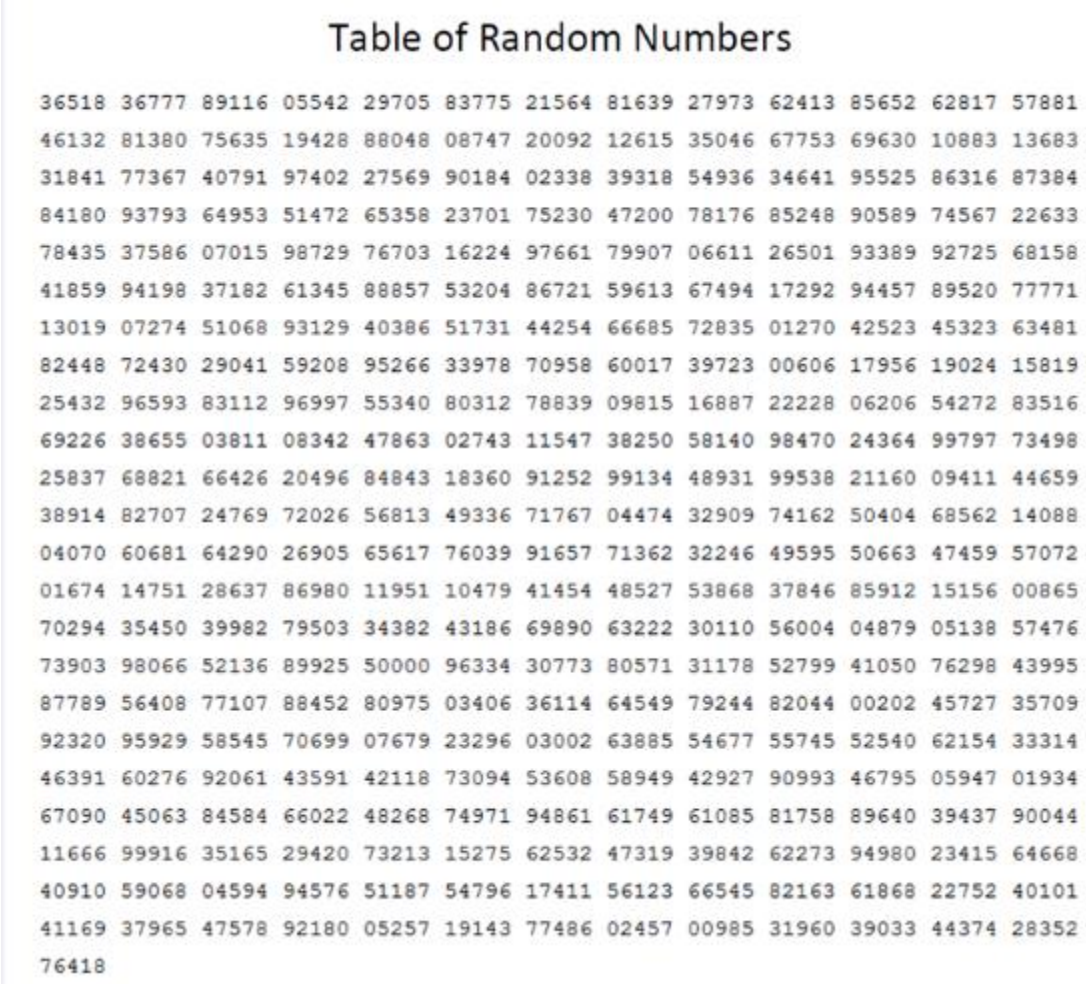


Table of Random Numbers

36518 36777 89116 05542 29705 83775 21564 81639 27973 62413 85652 62817 57881
46132 81380 75635 19428 88048 08747 20092 12615 35046 67753 69630 10883 13683
31841 77367 40791 97402 27569 90184 02338 39318 54936 34641 95525 86316 87384
84180 93793 64953 51472 65358 23701 75230 47200 78176 85248 90589 74567 22633
78435 37586 07015 98729 76703 16224 97661 79907 06611 26501 93389 92725 68158
41859 94198 37182 61345 88857 53204 86721 59613 67494 17292 94457 89520 77771
13019 07274 51068 93129 40386 51731 44254 66685 72835 01270 42523 45323 63481
82448 72430 29041 59208 95266 33978 70958 60017 39723 00606 17956 19024 15819
25432 96593 83112 96997 55340 80312 78839 09815 16887 22228 06206 54272 83516
69226 38655 03811 08342 47863 02743 11547 38250 58140 98470 24364 99797 73498
25837 68821 66426 20496 84843 18360 91252 99134 48931 99538 21160 09411 44659
38914 82707 24769 72026 56813 49336 71767 04474 32909 74162 50404 68562 14088
04070 60681 64290 26905 65617 76039 91657 71362 32246 49595 50663 47459 57072
01674 14751 28637 86980 11951 10479 41454 48527 53868 37846 85912 15156 00865
70294 35450 39982 79503 34382 43186 69890 63222 30110 56004 04879 05138 57476
73903 98066 52136 89925 50000 96334 30773 80571 31178 52799 41050 76298 43995
87789 56408 77107 88452 80975 03406 36114 64549 79244 82044 00202 45727 35709
92320 95929 58545 70699 07679 23296 03002 63885 54677 55745 52540 62154 33314
46391 60276 92061 43591 42118 73094 53608 58949 42927 90993 46795 05947 01934
67090 45063 84584 66022 48268 74971 94861 61749 61085 81758 89640 39437 90044
11666 99916 35165 29420 73213 15275 62532 47319 39842 62273 94980 23415 64668
40910 59068 04594 94576 51187 54796 17411 56123 66545 82163 61868 22752 40101
41169 37965 47578 92180 05257 19143 77486 02457 00985 31960 39033 44374 28352
76418

Table 3.1:Table of random numbers (Mathbitsnotebook, n.d.)

3.4.2 Sampling size determination

Samples of *P. xylostella* and parasitoids were collected from cabbages and kales in and around the original release sites of the parasitoids in the named counties with a view to recover parasitoids that were released in the different regions. Ten cabbages from each selected farm were randomly selected in each plot as previously done by Gichini *et al.* (2008).

3.5 Data Collection Methods

3.5.1 Sampling for *Plutella xylostella* and parasitoids on cultivated crucifers

The surveys were done in Kwale County: rainy season (June 2015, August 2015) and dry season (January 2016) and in Kitui, Kajiado, Kwale, Makueni and Machakos counties: rainy season (November 2015) and dry season (March 2016).

The farms sampled were those on cabbage production. Ten farms per region (Kitui, Kajiado, Kwale, Makueni and Machakos counties) were surveyed and the same farms were surveyed during each visit. However, less than ten farms were surveyed in Kwale county as a result of a massive decline in the number of crucifer farms available for sampling due to lack of irrigation water during the dry weather season in Jan 2016.

Ten randomly selected cabbages or kales per farm were sampled and their leaves checked for presence of *P. xylostella* larvae, pupa and parasitoid cocoons. The samples collected were counted, recorded and put in plastic containers having a cloth mesh at the top and lined with paper towel at the bottom to allow for ventilation and prevent condensation, respectively. Field numbers were assigned to each container. The number of samples collected, type/species collected, field number, host plant and collection date were recorded and the samples taken to the laboratory at *ICIPE*. Additionally, information on soil type and other pests found on the crop were recorded. The farms that were sampled were geo-referenced using the Global Positioning System (GPS, model Magellan® Triton™ 400).

The samples collected were taken to the laboratory and kept at room temperature (23 ± 2 ºC), 50-70 % relative humidity and a 12:12 hours (Light: Darkness) photoperiod. The *P. xylostella* larvae were fed on fresh cabbage leaves on daily basis. Emergence of either *P. xylostella* or parasitoid was monitored daily until no more emergence was observed. All the samples that emerged were identified, sexed and recorded. Identification and sexing of the braconid wasps collected was carried out using a Leica EZ4D microscope fitted with the Leica Application (LAS EZ 3.0.0) so as to facilitate viewing of their small ovipositor. Moreover, detailed features that identify *C. vestalis* were identified using the INT Key (Dallwitz et al., 1999). The adults that emerged from the field-collected samples were reared in Perspex cages (20 × 20 × 20 cm external dimensions) to establish colonies for both the host and parasitoids while all the dead parasitoids were stored in 95 % ethanol.

During the surveys on each sampled farm, questionnaires were used to collect information on type of cruciferous crop cultivated and variety, planting time, harvesting intervals, intercrops, type of manure and fertilizer applied and their last application dates, irrigation type, the pest management strategies applied by farmers, reason for pesticide application, frequency of their application, last application dates, change in the frequency of use of the management strategies and the farmers knowledge on use of natural enemies in controlling *P. xylostella*.

The South African strain of *C. vestalis* samples similar to which had been released in East Africa was provided by the Department of Agricultural Research Services (DARS) in Malawi. Twenty samples from each site were used for molecular work.

3.5.2 Rearing of *Plutella xylostella* and *Cotesia vestalis*

The emerging insects were reared to establish colonies for both the host and parasitoids. Parasitoids from each county were reared separately in Perspex cages to obtain a larger number for molecular work.

To establish a colony of *P. xylostella*, small aluminium foil strips were cut and some groves made on them. This was followed by smearing of crashed cabbage on the foil. These foils were then placed inside the oviposition cages of 20 x 20 x 20 cm and the emerging *P. xylostella* were introduced into the cages to oviposit on the aluminium foil for 24 hours. The *P. xylostella* were provided with 10% sugar solution on a ball of cotton wool that was placed on a Petri dish as their food. Thereafter, the aluminium foil containing the eggs were removed, cut into smaller pieces and placed on 8-week old cabbages for them to hatch and to ensure that the first instar larvae had access to food the moment they hatched.

The culture of *C. vestalis* was established from *C. vestalis* that merged from *P. xylostella* larvae and parasitoid cocoons collected from the field. The parasitoid culture were maintained on second and third instar DBM larvae on cabbages for 24hrs in laboratory rearing cages measuring 20 × 20 × 20 cm at 25±1°C, 65±5% RH and L12:D12 photoperiod. After the establishment of second to third instars larvae of *P. xylostella*, *C. vestalis* were put into the mating cage for 24 hours and honey streaked thinly on a paper sheet which was stuck on the sides of the cage using masking tape as a source of food and water was provided in glass vials with a cotton wick at the mouth. Second or third instars *P. xylostella* larvae were introduced into the *C. vestalis* cage and removed after 24 hrs. After removal of the plants, the larvae were put in lunch boxes (11.5cm diameter, 6cm high).The larvae in the lunch boxes were fed on cabbage leaves on daily basis until pupation.

The parasitoid cocoons were collected and placed in petri-dishes to await emergence. The environmental conditions in the laboratory were maintained at 25±1°C with relative humidity of 65 ± 5%. The photo to dark period was kept at 12: 12hours.

The emerging *C. vestalis* from each site were identified using insect identification keys(INT

key) and compared with the South African strain of *C. vestalis* samples.

Adults were stored in 100% ethanol for molecular study.

3.5.2 DNA Extraction, Amplification and Sequencing

3.5.2.1 DNA Extraction

The *C. vestalis* samples from Kitui, Kwale, Kajiado, Makueni were preserved in 95% ethanol. More samples of *Cotesia spp* similar to those that had been released in East Africa were sought from the Department of Agricultural Research Services (DARS) in Malawi. All the samples were stored at -20°C.The samples had their lateral, ventral and dorsal images taken using a microscope (Leica Application Suite).

Surface sterilization of the samples was done in 3% sodium hypochlorite and rinsed thrice in distilled water. After sterilization, the samples were put in labeled eppendorf tubes and stored at -20 °C awaiting DNA extraction.

DNA extraction was carried out using the ISOLATE II Genomic DNA Kit (Bioline, UK). Samples were homogenized in 1.5ml eppendorf tubes using a pestle in a 180 µl GL lysis buffer and 25µl proteinase K solution and vortexed. The homogenate was then incubated at 56°c for 3 hours. The samples were vortexed and 200µl of lysis buffer G3 added followed by vigorous vortexing and incubation at 70°C for 10 mins. DNA was extracted using 210µl of absolute ethanol. Binding of DNA was done in a spin column which was attached to a 2ml collection tube. After loading the sample into the spin column, it was centrifuged for 1 min at 11,000 x g and the flow-through was discarded. This was followed by washing with a wash buffer twice to remove residual ethanol. The first wash was done by adding 500µl of wash buffer GW1 and the second one by adding 600 µl of wash buffer GW2 and centrifuging for 1 min at 11,000 x g after each wash. The sample was then resuspended in 100µl elution buffer G that had been pre-heated (70°C), incubated for 1 min at room temperature and centrifuged again at 11,000 x g for 1 min. The DNA was then quantified using a nanodrop (Thermo Scientific© Nanodrop 2000™).

3.5.2.2 PCR amplification and Gel Electrophoresis

The 700bp fragment of the COI gene was amplified using universal primers; Forward primer-LCO_1490 (5'-GGTCAACAAATCATAAAGATATTG-3') and Reverse primer-HCO_2198 (5'-TAAACTTCAGGGTGACCAAAAAATCA-3') (Folmer et al. 1994) . The PCR was

done in a 20 µL reaction volume containing 5× My *Taq* Reaction Buffer (5 mM dNTPs, 15 mM $MgCl_2$, enhancers and stabilizers), 10 µ mole of each primer, 0.25 µL My *Taq* DNA polymerase (Bioline, UK), 0.5 mM $MgCl_2$ and 15 ng/µL DNA template. The reaction took place in the Nexus Mastercycler gradient (Eppendorf). The PCR cycling conditions were as follows: initial denaturation of 2 min at 95 °C, then 40 cycles of 30 sec at 95 °C, 40 sec annealing at 50.6 °C and 1 min at 72 °C, and a final elongation done at 72 °C for 10mins.

The PCR products were separated on a 1.2 % agarose gel stained with 10mg/mL of ethidium bromide. The gel electrophoresis was done at 80V for 40 mins. The DNA bands on the gel were analyzed and documented under ultraviolet illumination using the KETA GL imaging system trans-illuminator (Wealtec Corp). The PCR products with successfully amplified DNA were excised and purified using the Isolate II PCR and Gel Kit (Bioline, UK) as instructed by the manufacturer. The purified products were then sent to Macrogen Inc Europe Laboratory, in Netherlands for sequencing.

3.4 Data Analysis

The parasitism rates of *P. xylostella* by the solitary parasitoid, *C. vestalis* were calculated using the formula (Nofemela & Ktir, 2005) :

$$\%\, parasitism = \frac{Sum\ of\ \ Cotesia\ vestalis}{Total\ adults\ (C.vestalis\ +\ P.xylostella)} \text{x}100$$

All the samples that died before emergence were excluded from calculations of parasitism rates (Löhr *et al.*, 2007c). The datasets were checked for normality using the Shapiro-Wilk test in R studio. The data on *P. xylostella* and *C. vestalis* densities were normalized by log-transformation prior to analysis of variance. The data for every survey period was subjected to Analysis of Variance (ANOVA). The means having significant differences were separated using Tukey's Honest Significant Difference (HSD) test at 5% level of significance. All the analyses were done using the R Studio software version 2.15.1 (Studio, 2012). The data was then presented in form of graphs.

3.5 Sequence Analysis

Analysis of sequences was done by first examining the chromatograms in Chromas v 2.5.1 (Hall 1999), and ambiguous sites were corrected to produce a consensus sequence. The consensus sequences were compared to those available at GenBank using BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi) for species identification. Multiple alignment for the samples was done using Clustaw X version 2.1 (Thompson *et al.*, 1997). The general time reversible (GTR +G) model of nucleotide substitution was the best fit model according to the JModeltest 2.1.7 program (Darriba *et al.*, 2012). The consensus sequences were run in RAXml v8.2.0 (Stamatakis, 2014) to generate the best-scoring Maximum likelihood tree using the previously selected GTR+G model with a bootstrap test of 1000 replications (Saitou & Nei, 1987) and the phylogenetic tree construction was done and viewed using FigTree v1.4 (Rambaut, 2012).

The sequences were submitted to the Barcode of Life database (BOLD) and deposited in GenBank (Accession numbers: ABZ6416 and ADL5635). Storage of the DNA voucher specimens was done in the Arthropod Pathology Unit (APU) at *ICIPE*.

CHAPTER FOUR

RESULTS

4.1 Establishment and parasitism rates of *C. vestalis* on *P. xylostella*

During the June 2015 survey (cool and wet), the mean of *P. xylostella* densities in Lungalunga (12.67/farm) were twice the densities recorded in Matuga (6.55/farm). The *P. xylostella* ($F=2.081$; $df=2, 21$; $p= 0.15$) and *C. vestalis* ($F=0.403$; $df=2, 21$;$p= 0.673$) densities were not significantly different. On the other hand, the highest parasitism rates by *C. vestalis* were recorded in Lungalunga (5%) which was almost twice the parasitism rates in Matuga (2.78%) whereas the parasitism rates in Diani was at zero (Figure 4.1).

The August 2015 survey (hot and dry), had, *P. xylostella* densities in Matuga (17.95/farm) 2-fold higher than in Diani (9.5/farm) and Lungalunga (9.83/farm). Similary, both the *P. xylostella* ($F=1.006$; $df=2,32$; $p=0.377$) and *C. vestalis* ($F=1.042$; $df=2,32$; $p= 0.364$) densities were not significantly different. The parasitism rates were 3.51% and 3.27% in Diani and Matuga respectively.

In January 2016 (hot and dry), the highest *P. xylostella* density of 1.6/farm was recorded in Lungalunga while the lowest was in Matuga which had no record of *P. xylostella*. Comparison of the *P. xylostella* ($F=0.914$; $df=2,14$; $p=0.423$) and *C. vestalis* ($F= 1.102$; $df=2,14$; $p=0.359$) densities showed no significant difference. The parasitism rates by *C. vestalis* in Lungalunga was 18.20% while it was 14.29% in Diani (Table 4.1).

Generally, the highest *P. xylostella* densities (9.5-17.95/farm) were recorded in August (Figure 4.2). Additionally, the comparison of the *P. xylostella* densities between the different survey periods differed significantly ($F=18.86$; $df=2, 90$; $p<0.05$).

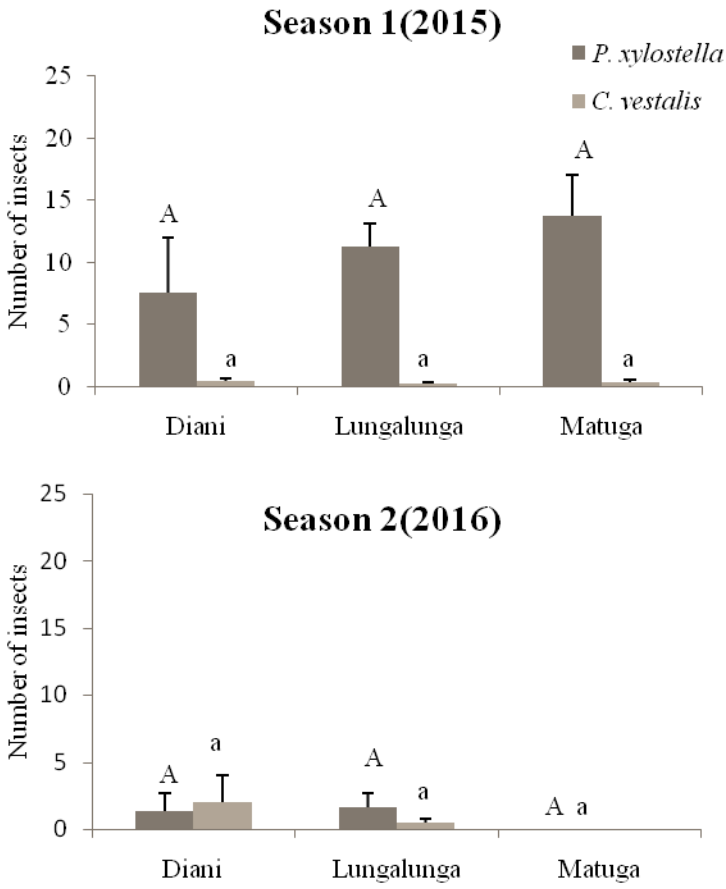


Figure 4.1: The number of *P. xylostella* per farm and its parasitoid *Cotesia vestalis* recorded in Kwale County in 2015 and 2016.

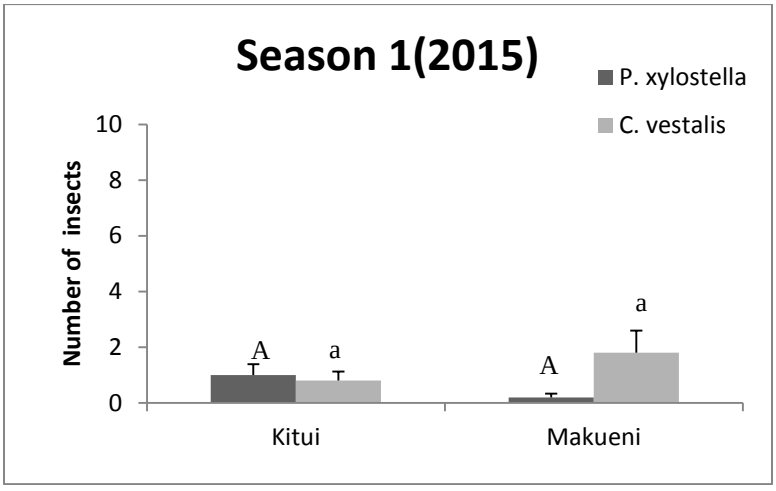
Table 4.1. The means of DBM+SE collected in Kwale county and parasitism rates+SE by Cotesia vestalis. Means+SE followed by same letters within a column are not significantly different at $P < 0.05$

Location	***Cotesia* Parasitism Means ±SE**	**DBM/plant Means ±SE**	**DBM/plant Means ±SE**	***Cotesia* Parasitism Means ±SE**	**DBM/plant Means ±SE**	***Cotesia* Parasitism Means ±SE**
	June 2015		Aug 2015		Jan 2016	
Diani	0	0	9.5±5.04a	3.41±2.63a	1.33±1.33a	14.29±14.29a
Lungalunga	12.67±3.27a	5.004±2.84a	9.83±2.54a	0	1.60±1.07a	18.20±10.08a
matuga	2.78±2.78a	6.55±2.09a	8.38.±3.60a	0.41±0.41a	0	0

In November 2015 (cool and wet season) survey, the *P. xylostella* density in Kitui (1/farm) was 5-fold compared with that in Makueni (0.2/farm) whereas the *C. vestalis* densities in Makueni (1.8/farm) was more than 2-fold higher than in Kitui (0.8/farm) as shown in Figure 4.2. There were no significant differences found between *P. xylostella* (F= 0.92; df=2,32; p=0.409) and *C.vestalis* (F=1.722; df=2,32; p=0.195) densities between the two regions . The parasitism rates in Kitui and Makueni were 20.92% and 37.86% respectively.

In March 2016 (hot and dry season), Kitui had the highest *P. xylostella* density (2.30/farm) which was over three times higher than in Makueni (0.6/farm). The densities of *P. xylostella* (F=1.119; df=1,18; p=0.304) and *C. vestalis* (F=3.6; df=1,18; p=0.074) were not significantly different during the survey (Figure 4.2). The parasitism rate in Kitui was 32.19% compared to the 15% parasitism rate recorded in Kitui.

There was no difference between the *P. xylostella* (F=1.873; df=1,78; p=0.175) and *C. vestalis* (F=3.029; df=1,78; p=0.0739) densities during the 2015 and 2016 survey in Kitui and Makueni counties. Overall, the highest parasitism rate was recorded in Kitui during the November 2015 survey (Table 4.2.)



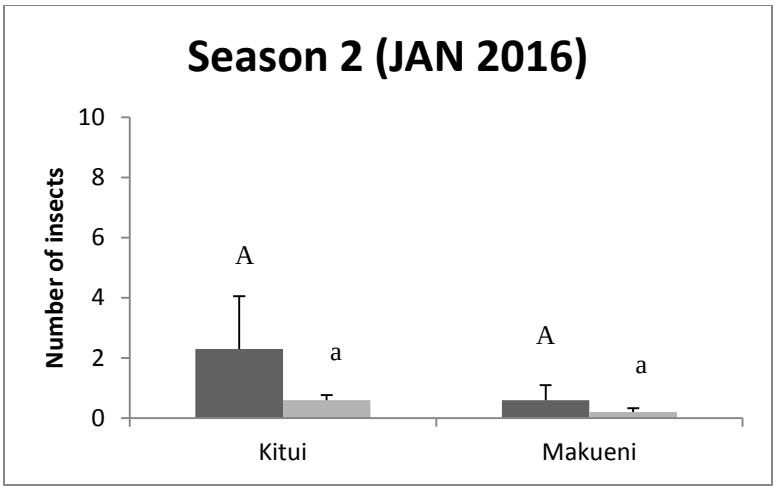


Figure 4.2: *P. xylostella* and *C. vestalis* densities for the different survey periods in Kitui and Makueni counties

Table 4.2: The means of DBM+SE collected in Kitui and Makueni county and parasitism rates+SE by *Cotesia vestalis*. Means+SE followed by same letters within a column are not significantly different at P < 0.05.

Location	DBM/plant Means ±SE	Cotesia Parasitism Means ±SE	DBM/plant Means ±SE	Cotesia Parasitism Means ±SE
	November 2015		March 2016	
Kitui	1.0±0.4a	13.83±6.84a	2.3±1.76a	25.77±10.48a
Makueni	0.2±0.13a	34.53±12.57a	0.6±0.50a	15.0±10.67a

A baseline survey on pest management practices indicated that 89.25% of the farm households interviewed in Kwale county were using pesticides. The farmers used a total of 18 different active ingredients of synthetic pesticides under different application regimes. Majority of the pesticides in Kwale county were pyrethroids, which constituted 75.47% of the total pesticides followed by neonicotinoids (8.49%) and carbamates (5.66%) (Table 4.3).

Based on the household data collected in Kitui, Makueni and Machakos, 78.57% of the farmers were spraying their vegetables. The most commonly used insecticides were pyrethroids (46.16%) whose usage was 3-fold higher than that of organophosphates (15.55%). Additionally, only 5.5% of the products used were micro-organism derived and 1.1% were plant derived products (Table 4.4).

According to the data on pesticide use and frequency of use collected from the questionnaires, only 10.75% of the farms in Kwale County were not using pesticides. The remaining majority of farms used a total of 18 different active ingredients of synthetic pesticides under different trade names. Majority of these were pyrethroids which constituted 75.47% of the total pesticides followed by neonicotinoids and carbamates which accounted for 8.49% and 5.66% respectively (Table 4.3).

Table 4.3:The various types of insecticide active ingredients used in Cruciferae farms and their frequency of usage in Kwale County.

KWALE COUNTY		
Active Ingredient	**Substance group**	**Usage Frequency (%)**
Lambda-Cyhalothrin	Pyrethroid	41.51
Alphacypermethrin	Pyrethroid	24.53
Deltamethrin	Pyrethroid	3.77
Beta-Cyfluthrin	Pyrethroid	3.77
Cypermethrin	Pyrethroid	1.89
Mancozeb	Carbamate	2.83
Carbosulfan	Carbamate	0.94
Methomyl	Carbamate	0.94
Propamocarbhydrochloride	Carbamate	0.94
Chlorpyrifos	Organophosphate	0
Dimethoate	Organophosphate	0
Diazinon	Organophosphate	0
Thiamethoxam	Neonicotinoid	3.77
Imidacloprid	Neonicotinoid	2.83
Acetamiprid	Neonicotinoid	1.89
Hydrochloride	Neonicotinoid	0
Lufenuron	Benzoylurea	3.77
Metalaxyl	Phenylamide	2.83

Fluopicolide	Benzamide	0.94
Carbendazim	Benzimidazole	0.94
Pyridaben	Pyridazinone	0.94
Sulphur	Fluoride	0.94
Hexaconazole	Triazole	0
Flubendiamide	Benzenedicarboxamide	0
Azoxystrobin	Strobilurin	0
Diafenthiuron	Thiourea	0
Emamectin benzoate	Micro-organism derived	0
Abamectin	Micro-organism derived	0
Azadirachtin	Plant derived	0
Bt	Microbial pesticide	0

Based on the data collected in Kajiado, Kitui, Makueni and Machakos, only 22% of the farms were not spraying their vegetables. Pyrethroids were the most used product which made up 46.39% of the total pesticides while organophosphates were second at 14.43% and neonicotinoids came in third at 12.37%. Additionally, only 4.44% of the products were micro-organism derived, 1.11% were plant derived products and 1.11% *Bt* (Table 4.4).

Table 4.4:The different pesticide active ingredients used in Cruciferae farms and their frequency of usage in eastern region (Kajiado, Kitui, Makueni and Machakos counties).

Active Ingredient	Insecticide class	Usage Frequency (%)
Lambda-cyhalothrin	Pyrethroid	16.67
Alpha-cyperpermethrin	Pyrethroid	13.33
Cypermethrin	Pyrethroid	12.22
Beta-cyfluthrin	Pyrethroid	3.33
Deltamethrin	Pyrethroid	1.11
Methomyl	Carbamate	5.56
Propamocarb	Carbamate	2.22
Mancozeb	Carbamate	1.11
Chlorpyrifos	Organophosphate	12.22
Dimethoate	Organophosphate	1.11
Diazinon	Organophosphate	2.22
Thiamethoxam	Neonicotinoids	4.44
Hydrochloride	Neonicotinoids	2.22
Imidacloprid	Neonicotinoids	3.33
Acetamiprid	Neonicotinoids	2.22
Emamectin benzoate	Micro-organism derived	1.11
Abamectin	Micro-organism derived	3.33
Hexaconazole	Triazole	2.22
Lufenuron	Benzoylurea	3.33
Flubendiamide	Benzenedicarboxamide	2.22
Azoxystrobin	Strobilurin	1.11
Diafenthiuron	Thiourea	1.11
Azadirachtin	Plant derived	1.11
Bt	Microbial pesticide	1.11

The change in frequency of pesticide application indicated that farmers were spraying more

frequently than the previous years as they complained of reduced efficacy of the pesticides which can be attributed to development of resistance over time. Pesticide application was weekly or fortnightly on routine basis while some only applied in presence of pests. The farmers also used two or more products either to manage *P. xylostella* or other pests and against diseases such as the leafspots and downy mildew.

The other common pests found were the cabbage aphids, bollworms, whiteflies ,thrips, cabbage loopers, cutworms and leafminers. Bollworms were ranked as the most abundant followed by whiteflies whereas aphids were the most abundant in Kajiado, Makueni, Kitui and Machakos counties followed by the whiteflies (Table 4.5).

Table 4.5: Ranking of other pests attacking crucifers in Kwale, Kajiado, Kitui, Machakos and Makueni counties)

Kwale County			
	Survey period		
Pests	June 2015	August 2015	Jan 2016
Bollworms	39.39	90.70	41.18
Whiteflies	-	2.33	23.53
Aphids	18.18	6.98	-
Cabbage loopers	3.03	-	11.76
Other caterpillars	-	-	5.88
Kajiado, Kitui, Machakos and Makueni counties			
	Nov 2015	Mar 2016	
Aphids	42.5	67.5	
Whiteflies	32.5	75	
Thrips	15	35	
Bollworms	5	15	
Cabbage loopers	5	5	
Leafminers	5	5	

Cutworms	2.5	-

Only 40% of the farmers were well informed about use of parasitoids in management of *P. xylostella* while the rest had little knowledge or no knowledge of parasitoids in management of *P. xylostella* in cabbage production.

Farmers were practicing intercropping whereby they intercropped crucifer crops with tomatoes, sweet pepper, black nightshade, amaranth, spinach, chillies or pumpkin. In Kwale county, 32.26% of the farms surveyed practiced intercropping as opposed to Kajiado, Kitui, Makueni and Machakos county where only 1.25% of the total farms surveyed intercropped their cruciferous vegetables with other crops (Table 4.6).

Production of vegetables relied on rainfall but some used irrigation especially in Kwale County whereby more than 90% of the farms surveyed in January 2016 were the ones under irrigation. Hand watering was the most used followed by drip irrigation as a way of conserving water in the semi-arid regions of Kajiado, Makueni, Kitui and Machakos counties . In Kajiado, Kitui, Makueni and Machakos counties, 78.75% of the farms surveyed were under irrigation during both survey periods (Table 4.6).

Table 4.6: Proportion of farm surveyed that practiced intercropping and used irrigation

	Farming practices						
	Kwale county				**Kajiado, Kitui, Machakos and Makueni**		
Survey period	**June 2015**	**Aug 2015**	**Jan 2016**	**Total**	**Nov 2015**	**Mar 2016**	**Total**
Intercropping (%)	9.09	51.16	29.41	32.26	2.5	0	1.25
Irrigation (%)	36.36	95.35	88.24	73.12	60	97.5	78.75

4.2 Molecular Identification

4.2.1 Quantification of DNA

The purity of the DNA based on the ratio of absorbance at 260 and 280nm was greater than 1.8 which is the ideal ratio for DNA that is quite pure. It ranged from 1.92 to 2.72. The nucleic acid concentration of the samples ranged between 30.8 to 146.3 ng/µl (Table 4.7).

Table 4.7: DNA concentration and quality for all the *Cotesia vestalis* samples.

Sample ID	Nucleic Acid Conc.	Unit	A260	A280	260/280
Kj6	138.1	ng/µl	2.763	1.38	2
Kj7	83.3	ng/µl	1.665	0.805	2.07
Kj8	116.4	ng/µl	2.329	1.178	1.98
Kj9	110.7	ng/µl	2.213	1.064	2.08
Kj10	107.6	ng/µl	2.152	1.123	1.92
Kj21	102.7	ng/µl	2.054	1.069	1.92
Kj22	145.9	ng/µl	2.918	1.476	1.98
Kj24	120.1	ng/µl	1.951	1.01	1.93
Kj25	66.8	ng/µl	1.336	0.668	2
Kt6	62.4	ng/µl	1.248	0.615	2.03
Kt7	41.9	ng/µl	0.838	0.369	2.27
Kt8	48.5	ng/µl	0.97	0.477	2.03
Kt9	48.2	ng/µl	0.965	0.454	2.13
Kt10	46.2	ng/µl	0.924	0.446	2.07
Kt26	45.9	ng/µl	0.917	0.432	2.12
Kt27	41.8	ng/µl	0.836	0.369	2.26
Kt28	39.3	ng/µl	0.785	0.286	2.74
Kt29	48.8	ng/µl	0.976	0.465	2.1
Kt30	31.8	ng/µl	0.637	0.25	2.55
Kw7	49.7	ng/µl	0.993	0.47	2.11
Kw8	69.5	ng/µl	1.391	0.687	2.02
Kw9	45.9	ng/µl	0.917	0.428	2.14

Table 4.7: (Continued)

Sample ID	Nucleic Acid Conc.	Unit	A260	A280	260/280
Kw10	51.9	ng/µl	1.037	0.501	2.07
Kw22	30.8	ng/µl	0.616	0.263	2.34
Kw23	34.7	ng/µl	0.695	0.308	2.26
Kw24	60	ng/µl	1.199	0.572	2.1
Kw25	72.5	ng/µl	1.451	0.496	2.93
Kw26	48.8	ng/µl	0.977	0.448	2.18
Mk1	118	ng/µl	2.36	1.053	2.24
Mk2	111.4	ng/µl	2.227	1.165	1.91
Mk3	98.9	ng/µl	1.977	0.98	2.02
Mk4	121	ng/µl	2.421	1.203	2.01
Mk5	146.3	ng/µl	2.926	1.499	1.95
Mk6	133.8	ng/µl	2.677	1.374	1.95
Mk7	127.4	ng/µl	2.548	1.325	1.92
Mk8	115.5	ng/µl	2.309	1.19	1.94
Mk9	86.8	ng/µl	1.737	0.898	1.94
Mk10	109.6	ng/µl	2.191	1.124	1.95
mw11	32.7	ng/µl	0.654	0.286	2.28
mw12	36.7	ng/µl	0.733	0.329	2.23
mw13	33.2	ng/µl	0.664	0.3	2.21
mw14	36.4	ng/µl	0.728	0.297	2.45
mw15	39.5	ng/µl	0.789	0.36	2.19
mw21	39.6	ng/µl	0.792	0.368	2.15
mw22	29.8	ng/µl	0.595	0.219	2.72
mw23	41.7	ng/µl	0.833	0.378	2.21
mw24	46.3	ng/µl	0.926	0.387	2.39
mw25	38.2	ng/µl	0.763	0.332	2.3

4.2.2 PCR Amplification

DNA from all the samples were successfully amplified with the Forward primer-LCO_1490 and Reverse primer-HCO_2198 resulting in PCR products of the same size indicating that the samples contained a single DNA molecule. Each band represents DNA of a specific molecular weight. The amplified products produced bands which were approximately 700bp (Figure 4.3).

Figure 4.3: Fragment patterns of the COI region (MtDNA) of *Cotesia vestalis* from Kajiado, Kitui, Kwale, Makueni and Machakos County separated on a 1% agarose gel

Lane 1-10 (Kajiado samples), Lane 10-19 (Kitui samples), Lane 20-28 (Kwale samples), Lane 29-38 (Makueni samples), Lane 39-48 (Malawi samples), L- 100 bp DNA ladder (Bioline).

4.2.3 Bioinformatics Analysis

BLAST results for the forty eight (48) samples had mitochondrion, complete genome as the best hit (E-Value 0.0) and had a 97-100% similarity to *Cotesia vestalis* samples of accession numbers FJ154897.1 (Wei *et al.*, 2010). However, Kw7 from Kwale was the only sample that had cytochrome oxidase subunit 1 (COI) gene as the best hit (E-value 0.0) with 98% identity to *Cotesia sp*. (Genbank accession: HM430398.1) (Alex Smith *et al.*, 2013) as shown in Table 4.8. This confirmed the identity of the parasitoid as *Cotesia vestalis*.

Table 4.8: NCBI BLAST Results of PCR Purified samples producing best hits to Nucleotide collection (nr/nt) database of Genbank

Sample ID	Location	Match to NCBI NR Database		Accession no	Similarity (%)	Species match
KJ6	Kajiado	mitochondrion, genome	complete	FJ154897	99%	*Cotesia vestalis*
KJ7	Kajiado	mitochondrion, genome	complete	FJ154897	99%	*Cotesia vestalis*
KJ8	Kajiado	mitochondrion, genome	complete	FJ154897	100%	*Cotesia vestalis*
KJ9	Kajiado	mitochondrion, genome	complete	FJ154897	100%	*Cotesia vestalis*
KJ10	Kajiado	mitochondrion, genome	complete	FJ154897	99%	*Cotesia vestalis*
KJ21	Kajiado	mitochondrion, genome	complete	FJ154897	99%	*Cotesia vestalis*
KJ22	Kajiado	mitochondrion, genome	complete	FJ154897	100%	*Cotesia vestalis*
KJ24	Kajiado	mitochondrion, genome	complete	FJ154897	98%	*Cotesia vestalis*
KJ25	Kajiado	mitochondrion, genome	complete	FJ154897	99%	*Cotesia vestalis*
Kt6	Kitui	mitochondrion, genome	complete	FJ154897	100%	*Cotesia vestalis*
Kt7	Kitui	mitochondrion, genome	complete	FJ154897	99%	*Cotesia vestalis*
Kt7	Kitui	mitochondrion, genome	complete	FJ154897	99%	*Cotesia vestalis*
Kt8	Kitui	mitochondrion, genome	complete	FJ154897	99%	*Cotesia vestalis*

Table 4.8: (Continued)

Kt9	**Kitui**	**mitochondrion, complete genome**	**FJ154897**	**100%**	***Cotesia vestalis***
Kt10	Kitui	mitochondrion, complete genome	FJ154897	100%	*Cotesia vestalis*
Kt26	Kitui	mitochondrion, complete genome	FJ154897	99%	*Cotesia vestalis*
Kt27	Kitui	mitochondrion, complete genome	FJ154897	100%	*Cotesia vestalis*
Kt28	Kitui	mitochondrion, complete genome	FJ154897	99%	*Cotesia vestalis*
Kt29	Kitui	mitochondrion, complete genome	FJ154897	99%	*Cotesia vestalis*
Kt30	Kitui	mitochondrion, complete genome	FJ154897	99%	*Cotesia vestalis*
Kw7	Kwale	CNCH1553 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial.	HM430398	98%	*Cotesia sp.*
Kw8	Kwale	mitochondrion, complete genome	FJ154897	100%	*Cotesia vestalis*
Kw9	Kwale	mitochondrion, complete genome	FJ154897	100%	*Cotesia vestalis*
Kw10	Kwale	mitochondrion, complete genome	FJ154897	99%	*Cotesia vestalis*
Kw22	Kwale	mitochondrion, complete genome	FJ154897	100%	*Cotesia vestalis*
Kw23	Kwale	mitochondrion, complete genome	FJ154897	100%	*Cotesia vestalis*

Table 4.8: (Continued)

Sample ID	Location	Match to NCBI NR Database	Accession no	Similarity (%)	Species match
Kw24	Kwale	mitochondrion, complete genome	FJ154897	100%	*Cotesia vestalis*
Kw25	Kwale	mitochondrion, complete genome	FJ154897	100%	*Cotesia vestalis*
Mk1	Makueni	mitochondrion, complete genome	FJ154897	100%	*Cotesia vestalis*
Mk2	Makueni	mitochondrion, complete genome	FJ154897	97%	*Cotesia vestalis*
Mk3	Makueni	mitochondrion, complete genome	FJ154897	98%	*Cotesia vestalis*
Mk4	Makueni	mitochondrion, complete genome	FJ154897	99%	*Cotesia vestalis*
Mk5	Makueni	mitochondrion, complete genome	FJ154897	100%	*Cotesia vestalis*
Mk6	Makueni	mitochondrion, complete genome	FJ154897	100%	*Cotesia vestalis*
Mk7	Makueni	mitochondrion, complete genome	FJ154897	99%	*Cotesia vestalis*
Mk8	Makueni	mitochondrion, complete genome	FJ154897	100%	*Cotesia vestalis*
Mk9	Makueni	mitochondrion, complete genome	FJ154897	100%	*Cotesia vestalis*
Mk10	Makueni	mitochondrion, complete genome	FJ154897	99%	*Cotesia vestalis*
Mw11	Malawi	mitochondrion, complete genome	FJ154897	99%	*Cotesia vestalis*

Table 4.8: (Continued)

Sample ID	Location	Match to NCBI NR Database	Accession no	Similarity (%)	Species match
Mw12	Malawi	mitochondrion, complete genome	FJ154897	99%	*Cotesia vestalis*
Mw13	Malawi	mitochondrion, complete genome	FJ154897	99%	*Cotesia vestalis*
Mw14	Malawi	mitochondrion, complete genome	FJ154897	99%	*Cotesia vestalis*
Mw15	Malawi	mitochondrion, complete genome	FJ154897	99%	*Cotesia vestalis*
Mw21	Malawi	mitochondrion, complete genome	FJ154897	99%	*Cotesia vestalis*
Mw22	Malawi	mitochondrion, complete genome	FJ154897	99%	*Cotesia vestalis*
Mw23	Malawi	mitochondrion, complete genome	FJ154897	99%	*Cotesia vestalis*
Mw24	Malawi	mitochondrion, complete genome	FJ154897	99%	*Cotesia vestalis*
Mw25	Malawi	mitochondrion, complete genome	FJ154897	99%	*Cotesia vestalis*

Key: Kj-Kajiado, Kt-Kitui, Kw-Kwale, Mk-Makueni, Mw-Malawi

4.2.4 Multiple Sequence Alignment

All the aligned sequences showed a high degree of conserved residues among the forty eight samples (Figure 4.4).

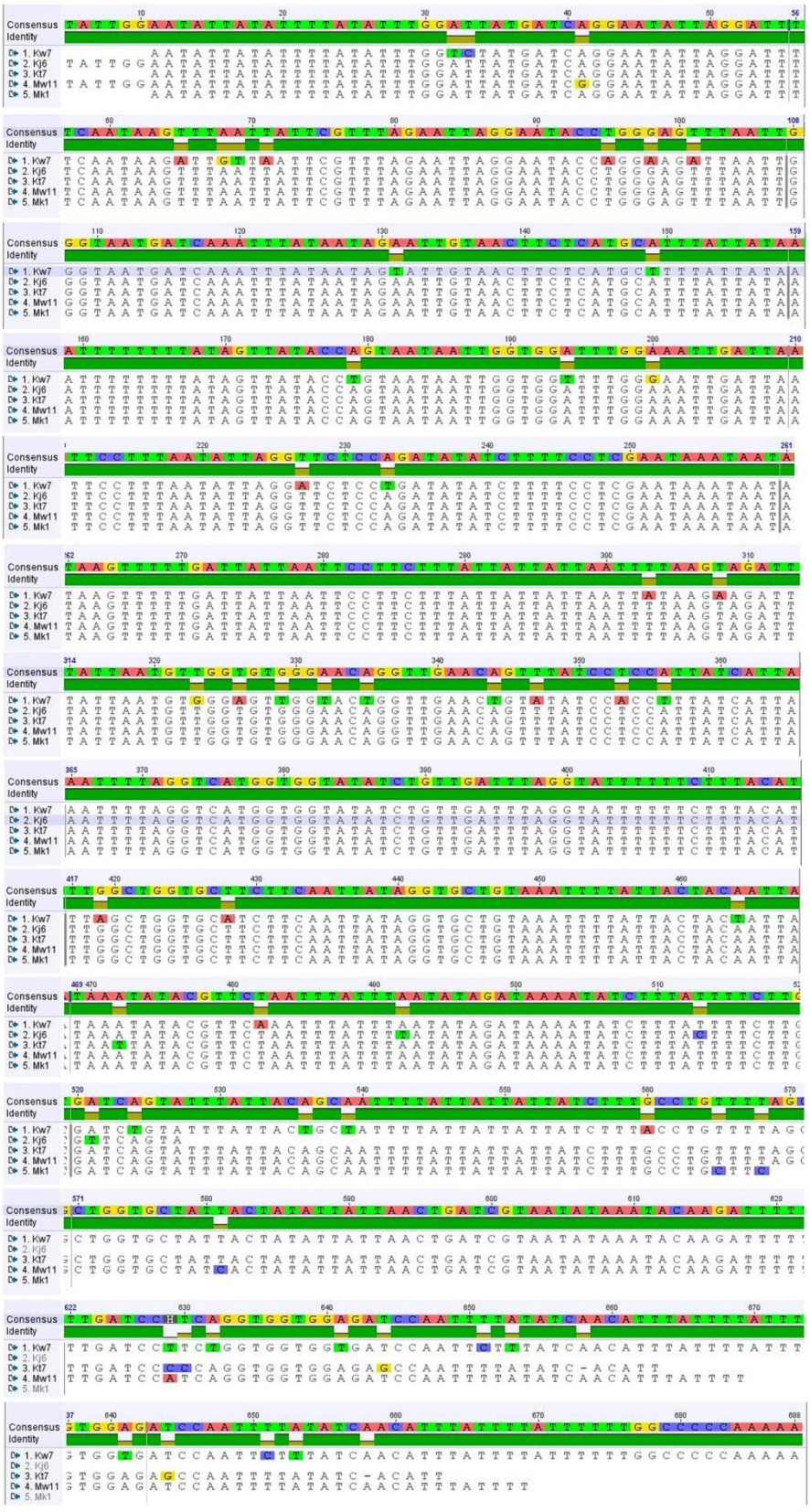


Figure 4.4: Alignment of *Cotesia vestalis* sequences. using clustalX. Conserved regions are marked with asterisks below the sequences and the numbers on the right indicate the position of the last nucleotide in the alignment. The sequences were sampled from five regions denoted as follows: Kj-Kajiado, Kt-Kitui, Kw-Kwale, Mw-Malawi and Mk-Makueni.

4.2.5 Phylogenetic analysis

This phylogram (Figure 4.5) showed samples of *C. vestalis* that separated into two major clusters. The first cluster consisted of only one sample (Kw7) collected from Kwale County while all the other samples fell under the second cluster. The second cluster had further sub-clustering which comprised of samples from Kitui, Kajiado. Makueni, Malawi and Kwale. The sub-cluster containing two samples (Mw11 and Mw23 from Malawi) had the highest bootstrap value of 99%.

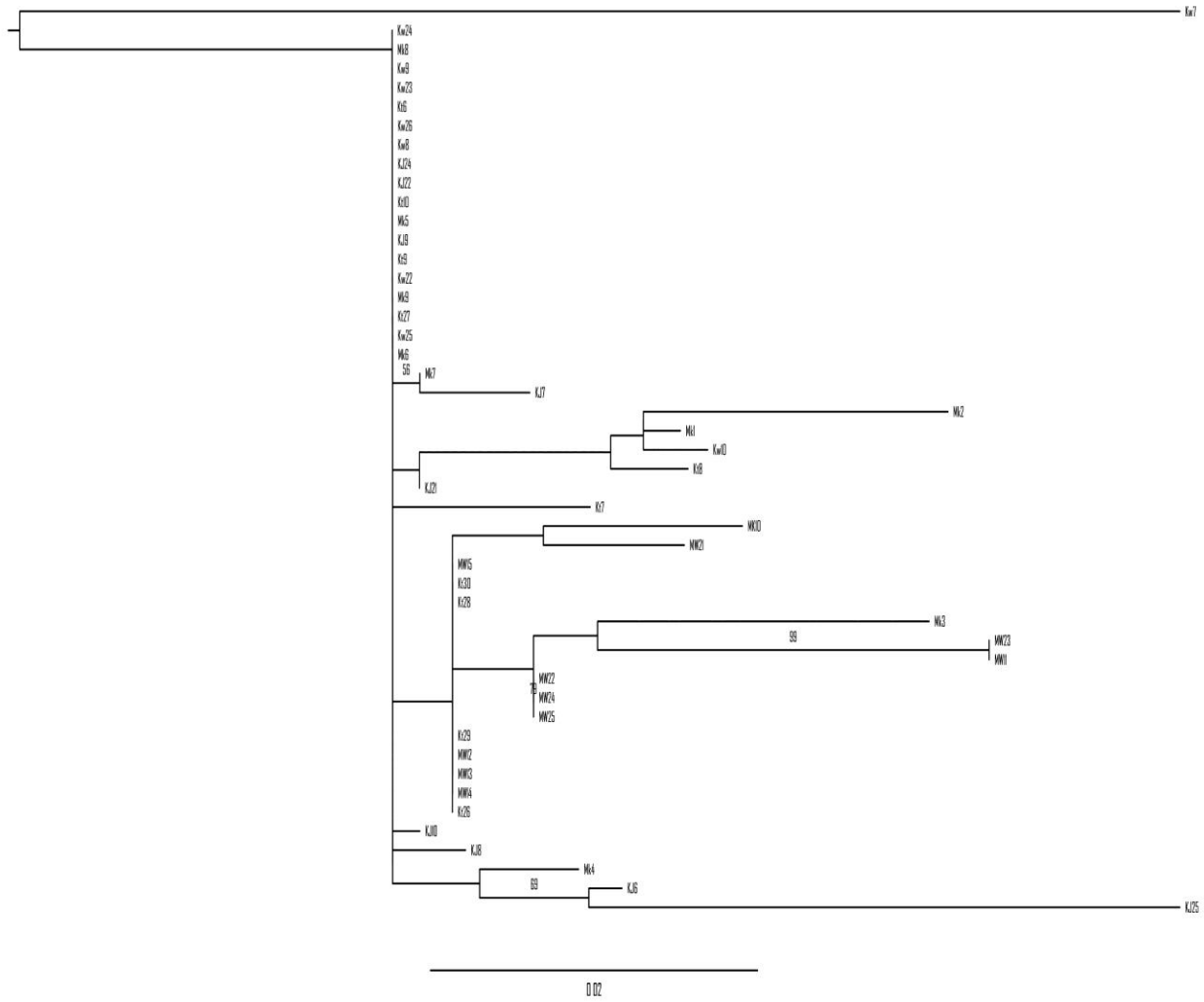


Figure 4.5: Phylogenetic tree of *Cotesia vestalis* inferred from COI sequences of 48 samples collected from Kitui, Kajiado. Makueni, Malawi and Kwale using GTR + G model. The bootstrap support values (100 replicates) are represented by values designated at the tree nodes and are only shown for branches with support of above 50% bootstrap values.

4.2.6 Genetic distances and Distance Summary

The pairwise genetic distances based on a kimura-two-parameter algorithm (Tamura *et al.*, 2011) was used to estimate the divergent distances between the different sequences. The highest nucleotide distance was between kw and Mk with a value of 0.018 while the distance between Mw and Kt was the least at 0.008 (Table 4.8). This indicated that Mw and Kt samples had a closer relationship than that between samples Kw and Mk.

Table 4.8: Estimates of Evolutionary Divergence between samples from Kitui, Kajiado. Makueni, Malawi and Kwale generated by Mega 6 Program.

Sample	Kt	Mw	Kj	Mk	Kw
Kt	-				
Mw	0.008	-			
Kj	0.010	0.010	-		
Mk	0.011	0.012	0.014	-	
Kw	0.014	0.012	0.014	0.018	-

The distance summary done in bold showed a within- species mean distance of 23.51%, a 75% maximum distance and 0% min distance (Table 4.9). The alignment option used was kimura-2-parameter (Tamura 2011).

Table 4.9: The distribution of sequence divergence of *C. vestalis* samples

					Species Details	**Genus Details**	**Family details**
	N	Taxa	Comparisons	Min Dist (%)	Mean Dist(%)	Max Dist(%)	SE Dist (%)
Within Species	48	1	1128	0	23.51	75	0.03
Within Genus	0	0	0	0	0	0	0
Within Family	0	0	0	0	0	0	0

CHAPTER FIVE

DISCUSSION

Low parasitism rates were recorded during the survey with even records of as low as 0% parasitism by *C. vestalis*. Generally, the relationship between the *P. xylostella* densities and the parasitism was inversely proportional. In contrast, in Eastern cape (South Africa), *C. vestalis* showed its potential as a biological control agent with parasitism rates ranging from 10 to 80% and even 100% when the *P. xylostella* moth numbers were low (Smith & Villet, 2001) and they were effective in management if p. xylostella in Ethiopia (Ayalew *et al.*, 2002) and China (Liu *et al.*, 2000).

The low parasitism rates may be as a result of extensive use of pesticides as farmers continued spraying on calendar basis using synthetic insecticides either weekly or fortnightly. The success of classical biological control programs is often reduced by widespread use of broad-spectrum insecticides (Bordat, 2004). Fields with minimum pesticide usage or organically managed ones were reported to have higher densities of parasitoids as compared to fields that were sprayed (Ayalew *et al.*, 2002; Kfir, 2004; Rowell *et al.*, 2005). On the other hand, only 16.18% of the total farms surveyed were not using insecticides.

Only 0.53% of the farmers used plant based insecticides (Azadirachtin) and 1.05% *Bacillus thurigiensis* which are compatible with the use of natural enemies. When *C. vestalis* was introduced in St. Helena (South Africa) and farmers advised to replace chemical insecticides with *B. thuringiensis* to maximize opportunities for parasitoid establishment, the result was high parasitism levels (27.7-80%) and effective suppression of DBM by both the native and introduced biocontrol agents (Kfir & Thomas, 2001). The IPM technology which involved augmentative parasitoid releases, judicious spraying using selective insecticides with strong support from extension efforts resulted in successful establishment of *C. vestalis*, reduced *P. xylostella* densities, increased yields and reduced production costs in the Philippines lowlands (Rowell *et al.*, 2005; Jankowski *et al.*, 2007). Generally, as long as broad-spectrum insecticide use is curtailed, *D. semiclausum* and *D. collaris* establish in cooler, temperate climates and *C. vestalis* and *O. sokolowskii* perform better in warmer climates (Endersby & Ridland, 2004;Shelton, 2001; Srinivasan et al., 2011). Successful establishment of parasitoids requires use of insecticides that are compatible with these natural enemies (Grzywacz *et al.*, 2010; Srinivasan *et al.*, 2011).

There were records of other pests in crucifer farms recorded during the survey. Therefore farmers widely used broad-spectrum insecticides not only to manage *P. xylostella* but also other pests including the aphids, whiteflies and the bollworms which were common in the crucifer fields. Some programs used in management of pests are skewed towards the specific management of the *P. xylostella* and lack the necessary control technological inputs to manage the other pests. Similar observations of other cabbage pests such as aphids during dry seasons which necessitated use of pesicides were reported (Oruko & Ndun'gu, 2001) including presence of cabbage webworm, *Hellula undalis,* and *Spodoptera exigua in cabbage production in* Malaysia (Sivapragasam, 2004). Therefore, a holistic approach to tackle the other occasional and recurrent pests will be crucial in order to maximize yields.

Farmers used various classes of insecticides and in Kwale county, pyrethroids constituted 75.47% of the total pesticides followed by neonicotinoids and carbamates which accounted for 8.49% and 5.66% respectively. DBM has been reported to show significant resistance to almost every insecticide applied in the field including spinosyns(J. Z. Zhao *et al.,* 2002 ;Sparks *et al.,* 2012) , indoxacarb (Sayyed and Wright 2006), abamectin, carbamates organophosphates, pyrethroids (Girling *et al.,* 2011; Li *et al.,* 2012; Pu *et al.,* 2010) and the diamides(Troczka *et al.,* 2017).

Overreliance of rainfall for production which necessitate breaks and discontinuous vegetable production could also be a factor behind the poor establishment of *C. vestalis* in the Kajiado, Kitui, Kwale, Machakos and Makueni counties. A similar observation was reported on establishment of *Diadegma semimiclausum* in Kenya (Löhr *et al.,* 2007). Farmers who practiced irrigation mainly focused on hand watering and drip irrigation which was the most effective considering the inavailability of water especially during the dry seasons.

Lack of knowledge by farmers on the use of biological control in management of *P. xylostella* is also a challenge. This shows that there exists a wide gap in relation to farmers knowledge on pest management which can be narrowed through training to create awareness of natural enemies and use of environmentally friendly selective insecticides that do not harm natural enemies (Badenes-Perez & Shelton, 2006).

The parasitoid samples collected had over 98% identity to *Cotesia vestalis*.The COI gene is highly conserved as revealed by multiple sequence alignment and can be used as a viable marker in confirming *Cotesia* establishment upon release in study sites. Similar studies

reported comparison of partial COI gene sequences of *C. flavipes* collected from sugarcane in Ethiopia and specimens from other African countries to determine the origin of the Ethiopian population (Assefa *et al.*, 2008) and primers amplified 500 bp long sequences that showed a high degree of homology within species (> 98%) and the samples could be confidently assigned to a species, regardless of their origin.

CHAPER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

1. In conclusion, this study shows that *Cotesia vestalis* has established in Kajiado, Kitui, Kwale, Machakos and Makueni counties of Kenya. However, its contribution in management of *Cotesia vestalis* is low.

2. The molecular identification of the samples collected confirmed that they were *Cotesia vestalis.* Therefore, the COI gene is a reliable marker for identifying *Cotesia spp.*

3. There are cultural practices that affect the establishment of *Cotesia vestalis* such as irrigation, intercropping, and use of pesticides on regular basis.

6.2 Recommendations

1. Farmers should adopt judicious spraying using selective insecticides and increase usage of *Bt*-based and plant-based products so as to conserve the natural enemies and enhance establishment of introduced parasitoids.

2. Cultural practices such as intercropping and use of trap crops such as mustard will be very helpful in management of *P. xylostella* and conservation of natural enemies.

3. Stronger links between research and extension would be very helpful in informing the farmers on use of safe insect management measures against pests and would go a long way in farmer awareness on parasitoids.

4. Carry out a follow-up study on the impact of *Cotesia vestalis* after implementation of IPM with strong focus on reduction of chemical pesticides

REFERENCES

AAsman, K. (2002). Trap cropping effect on oviposition behaviour of the leek moth Acrolepiopsis assectella and the diamondback moth Plutella xylostella. *Entomologia Experimentalis et Applicata, 105*(2), 153–164.

Alam, M. M. (1990). Diamondback moth and its natural enemies in Jamaica and some other Caribbean Islands. *Proceedings of the Second International Workshop Tainan, Taiwan*. http://web.entomology.cornell.edu/shelton/diamondback-moth/pdf/1990papers/1990DBM26.pdf

Alex Smith, M., Fernández-Triana, J.L., Eveleigh, E., Gómez, J., Guclu, C., Hallwachs, W., Hebert, P.D.N., Hrcek, J., Huber, J.T., Janzen, D. & others. (2013). DNA barcoding and the taxonomy of Microgastrinae wasps (Hymenoptera, Braconidae): impacts after 8 years and nearly 20 000 sequences. *Mol. Ecol. Resour.* 13, 168–176.

Alizadeh, M., Rassoulian, G.R., Karimzadeh, J., Hosseini-Naveh, V. & Farazmand, H. (2011). Biological study of Plutella xylostella (L.)(Lep: Plutellidae) and it's solitary endoparasitoid, Cotesia vestalis (Haliday)(Hym. Braconidae) under laboratory conditions. *Pak. J. Biol. Sci. PJBS* 14, 1090–1099.

Anon, 2022. URL: https://www.greenlife.co.ke/diamondback-moth/

Anon. mathbitsnoteboo.com.

https://mathbitsnotebook.com/Algebra2/Statistics/STrandomtable.html

Assefa, Y., Mitchell, A., Conlong, D.E. & Muirhead, K.A. (2008). Establishment of Cotesia flavipes (Hymenoptera: Braconidae) in sugarcane fields of Ethiopia and origin of founding population. *J. Econ. Entomol.* 101, 686–691.

Ayalew, G., Baumgärtner, J., Ogol, K.C. & Löhr, B. (2006). Analysis of population dynamics of diamondback moth,(Plutella xylostella (Lepidoptera: Plutellidae)) at two sites in central Ethiopia, with particular reference to parasitism. *Biocontrol Sci. Technol.* 16, 607–618.

Ayalew, G., LORH, B., Baumgartner, J., & Ogol, C. (2002). Diamondback moth, Plutella xylostella (L.)(Lepidoptera: Plutellidae) and its parasitoids in Ethiopia. *Proceedings of the International Symposium*, 21–24.

Azidah, A.A., Fitton, M.G. & Quicke, D.L.J. (2000). Identification of the Diadegma species (Hymenoptera: Ichneumonidae, Campopleginae) attacking the diamondback moth, Plutella xylostella (Lepidoptera: Plutellidae). *Bull. Entomol. Res.* 90, 375–389.

Badenes-Perez, F.R., Nault, B.A. & Shelton, A.M. (2005). Manipulating the attractiveness and suitability of hosts for diamondback moth (Lepidoptera: Plutellidae). *J. Econ. Entomol.* 98, 836–844.

Badenes-Perez, F.R. & Shelton, A.M. (2006). Pest management and other agricultural practices among farmers growing cruciferous vegetables in the Central and Western highlands of Kenya and the Western Himalayas of India. *Int. J. Pest Manag.* 52, 303–315.

Bai, S.-F., Li, X., Chen, X.-X., Cheng, J.-A. & He, J.-H. (2011). Interspecific competition between two endoparasitoids Cotesia vestalis (Hymenoptera: Braconidae) and Oomyzus sokolowskii (Hymenoptera: Eulophidae). *Arch. Insect Biochem. Physiol.* 76, 156–167.

Bale, J.S., Van Lenteren, J.C. & Bigler, F. (2008). Biological control and sustainable food production. *Philos. Trans. R. Soc. B Biol. Sci.* 363, 761–776.

Banks, J.E. & Ekbom, B. (1999). Modelling herbivore movement and colonization: pest management potential of intercropping and trap cropping. *Agric. For. Entomol.* 1, 165–170.

Behura, S.K. (2006). Molecular marker systems in insects: current trends and future avenues. *Mol. Ecol.* 15, 3087–3113.

Bommarco, R., Miranda, F., Bylund, H. & Björkman, C. (2011). Insecticides suppress natural enemies and increase pest damage in cabbage. *J. Econ. Entomol.* 104, 782–791.

Bopape, M.J., Nofemela, R.S., Mosiane, M.S. & Modise, D.M. (2014). Effects of a selective and a broad-spectrum insecticide on parasitism rates of Plutella xylostella (L.)(Lepidoptera: Plutellidae) and species richness of its primary parasitoids. *Afr. Entomol.* 22, 115–126.

Bordat, D. (2004). Improving Biocontrol of Plutella xylostella. *Improv. Biocontrol Plutella Xylostella* 1–274.

Braun, L., Olfert, O., Soroka, J., Mason, P., Dosdall, L. M., & Kirk, A. A. (2004). Diamondback moth biocontrol activities in Canada. *Improving Biocontrol of Plutella Xylostella. Proc Int Symp p*, 144–146.

Capinera, J. (2001). Handbook of vegetable pests. *Choice Reviews Online*, *39*(04), 39–219339–2193. https://doi.org/10.5860/choice.39-2193.

Capinera, J. L. (2002). Diamondback Moth, Plutella xylostella (Linnaeus)(Insecta: Lepidoptera: Plutellidae): EENY-119/IN276, rev. 5/2000. *EDIS*, *2002*(8).

Caterino, M. S., Cho, S., & Sperling, F. A. (2000). The current state of insect molecular systematics: A thriving Tower of Babel. *Annual Review of Entomology*, *45*(1), 1–54.

Chauhan, U. & Sharma, K.C. (2004). Status of biocontrol agents of Plutellaxylostella (L)(Lepidoptera: Yponomeutidae) in hilly régions of the north-west Himalayas, India. *Improv. Biocontrol Plutella Xylostella* 153.

Charleston, D. S., & Kfir, R. (2000). The possibility of using Indian mustard, Brassica juncea, as a trap crop for the diamondback moth, Plutella xylostella, in South Africa. *Crop Protection*, *19*(7), 455–460.

Cherry, A. (2004). Public-private partnerships for development and implementation of entomopathogenic viruses as bioinsecticides for key lepidopteran pests in Ghana and Benin, West Africa. Final Technical Report. http://agris.fao.org/agris-search/search.do?recordID=GB2012104280

Christopher, H. (2020). Pest status and management options for *plutella xylostella l. (lepidoptera: Plutellidae)* in Iringa and Morogoro, Tanzania [Thesis, Sokoine

University of Agriculture]. http://www.suaire.sua.ac.tz/handle/123456789/3679

Cobblah, M.A., Owusu, E.O., Osae, M.Y. & others. (2012). Parasitism of Plutella xylostella (L.)(Lepidoptera: Plutellidae) by Cotesia plutellae Kurdjumov (Hymenoptera: Braconidae) and parasitoid development on Brassica oleracea L. sprayed with insecticides. *Ghana J. Hortic.* 10, 77–86.

Cox, A.J. & Hebert, P.D. (2001). Colonization, extinction, and phylogeographic patterning in a freshwater crustacean. *Mol. Ecol.* 10, 371–386.

Dallwitz, M. J., Paine, T. A., & Zurcher, E. J. (1999). User's guide to the DELTA Editor. H ttp. *Delta-Intkey. Com*.

Darriba, D., Taboada, G.L., Doallo, R. & Posada, D. (2012). jModelTest 2: more models, new heuristics and parallel computing. *Nat. Methods* 9, 772–772.

DeAngelis, D. L., & Waterhouse, J. C. (1987). Equilibrium and nonequilibrium concepts in ecological models. *Ecological Monographs*, *57*(1), 1–21.

Delvare, G., Kirk, A.A. & Bordet, D. (2004). The taxonomic status and role of Hymenoptera in biological control of DBM, Plutella xylostella (L.)(Lepidoptera: Plutellidae). In *Improving biocontrol of Plutella xylostella–Proceedings of the international symposium. CIRAD, Montpellier, France*: 17–49.

Devine, G.J. & Furlong, M.J. (2007). Insecticide use: contexts and ecological consequences. *Agric. Hum. Values* 24, 281–306.

Dobson, H., Cooper, J., Manyangarirwa, W. & Chiimba, W. (2002). Integrated vegetable pest management.

Dowton, M. & Austin, A.D. (1994). Molecular phylogeny of the insect order Hymenoptera: Apocritan relationships. *Proc. Natl. Acad. Sci.* 91, 9911–9915.

Elizabeth, S. & Zira, D.Y. (2009). Awareness and effectiveness of vegetable technology information packages by vegetable farmers in Adamawa State, Nigeria. *Afr. J. Agric. Res.* 4, 065–070.

Endersby, N.M. & Ridland, P.M. (2004). The management of diamondback moth and other crucifer pests. *Reg. Inst. Gosford Aust.*

Ezena, G.N. (2015). Exploiting the Insecticidal Potential of the Invasive Siam Weed, *Chromolaena Odorata* L.(Asteraceae) In the Management of Major Pests of Cabbage, *Brassica Oleracea Var Capitata* and Their Natural Enemies for Enhanced Yield in the Moist Semi-Deciduous Agro-Ecological Zone of Ghana. University of Ghana.

Food and Agriculture Organization of the United Nations (FAO). 2014. http://www.fao.org/faostat.

Fitton, M., & Walker, A. (1992). Hymenopterous parasitoids associated with diamondback moth: the taxonomic dilemma. Diamondback Moth and Other Crucifer Pests : Proceedings of the Second International Workshop, Tainan, Taiwan, 10-14 December 1990 / N.S. *Talekar*, Editor. http://agris.fao.org/agris-search/search.do?recordID=US201301766491

Furlong, M.J., Ju, K.H., Su, P.W., Chol, J.K., Il, R.C. & Zalucki, M.P. (2008). Integration of endemic natural enemies and Bacillus thuringiensis to manage insect pests of Brassica crops in North Korea. *Agric. Ecosyst. Environ.* 125, 223–238.

Furlong, M.J., Shi, Z., Liu, S.-S. & Zalucki, M.P. (2004). Evaluation of the impact of natural enemies on Plutella xylostella L.(Lepidoptera: Yponomeutidae) populations on commercial Brassica farms. *Agric. For. Entomol.* 6, 311–322.

Furlong, M.J., Wright, D.J. & Dosdall, L.M. (2013). Diamondback moth ecology and management: problems, progress, and prospects. *Annu. Rev. Entomol.* 58, 517–541.

Gariepy, T.D., Kuhlmann, U., Gillott, C. & Erlandson, M. (2007). Parasitoids, predators and PCR: the use of diagnostic molecular markers in biological control of Arthropods. *J. Appl. Entomol.* 131, 225–240.

Gasparich, G.E., Silva, J.G., Han, H.-Y., Mcpheron, B.A., Steck, G.J. & Sheppard, W.S. (1997). Population genetic structure of Mediterranean fruit fly (Diptera: Tephritidae) and implications for worldwide colonization patterns. *Ann. Entomol. Soc. Am.* 90, 790–797.

Gichini, G., Löhr, B., Rossbach, A., Nyambo, B. & Gathu, R. (2008). Can low release numbers lead to establishment and spread of an exotic parasitoid: The case of the diamondback moth parasitoid, Diadegma semiclausum (Hellén), in East Africa. *Crop Prot.* 27, 906–914.

Girling, R.D., Stewart-Jones, A., Dherbecourt, J., Staley, J.T., Wright, D.J. & Poppy, G.M. (2011). Parasitoids select plants more heavily infested with their caterpillar hosts: a new approach to aid interpretation of plant headspace volatiles. *Proc. R. Soc. Lond. B Biol. Sci.* rspb20102725.

Glare, T.R. (2000). Bacillus thuringiensis. *Biology, Ecology and Safety*.

Greenstone, M.H. (2006). Molecular methods for assessing insect parasitism. *Bull. Entomol. Res.* 96, 1–13.

Grossrieder, M., Kiefer, B., Kang, S. I., & Kuhlmann, U. (2005). Case study: Knowledge transfer in cabbage IPM through farmer participatory training in DPR Korea. *Second International Symposium on Biological Control of Arthropods*, *1*, 318–332. http://www.bugwood.org/arthropod2005/vol1/6d.pdf

Grzywacz, D., Parnell, M., Kibata, G., Oduor, G., Ogutu, W., Miano, D. & Winstanley, D. (2001). The development of endemic baculoviruses of Plutella xylostella (diamondback moth, DBM) for control of DBM in East Africa. In *The Management of Diamondback Moth and other Cruciferous Pests (Proceedings of the Fourth International Workshop on Diamondback Moth, Melbourne University)*: 179–183. Citeseer.

Grzywacz, D., Rossbach, A., Rauf, A., Russell, D.A., Srinivasan, R. & Shelton, A.M. (2010). Current control methods for diamondback moth and other brassica insect pests and the prospects for improved management with lepidopteran-resistant Bt vegetable brassicas in Asia and Africa. *Crop Prot.* 29, 68–79.

Hajibabaei, M., Janzen, D.H., Burns, J.M., Hallwachs, W. & Hebert, P.D. (2006). DNA barcodes distinguish species of tropical Lepidoptera. *Proc. Natl. Acad. Sci. U. S. A.* 103, 968–971.

Haque, M. A., Canning, E. U., & Wright, D. J. (1999). Entomopathogenicity of Vairimorpha sp. (Microsporidia) in the diamondback moth, Plutella xylostella (Lepidoptera: Yponomeutidae). *Bulletin of Entomological Research*, *89*(2), 147–152. https://doi.org/10.1017/S0007485399000231

Harvey, J.A., Gols, R. & Strand, M.R. (2009). Intrinsic competition and its effects on the survival and development of three species of endoparasitoid wasps. *Entomol. Exp. Appl.* 130, 238–248.

Haseeb, M., Liu, T.-X. & Jones, W.A. (2004). Effects of selected insecticides on Cotesia plutellae, endoparasitoid of Plutella xylostella. *BioControl* 49, 33–46.

Hebert, P.D., Cywinska, A., Ball, S.L. & others. (2003). Biological identifications through DNA barcodes. *Proc. R. Soc. Lond. B Biol. Sci.* 270, 313–321.

Hermansson, J. (2016). Biology of the diamondback moth (Plutella xylostella) and its future impact in Swedish oilseed rape production.

Hill, T.A. & Foster, R.E. (2000). Effect of insecticides on the diamondback moth (Lepidoptera: Plutellidae) and its parasitoid Diadegma insulare (Hymenoptera: Ichneumonidae). *J. Econ. Entomol.* 93, 763–768.

Hines, R. L., & Hutchison, W. D. (2001). Evaluation of action thresholds and spinosad for lepidopteran pest management in Minnesota cabbage. *Journal of Economic Entomology*, *94*(1), 190–196.

Hiroyoshi, S., Harvey, J.A., Nakamatsu, Y., Nemoto, H., Mitsuhashi, J., Mitsunaga, T. & Tanaka, T. (2017). Potential host range of the larval Endoparasitoid Cotesia vestalis (= plutellae)(Hymenoptera: Braconidae). *Int. J. Insect Sci.* 9, 1179543317715623.

Hoy, M. A., Jeyaprakash, A., Morakote, R., Lo, P. K. C., & Nguyen, R. (2000). Genomic Analyses of Two Populations of Ageniaspis citricola (Hymenoptera: Encyrtidae) Suggest That a Cryptic Species May Exist. *Biological Control*, *17*(1), 1–10. https://doi.org/10.1006/bcon.1999.0775

Huber, J. T., Darbyshire, S., Bissett, J., & Foottit, R. G. (2001). Taxonomy and biological control. In *Biological Control Programmes in Canada, 1981-2000* (pp. 14–22). CABI Publishing Wallingford UK.

Hufbauer, R.A., Bogdanowicz, S.M. & Harrison, R.G. (2004). The population genetics of a biological control introduction: mitochondrial DNA and microsatellie variation in native and introduced populations ofAphidus ervi, a parisitoid wasp. *Mol. Ecol.* 13, 337–348.

Hutchison, W. D., Burkness, E. C., Pahl, G., & Hurley, T. M. (2001). Integrating novel technologies for cabbage IPM in the USA: Value of on-farm research. *Proc. 4th International Workshop on the Management of Diamondback Moth*, 371–379.

Huber, J. T., Darbyshire, S., Bissett, J., & Foottit, R. G. (2001). Taxonomy and biological control. In *Biological Control Programmes in Canada, 1981-2000* (pp. 14–22). CABI Publishing Wallingford UK.

Hutchison, W. D., Burkness, E. C., Pahl, G., & Hurley, T. M. (2001). Integrating novel technologies for cabbage IPM in the USA: Value of on-farm research. *Proc. 4th International Workshop on the Management of Diamondback Moth*, 371–379.

Idris, A.B. & Grafius, E. (2001). Evidence of Diadegma insulare (Cresson), a parasitoid of diamondback moth, Plutella xylostella (L.), present in various habitats. *Pak. J. Biol. Sci.* 1, 742Á.

Idris, A.B., Zainal-Abidin, B.A.H., Sajap, A.S., Noran, A.M. & Hussan, A.K. (2002). Mortality of diamondback moth larvae infected with Nosema bombycis Negali. *Malays. Appl. Biol.* 31, 79–82.

Jankowski, A., Mithöfer, D., Löhr, B., & Weibel, H. (2007). Economics of biological control in cabbage production in two countries in East Africa. *Utilisation of Diversity in Land Use Systems: Sustainable and Organic Approaches to Meet Human Needs. Proceedings of the Conference on International Agricultural Research for Development 'Tropentag*. http://www.tropentag.de/2007/abstracts/full/348.pdf

Jiang, T., Wu, S., Yang, T., Zhu, C. & Gao, C. (2015). Monitoring field populations of

Plutella xylostella (Lepidoptera: Plutellidae) for resistance to eight insecticides in China. *Fla. Entomol.* 65–73.

Kahuthia-Gathu, R. (2007). Importance of wild crucifers for diamondback moth, Plutella xylostella L.(Lepidoptera: Plutellidae) and its parasitoids in Kenya.

Kahuthia-Gathu, R. (2012). Seasonal incidence of Plutella xylostella (Lepidoptera: Plutellidae) and its associated natural enemies in major crucifer growing areas of Kenya. *Journal of Applied Biosciences*, *46*, 3103–3112.

Kawaguchi, M. & Tanaka, T. (1999a). Biological characteristics of a larval endoparasitoid, Cotesia plutellae (Hymenoptera: Braconidae): Host stage preference, subsequent sex ratio of progeny and mate location of males. *Appl. Entomol. Zool.* 34, 213–221.

Kfir, R. (1997a). Parasitoids ofPlutella xylostella (Lep.: Plutellidae) in South Africa: An annotated list. *Entomophaga* 42, 517–523.

Kfir, R. (1997b). The diamondback moth with special reference to its parasitoids in South Africa. *The Management of Diamondback Moth and Other Crucifer Pests*, 54–60.

Kfir, R. (1998). Origin of the diamondback moth (Lepidoptera: Plutellidae). *Ann. Entomol. Soc. Am.* 91, 164–167.

Kfir, R. (2004). Effect of parasitoid elimination on populations of diamondback moth in cabbage. *The Management of Diamondback Moth and Other Crucifer Pests: Proceedings of the 4th International Workshop. The Regional Institute Ltd, Gosford NSW, Australia*, 197–205. http://www.regional.org.au/au/esa/ws/contributed/6/kfir.htm

Kfir, R. & Thomas, J. (2001). Biological control of diamondback moth in the island of St Helena. *Biocontrol News Inf.* 22, 76N.

Khaliq, A., Attique, M. N. R., & Sayyed, A. H. (2007). Evidence for resistance to pyrethroids and organophosphates in Plutella xylostella (Lepidoptera: Plutellidae) from Pakistan. *Bulletin of Entomological Research, 97*(2), 191–200. https://doi.org/10.1017/S0007485307004877

Kibata, G.N. (1997). The diamondback moth: a problem pest of brassica crops in Kenya.

Manag. Diamondback Moth Crucif. Pests 47–53.

Kirk, A.A., Mercadier, G., Bordat, D., Delvare, G., Pichon, A., Arvanitakis, L., Goudegnon, A.E. & Rincon, C. (2004). Variability in Plutella and its natural enemies: implications for biological control. *Europe* 5, 0.

Kwon, M., Lee, S.-H. & Lin, M.-Y. (2006). An Evidence to Use Cotes/a plutellae (Kurdjumov) (Hymenoptera: Braconidae) as a Field Control Agent against Diamondback Moth, Plutella xylostella L (Lepidoptera: Plutellidae). *J. Asia-Pac. Entomol.* 9, 55–59.

Legaspi Jr, B.C., Liu, T.X. & Sparks Jr, A. (2000). Occurrence of the diamondback moth and its parasitoids in the Lower Rio Grande Valley of Texas. *Subtrop. Plant Sci. J. Rio Gd. Val. Hortic. Soc.*

Li, Z., Zalucki, M.P., Bao, H., Chen, H., Hu, Z., Zhang, D., Lin, Q., Yin, F., Wang, M. & Feng, X. (2012). Population Dynamics and "Outbreaks" of Diamondback Moth (Lepidoptera: Plutellidae) in Guangdong Province, China: Climate or Failure of Management? *J. Econ. Entomol.* 105, 739–752.

Lin, Y.W., Wu, G. & Miyata, T. (2007). Insecticide susceptibility of surviving Cotesia plutellae (Hym: Braconidae) and Diaeretiella rapae (M'Intosh)(Hym: Aphidiidae) as affected by sublethal insecticide dosages on host insects. *Pest Manag. Sci.* 63, 841–850.

Liu, S., Wang, X., Guo, S., He, J. & Shi, Z. (2000a). Seasonal abundance of the parasitoid complex associated with the diamondback moth, Plutella xylostella (Lepidoptera: Plutellidae) in Hangzhou, China. *Bull. Entomol. Res.* 90, 221–231.

Liu, S., Wang, X., Shi, Z. & Gebremeskel, F.B. (2001). The biology of Diadromus collaris (Hymenoptera: Ichneumonidae), a pupal parasitoid of Plutella xylostella (Lepidoptera: Plutellidae), and its interactions with Oomyzus sokolowskii (Hymenoptera: Eulophidae). *Bull. Entomol. Res.* 91, 461–469.

Lohr, B. & Gathu, R. (2002). Towards biocontrol-based IPM for diamondback moth in

eastern and southern Africa. In *Integrated pest management conference for sub-Saharan Africa*: 8–12.

Löhr, B., Gathu, R., Kariuki, C., Obiero, J. & Gichini, G. (2007). Impact of an exotic parasitoid on Plutella xylostella (Lepidoptera: Plutellidae) population dynamics, damage and indigenous natural enemies in Kenya. *Bull. Entomol. Res.* 97, 337–350.

Lohr, B. & Kfir, R. (2004). Diamondback moth Plutella xylostella (L.) in Africa. A review with emphasis on biological control. *Improv. Biocontrol Plutella Xylostella* 2, 5707.

Long, K.D. & Dzung, D.T. (2014). Synopsis of Cotesia species, biological agents for pest control on vegetables in Vietnam, with description of one new species. *Tijdschr. Voor Entomol.* 157, 83–93.

Luchen, S. W. S. (2012). *Effects of intercropping Cabbage with Alliums and Tomato, on the incidence of the Diamondback moth, plutella xylostella (L.)* [Thesis]. http://dspace.unza.zm/handle/123456789/1858

MacDonald, C. & Loxdale, H. (2004). Molecular markers to study population structure and dynamics in beneficial insects (predators and parasitoids). *Int. J. Pest Manag.* 50, 215–224.

Macharia, I., Löhr, B. & De Groote, H. (2005). Assessing the potential impact of biological control of Plutella xylostella (diamondback moth) in cabbage production in Kenya. *Crop Prot.* 24, 981–989.

Malysh, J.M., Kazartsev, I.A., Frolov, A.N., Zverev, A.A. & Tokarev, Y.S. (2015). Molecular detection of Cotesia vestalis (Hymenoptera: Braconidae) in the beet webworm Loxostege sticticalis L.(Lepidoptera: Crambidae). *J. Appl. Entomol.*

Marchioro, C.A. & Foerster, L.A. (2011). Development and survival of the diamondback moth, Plutella xylostella (L.)(Lepidoptera: Yponomeutidae) as a function of temperature: effect on the number of generations in tropical and subtropical regions. *Neotrop. Entomol.* 40, 533–541.

Mason, J.M., Matthews, G.A. & Wright, D.J. (1999). Evaluation of spinning disc technology

for the application of entomopathogenic nematodes against a foliar pest. *J. Invertebr. Pathol.* 73, 282–288.

Mason, W.R.M. (1981). The polyphyletic nature of Apanteles Foerster (Hymenoptera: Braconidae): A phylogeny and reclassification of Microgastrinae. *Mem. Entomol. Soc. Can.* 113, 1–147.

Massomo, S.M., Mabagala, R.B., Mortensen, C.N., Hockenhull, J. & Swai, I.S. (2005). Cabbage production in Tanzania: challenges faced by smallholder farmers in the management of black rot disease. *J. Sustain. Agric.* 26, 119–141.

Miranda Ortiz, F. (2011). *Biological control of diamondback moth*. https://pub.epsilon.slu.se/8073/1/miranda_f_110414.pdf

Monnerat, R.G., Kirk, A.A. & Bordat, D. (2002). Biology of Diadegma sp.(Hymenoptera: Ichneumonidae), a parasitoid of Plutella xylostella (L.)(Lepidoptera: Yponomeutidae), from reunion Island. *Neotrop. Entomol.* 31, 271–274.

Morallo-Rejesus, B., Inocencio, E., Eusebio, J., & Barroga, S. (1997). Technology transfer of Cotesia-based IPM for diamondback moth on lowland crucifers in the Philippines. *The Management of Diamondback Moth and Other Crucifer Pests, Proceedings of the Third International Workshop*, *29*, 267–275. http://web.entomology.cornell.edu/shelton/diamondback-moth/pdf/1996papers/1996DBM46.pdf

Moritz, C. & Cicero, C. (2004). DNA barcoding: promise and pitfalls. *PLoS Biol* 2, e354.

Munthali, D.C. (2009). Evaluation of cabbage varieties for resistance to the cabbage aphid. *Afr. Entomol.* 17, 1–7.

Nofemela, R.S. (2013). The effect of obligate hyperparasitoids on biological control: differential vulnerability of primary parasitoids to hyperparasitism can mitigate trophic cascades. *Biol. Control* 65, 218–224.

Nofemela, R. S., & Kfir, R. (2008). Diadegma mollipla parasitizing Plutella xylostella: Host instar preference and suitability. *Entomologia Experimentalis et Applicata*, *126*(1), 9–17.

Nofemela, R.S. & Ktir, R. (2005). The role of parasitoids in suppressing diamondback moth, PIuteIIa xylostella (L.)(Lepidoptera: Plutellidae), populations on unspraved cabbage in the North West Province of South Africa. *Afr. Entomol.* 13, 71–83.

Nofemela, S.R. (2004). Studies on parasitoids of the diamondback moth, Plutella xylostella (L.)(Lepidoptera: Plutellidae). *South Afr. M Sc Thesis Rhodes Univ. Grahamst. South Afr.*

Nyambo, B., Gichini, G., Obiero, J., & Njumwa, G. (2008). Re-distribution of ex-South African strain of Cotesia plutellae Kurdjumov (Hymenoptera, Braconidae) in Africa for control of diamondback moth. *Proc. XXII International Congress of Entomol*, 6–12.

Nyambo, B. & Pekke, A. (1995). Brassica planning workshop for East and Southern Africa Region, Lilongwe-Malawi, 15-18 May 1995. *GTZ-IPM Hortic. Nairobi*.

Obeng-Ofori, D. (1998). Pests of field, plantation and vegetable crops. *Their Biol. Damage Control*.

Obeng-Ofori, D., Danquah, E.Y. & Ofosu-Anim, J. (2007). *Vegetable and Spice Crop Production in West-Africa*. City Publishers.

Oruko, L. & Ndun'gu, B. (2001). Final Socio-Economic Report for Peri-urban Vegetable IPM Thematic Cluster. A report to the DFID Crop Protection Programme. *Nat. Resour. Int. Aylesford Kent UK*.

Oruku, L. & Ndung'u, B. (2001). Final socio-economic report for the peri-urban vegetable IPM thematic cluster. *CABI Afr. Reg. Cent. Rep. Nairobi* 49.

Pratissoli, D., Polanczyk, R.A., Holtz, A.M., Dalvi, L.P., Silva, A.F. & Silva, L.N. (2008). Selection of Trichogramma species for controlling the Diamondback moth. *Hortic. Bras.* 26, 259–261.

Pu, X., Yang, Y., Wu, S. & Wu, Y. (2010). Characterisation of abamectin resistance in a field-evolved multiresistant population of Plutella xylostella. *Pest Manag. Sci.* 66, 371–378.

Raemaekers, R.H. & others. (2001). *Crop production in tropical Africa.* DGIC.

Rambaut, A. (2012). FigTree v1. 4 2006-2012. *Institute of Evolutionary Biology, University of Edinburgh, Edinburgh*.

Rami, K. (2005). *The impact of parasitoids on Plutella xylostella populations in South Africa and the successful biological control of the pest on the island of St. Helena*. http://www.academia.edu/download/39268858/3c.pdf

Reddy, G.V.P., Tabone, E. & Smith, M.T. (2004). Mediation of host selection and oviposition behavior in the diamondback moth Plutella xylostella and its predator Chrysoperla carnea by chemical cues from cole crops. *Biol. Control* 29, 270–277.

Rincon, C., Arvanitakis, L., Kirk, A.A., Bordat, D., Kirk, A.A. & Bordat, D. (2004). Morphological and genetic differentiation of five populations of Cotesia plutellae (Hymenoptera: Braconidae), a parasitoid of Plutella xylostella (Lepidoptera: Plutellidae) from different geographical origins. In *Improving biocontrol of Plutella xylostella. Proceedings of the International Symposium, 21–24 October 2002*: 229–236. Montpellier France.

Rowell, B., Bunsong, N., Satthaporn, K., Phithamma, S. & Doungsa-Ard, C. (2005). Hymenopteran parasitoids of Diamondback moth (Lepidoptera: Ypeunomutidae) in northern Thailand. *J. Econ. Entomol.* 98, 449–456.

Russo, C.A., Takezaki, N. & Nei, M. (1996). Efficiencies of different genes and different tree-building methods in recovering a known vertebrate phylogeny. *Mol. Biol. Evol.* 13, 525–536.

Saitou, N. & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol. Biol. Evol.* 4, 406–425.

Sandur, S. (2004). Implications of Diamondback moth control for Indian cabbage farmers. Report for Cimbaa. *Http://www. Cimbaa. Org/Downloads/Cimbaa_indiandbm_reportfinal-% 20apr05. Pdf.*

Sarfraz, M. (2004). Interaction between diamondback moth and Bacillus thuringiensis. *Outlooks on Pest Management*, *15*(4), 167.

Sarfraz, M. & Keddie, B.A. (2005). Conserving the efficacy of insecticides against Plutella xylostella (L.)(Lep., Plutellidae). *J. Appl. Entomol.* 129, 149–157.

Schippers, R. R., & others. (2000). African indigenous vegetables: An overview of the cultivated species. http://www.cabdirect.org/abstracts/20036793144.html

Shaw, M.R. (2003). Revised synonymy in the genus Cotesia (Hymenoptera: Braconidae: Microgastrinae): the identity of Microgaster vestalis Haliday, 1834, as a senior synonym of Apanteles plutellae Kurdjumov, 1912. *Entomol. Gaz.* 54, 187–190.

Shelton, A.M. (2001). Regional outbreaks of diamondback moth due to movement of contaminated plants and favourable climatic conditions. In *Proc. IV Int. Workshop "Management of Diamondback Moth and other Crucifer Pests". Melbourne*: 26–29.

Shelton, A.M., Sances, F.V., Hawley, J., Tang, J.D., Boune, M., Jungers, D., Collins, H.L. & Farias, J. (2000). Assessment of insecticide resistance after the outbreak of diamondback moth (Lepidoptera: Plutellidae) in California in 1997. *J. Econ. Entomol.* 93, 931–936.

Shi, Z., Guo, S., Lin, W. & Liu, S. (2004a). Evaluation of selective toxicity of five pesticides against Plutella xylostella (Lep: Plutellidae) and their side-effects against Cotesia plutellae (Hym: Braconidae) and Oomyzus sokolowskii (Hym: Eulophidae). *Pest Manag. Sci.* 60, 1213–1219.

Shi, Z., Liu, S. & Li, Y. (2002). Cotesia plutellae parasitizing Plutella xylostella: host-age dependent parasitism and its effect on host development and food consumption. *BioControl* 47, 499–511.

Shi, Z.-H., Li, Q.-B. & Li, X. (2004). Interspecific competition between Diadegma semiclausum Hellen (Hym., Ichneumonidae) and Cotesia plutellae

(Kurdjumov)(Hym., Braconidae) in parasitizing Plutella xylostella (L.)(Lep., Plutellidea). *J. Appl. Entomol.* 128, 437–444.

Silva-Aguayo, G. (2007). Botanical insecticides in Radcliffe's IPM world textbook. *Univ. Minn. USA*.

Sivapragasam, A. (2004). Brassica IPM adoption: progress and constraints in south-east Asia. *Manag. Diamondback Moth Crucif. Pests MARDI Malays.* 11–18.

Smith, T.J. & Villet, M.H. (2001). Parasitoids associated with the diamondback moth, Plutella xylostella (L.), in the Eastern Cape, South Africa. In *The management of diamondback moth and other crucifer pests. Proceedings of the Fourth International Workshop, 26Á/29 November*: 249Á.

Smith, T.J. & Villet, M.H. (2003). Parasitoids associated with the diamondback moth, Plutella xylostella (L.), in the Eastern Cape, South Africa. In *Proceedings of the Fourth International Workshop on The Management of Diamond-back moth and other crucifer pests*.

Soyelu, O. J. (2010). Effect of Nutrition on *Cotesia Plutellae* (Hymenoptera: Braconidae) and Its Parasitism on the Diamondback Moth, *Plutella Xylostella* (Lepidoptera: Plutellidae) [PhD Thesis]. University of Fort Hare,. 2010.

Sparks, T.C., Dripps, J.E., Watson, G.B. & Paroonagian, D. (2012). Resistance and cross-resistance to the spinosyns–a review and analysis. *Pestic. Biochem. Physiol.* 102, 1–10.

Srinivasan, R., M. Shelton, A. & L. Collins, H. (2011). *The Sixth International Workshop on Management of the Diamondback Moth and Other Crucifer Insect Pests*. AVRDC-WorldVegetableCenter.

Stamatakis, A. (2014). RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* 30, 1312–1313.

Stein, E. D., White, B. P., Mazor, R. D., Jackson, J. K., Battle, J. M., Miller, P. E., Pilgrim, E. M., & Sweeney, B. W. (2013). *Does DNA barcoding improve performance of*

traditional stream bioassessment metrics? https://www.bioone.org/doi/full/10.1086/674782

Stouthamer, R. (2006). 11 Molecular Methods for the Identification of Biological Control Agents at the Species and Strain Level. *Environ. Impact Invertebr. Biol. Control Arthropods Methods Risk Assess.* 10, 187.

Studio, R. (2012). R Studio. *Boston MA*.

Talekar, N. S. (2004). Biological control of diamondback moth in Asia. *Improving Biocontrol of Plutella Xylostella. CIRAD, Montpellier, France*, 103–113.

Talekar, N.S. & Shelton, A.M. (1993). Biology, ecology, and management of the diamondback moth. *Annu. Rev. Entomol.* 38, 275–301.

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M. & Kumar, S. (2011). MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol. Biol. Evol.* 28, 2731–2739.

Tewari, J. P., & Mithen, R. F. (1999). Diseases. *Biology of Brassica Coenospecies. Elsevier, Amsterdam*, 375–411.

Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F. & Higgins, D.G. (1997). The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res.* 25, 4876–4882.

Tian, S.-P., Zhang, J.-H., Yan, Y.-H. & Wang, C.-Z. (2008). Interspecific competition between the ichneumonid Campoletis chlorideae and the braconid Microplitis mediator in their host Helicoverpa armigera. *Entomol. Exp. Appl.* 127, 10–19.

Traugott, M., Zangerl, P., Juen, A., Schallhart, N. & Pfiffner, L. (2006). Detecting key parasitoids of lepidopteran pests by multiplex PCR. *Biol. Control* 39, 39–46.

Troczka, B.J., Williamson, M.S., Field, L.M. & Davies, T.E. (2017). Rapid selection for resistance to diamide insecticides in Plutella xylostella via specific amino acid polymorphisms in the ryanodine receptor. *Neurotoxicology* 60, 224–233.

Van Lenteren, J.C., Babendreier, D., Bigler, F., Burgio, G., Hokkanen, H.M.T., Kuske, S.,

Loomans, A.J.M., Menzler-Hokkanen, I., Van Rijn, P.C.J., Thomas, M.B. & others. (2003). Environmental risk assessment of exotic natural enemies used in inundative biological control. *BioControl* 48, 3–38.

Van Nouhuys, S. & Punju, E. (2010). Coexistence of competing parasitoids: which is the fugitive and where does it hide? *Oikos* 119, 61–70.

Varela, A. M., Seif, A., & Lohr, B. (2003). *A guide to IPM in brassicas production in east and southern Africa*. ICIPE Science Press.

Vrijenhoek, R. (1994). DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Mol. Mar. Biol. Biotechnol.* 3, 294–299.

Wagener, B., Reineke, A., Löhr, B. & Zebitz, C.P.W. (2004). A PCR-based approach to distinguish important Diadegma species (Hymenoptera: Ichneumonidae) associated with diamondback moth, Plutella xylostella (Lepidoptera: Plutellidae). *Bull. Entomol. Res.* 94, 465–471.

Wakeham, A.J. & Kennedy, R. (2010). Risk assessment methods for the ringspot pathogen Mycosphaerella brassicicola in vegetable Brassica crops. *Plant Dis.* 94, 851–859.

Wambani, H., Nyambati, E.M., Kamidi, M., Mulati, J. & others. (2007). Participatory evaluation of cabbage varieties as a source of food and income for smallholder farmers in north western Kenya. In *Proceedings of the African Crop Science Conference*: 355–357.

Warwick, H.R.I., Wellesbourne, W. & CV359EF, U. (2010). Control of Diamond back Moth (Plutella xylostella) on Cabbage (Brassica oleracea var capitata) using Intercropping with Non-Host Crops" E. Asare-Bediako," AA Addo-Quaye and A. Mohammed" Department of Crop Science, University of Cape Coast, Cape Coast, Ghana. *Am J Food Technol* 5, 269–274.

Webb, S.E. (2004). Insect management for Crucifers (Cole Crops)(Broccoli, Cabbage, Cauliflower, Collards, Kale, Mustard, Radishes, Turnips). *Fla. Coop. Ext. Serv. Circ.*

ENY-464 Univ. Fla. Inst. Food Agric. Sci.

Wei, S., Shi, M., Sharkey, M.J., van Achterberg, C. & Chen, X. (2010). Comparative mitogenomics of Braconidae (Insecta: Hymenoptera) and the phylogenetic utility of mitochondrial genomes with special reference to Holometabolous insects. *BMC Genomics* 11, 1.

Whitfield, J.B., Mardulyn, P., Austin, A.D. & Dowton, M. (2002). Phylogenetic relationships among microgastrine braconid wasp genera based on data from the 16S, COI and 28S genes and morphology. *Syst. Entomol.* 27, 337–359.

Xia, Y., Lu, Y., Shen, J., Gao, X., Qiu, H. & Li, J. (2014). Resistance monitoring for eight insecticides in Plutella xylostella in central China. *Crop Prot.* 63, 131–137.

Yu, R.-X., Shi, M., Huang, F. & Chen, X.-X. (2008). Immature development of Cotesia vestalis (Hymenoptera: Braconidae), an endoparasitoid of Plutella xylostella (Lepidoptera: Plutellidae). *Ann. Entomol. Soc. Am.* 101, 189–196.

Zalucki, M.P., Shabbir, A., Silva, R., Adamson, D., Shu-Sheng, L. & Furlong, M.J. (2012). Estimating the economic cost of one of the world's major insect pests, Plutella xylostella (Lepidoptera: Plutellidae): just how long is a piece of string? *J. Econ. Entomol.* 105, 1115–1129.

Zhao, J.Z., Li, Y.X., Collins, H.L., Gusukuma-Minuto, L., Mau, R.F.L., Thompson, G.D. & Shelton, A.M. (2002). Monitoring and characterization of diamondback moth (Lepidoptera: Plutellidae) resistance to spinosad. *J. Econ. Entomol.* 95, 430–436.

APPENDICES

Appendix 1: Farm Survey Questionnaire

Field Data sheet for *Cotesia vestalis* pilot site (Kenya, Kajiado, Kitui, Machakos, Makueni)

County _____ Sub-County _____ Division _____Location_____Village _____

Farmer's name _____Farmer's no_____

Collection no _____ Field no _____

Date _____

Latitude _____ Longitude _____

Altitude_____

Crop_____Variety_____Planting date _____

Crop stage _____ Harvesting interval _____

Pesticides used to control DBM _____

Last application _____ Application frequency _____

Pesticide application frequency in 2014/season _____

Current pesticide application frequency/season _____

Pesticides used to control aphids _____

Last application _____ Application frequency _____

Management _____ Approximate farm acreage on kale/cabbage _____

Irrigation type used _____ Soil type _____

Manure used _____ Last application _____

Fertilizer used _____ Last application _____

Fertilizer application interval _____

Other related crucifers _____

Farmer's knowledge on natural enemies _____

_____

_____

_____

Appendix 2

>Kj6

AAGATATTGGAATATTATATTTTATATTTGGATTATGATCAGGAATATTAGGATTTTCAATAAGTTTAATTATTCGTTTAGAA
TTAGGAATACCTGGGAGTTTAATTGGTAATGATCAAATTTATAATAGAATTGTAACTTCTCATGCATTTATTATAATTTTTTTT
ATAGTTATACCAGTAATAATTGGTGGATTTGGAAATTGATTAATTCCTTTAATATTAGGTTCTCCAGATATATCTTTTCCTCGA
ATAAATAATATAAGTTTTTGATTATTAATTCCTTCTTTATTATTATTAATTTTAAGTAGATTTATTAATGTTGGTGTGGGAACA
GGTTGAACAGTTTATCCTCCATTATCATTAATTTTAGGTCATGGTGGTATATCTGTTGATTTAGGTATTTTTTCTTTACATTTG
GCTGGTGCTTCTTCAATTATAGGTGCTGTAAATTTTATTACTACAATTATAAATATACGTTCTAATTTATTTTATATAGATAAA
ATATCTTTACTTTCTTGTTCAGTA

>Kj7

AATATTATATTTTATATTTGGATTATGATCAGGAATATTAGGATTTTCAATAAGTTTAATTATTCGTTTAGAATTAGGAATAC
CTGGGAGTTTAATTGGTAATGATCAAATTTATAATAGAATTGTAACTTCTCATGCATTTATTATAATTTTTTTTATAGTTATAC
CAGTAATAATTGGTGGATTTGGAAATTGATTAATTCCTTTAATATTAGGTTCTCCAGATATATCTTTTCCTCGAATAAATAAT
ATAAGTTTTTGATTATTAATTCCTTCTTTATTATTATTAATTTTAAGTAGATTTATTAATGTTGGTGTGGGAACAGGTTGAACA
GTTTATCCTCCATTATCATTAATTTTAGGTCATGGTGGTATATCTGTTGATTTAGGTATTTTTTCTTTACATTTGGCTGGTGCTT
CTTCAATTATAGGTGCTGTAAATTTTATTAATACAATTATAAATATACGTTCTAATTTATTTAATATAGATAAAATATCTTTAT
TTTCTTGATCAGTATTTATTACAGCAATTTTATTATTATTATCTTTGCCTGTTTTAGCTGGTGCTATTACCATATTATTATCTGA
GCGTAATATAAATACAAGATTTTTTGATCCATCAGGTGGTGGAGATCCAATTTTATATCAACATTTTTTTT

>Kj8

AAGATATTGGAATATTATATTTTATATTTGGATTATGATCAGGAATATTAGGATTTTCAATAAGTTTAATTATTCGTTTAGAA
TTAGGAATACCTGGGAGTTTAATTGGTAATGATCAAATTTATAATAGAATTGTAACTTCTCATGCATTTATTATAATTTTTTTT
ATAGTTATACCAGTAATAATTGGTGGATTTGGAAATTGATTAATTCCTTTAATATTAGGTTCTCCAGATATATCTTTTCCTCGA
ATAAATAATATAAGTTTTTGATTATTAATTCCTTCTTTATTATTATTAATTTTAAGTAGATTTATTAATGTTGGTGTGGGAACA
GGTTGAACAGTTTATCCTCCATTATCATTAATTTTAGGTCATGGTGGTATATCTGTTGATTTAGGTATTTTTTCTTTACATTTG
GCTGGTGCTTCTTCAATTATAGGTGCTGTAAATTTTATTACTACAATTATAAATATACGTTCTAATTTATTTAATATAGATAAA
ATATCTTTATTTTCTTGATCAGTATTTATTACAGCAATTTTATTATTATTATCTTTGCCTGTCGTAG

>Kj9

AATATTATATTTTATATTTGGATTATGATCAGGAATATTAGGATTTTCAATAAGTTTAATTATTCGTTTAGAATTAGGAATAC
CTGGGAGTTTAATTGGTAATGATCAAATTTATAATAGAATTGTAACTTCTCATGCATTTATTATAATTTTTTTTATAGTTATAC
CAGTAATAATTGGTGGATTTGGAAATTGATTAATTCCTTTAATATTAGGTTCTCCAGATATATCTTTTCCTCGAATAAATAAT
ATAAGTTTTTGATTATTAATTCCTTCTTTATTATTATTAATTTTAAGTAGATTTATTAATGTTGGTGTGGGAACAGGTTGAACA
GTTTATCCTCCATTATCATTAATTTTAGGTCATGGTGGTATATCTGTTGATTTAGGTATTTTTTCTTTACATTTGGCTGGTGCTT
CTTCAATTATAGGTGCTGTAAATTTTATTACTACAATTATAAATATACGTTCTAATTTATTTAATATAGATAAAATATCTTTAT
TTTCTTGATCAGTATTTATTACAGCAATTTTATTATTATTATCTTTGCCTGTTTTAGCTGGTGCTATTACTATATTATTAACTGA
TCGTAATATAAATACAAGATTTTTTGATCCATCAGGTGGTGGAGATCCAATTTTATATCAACATTTATTTT

>Kj10

AATATTATATTTTATATTTGGATTATGATCAGGAATATTAGGATTTTCAATAAGTTTAATTATTCGTTTAGAATTAGGAATAC
CTGGGAGTTTAATTGGTAATGATCAAATTTATAATAGAATTGTAACTTCTCATGCATTTATTATAATTTTTTTTATAGTTATAC
CAGTAATAATTGGTGGATTTGGAAATTGATTAATTCCTTTAATATTAGGTTCTCCAGATATATCTTTTCCTCGAATAAATAAT
ATAAGTTTTTGATTATTAATTCCTTCTTTATTATTATTAATTTTAAGTAGATTTATTAATGTTGGTGTGGGAACAGGTTGAACA
GTTTATCCTCCATTATCATTAATTTTAGGTCATGGTGGTATATCTGTTGATTTAGGTATTTTTTCTTTACATTTGGCTGGTGCTT
CTTCAATTATAGGTGCTGTAAATTTTATTACTACAATTATAAATATACGTTCTAATTTATTTAATATAGATAAAATATCTTTAT
TTTCTTGATCAGTATTTATTACAGCAATTTTATTATTATTATCTTTGCCTGTTTTAGCTGGTGCTATTACTATATTATTAACTGA
TCGTAATATAAATACAAGATTTTTTGATCCATCAGGTGGTGGAGATCCAATTTTAAATCAACATTTATTT

>Kj21

AATATTATATTTTATATTTGGATTATGATCAGGAATATTAGGATTTTCAATAAGTTTAATTATTCGTTTAGAATTAGGAATAC

CTGGGAGTTTAATTGGTAATGATCAAATTTATAATAGAATTGTAACTTCTCATGCATTTATTATAATTTTTTTTATAGTTATAC
CAGTAATAATTGGTGGATTTGGAAATTGATTAATTCCTTTAATATTAGGTTCTCCAGATATATCTTTTCCTCGAATAAATAAT
ATAAGTTTTTGATTATTAATTCCTTCTTTATTATTATTAATTTTAAGTAGATTTATTAATGTTGGTGTGGGAACAGGTTGAACA
GTTTATCCTCCATTATCATTAATTTTAGGTCATGGTGGTATATCTGTTGATTTAGGTATTTTTTCTTTACATTTGGCTGGTGCTT
CTTCAATTATAGGTGCTGTAAATTTTATTACTACAATTATAAATATACGTTCTAATTTATTTAATATAGATAAAATATCTTTAT
TTTCTTGATCAGTATTTATTACAGCAATTTTATTATTATTATCTTTGCCTGTTTTACCTGGTGCTATTACTATATTATTAACTGA
TCGTAATATAAATACAAGATTTTTTGATCCATCAGGTGGTGGAGATCCAATTTTATATCAACATTTATTTT
>Kj22
AATATTATATTTTATATTTGGATTATGATCAGGAATATTAGGATTTTCAATAAGTTTAATTATTCGTTTAGAATTAGGAATAC
CTGGGAGTTTAATTGGTAATGATCAAATTTATAATAGAATTGTAACTTCTCATGCATTTATTATAATTTTTTTTATAGTTATAC
CAGTAATAATTGGTGGATTTGGAAATTGATTAATTCCTTTAATATTAGGTTCTCCAGATATATCTTTTCCTCGAATAAATAAT
ATAAGTTTTTGATTATTAATTCCTTCTTTATTATTATTAATTTTAAGTAGATTTATTAATGTTGGTGTGGGAACAGGTTGAACA
GTTTATCCTCCATTATCATTAATTTTAGGTCATGGTGGTATATCTGTTGATTTAGGTATTTTTTCTTTACATTTGGCTGGTGCTT
CTTCAATTATAGGTGCTGTAAATTTTATTACTACAATTATAAATATACGTTCTAATTTATTTAATATAGATAAAATATCTTTAT
TTTCTTGATCAGTATTTATTACAGCAATTTTATTATTATTATCTTTGCCTGTTTTAGCTGGTGCTATTACTATATTATTAACTGA
TCGTAATATAAATACAAGATTTTTTGATCCATCAGGTGGTGGAGATCCAATTTTATATCAACATTTATTTT
>Kj24
AATATTATATTTTATATTTGGATTATGATCAGGAATATTAGGATTTTCAATAAGTTTAATTATTCGTTTAGAATTAGGAATAC
CTGGGAGTTTAATTGGTAATGATCAAATTTATAATAGAATTGTAACTTCTCATGCATTTATTATAATTTTTTTTATAGTTATAC
CAGTAATAATTGGTGGATTTGGAAATTGATTAATTCCTTTAATATTAGGTTCTCCAGATATATCTTTTCCTCGAATAAATAAT
ATAAGTTTTTGATTATTAATTCCTTCTTTATTATTATTAATTTTAAGTAGATTTATTAATGTTGGTGTGGGAACAGGTTGAACA
GTTTATCCTCCATTATCATTAATTTTAGGTCATGGTGGTATATCTGTTGATTTAGGTATTTTTTCTTTACATTTGGCTGGTGCTT
CTTCAATTATAGGTGCTGTAAATTTTATTACTACAATTATAAATATACGTTCTAATTTATTTAATATAGATAAAATATCTTTAT
TTTCTTGATCAGTATTTATTACAGCAATTTTATTATTATTATCTTTGCCTGTTTTAGCTGGTGCTATTACTATATTATTAACTGA
TCGTAATATAAATACAAGATTTTTTGATCCATCAGGTGGTGGAGATCCAATTTTATATCAACATTTATTTT
>Kj25
AAGATATTGGAATATTATATTTTATATTTGGATTATGATCAGGAATATTAGGATTTTCAATAAGTTTAATTATTCGTTTAGAA
TTAGGAATACCTGGGAGTTTAATTGGTAATGATCAAATTTATAATAGAATTGTAACTTCTCATGCATTTATTATAATTTTTTTT
ATAGTTATACCCGTAATAATTGGTGGATTTGGAAATTGATTAATTCCTTTAATATTAGGTTCTCCAGATATATCTTTTCCTCGA
ATAAATAATATAAGTTTTTGATTATTAATTCCTTCTTTATTATTATTAATTTTAAGTAGATTTATTAATGTTGGTGTGGGAACA
GGTTGAACAGTTTATCCTCCATTATCATTAATTTTAGGTCAGGGTGGTATATCTGTTGATTTAGGTATTTTTTCTTTACATTTG
GCTGGTGCTTTTTCAATTATAGGTGCTGTAAATTTTCTTACTACAATTATAAATATACGTTGTAATTTATTTTATTTTGATCCA
ATATCTTTATTTTCTTGTTCACGACTTCTTTACGAAAA
>Kt6
AATATTATATTTTATATTTGGATTATGATCAGGAATATTAGGATTTTCAATAAGTTTAATTATTCGTTTAGAATTAGGAATAC
CTGGGAGTTTAATTGGTAATGATCAAATTTATAATAGAATTGTAACTTCTCATGCATTTATTATAATTTTTTTTATAGTTATAC
CAGTAATAATTGGTGGATTTGGAAATTGATTAATTCCTTTAATATTAGGTTCTCCAGATATATCTTTTCCTCGAATAAATAAT
ATAAGTTTTTGATTATTAATTCCTTCTTTATTATTATTAATTTTAAGTAGATTTATTAATGTTGGTGTGGGAACAGGTTGAACA
GTTTATCCTCCATTATCATTAATTTTAGGTCATGGTGGTATATCTGTTGATTTAGGTATTTTTTCTTTACATTTGGCTGGTGCTT
CTTCAATTATAGGTGCTGTAAATTTTATTACTACAATTATAAATATACGTTCTAATTTATTTAATATAGATAAAATATCTTTAT
TTTCTTGATCAGTATTTATTACAGCAATTTTATTATTATTATCTTTGCCTGTTTTAGCTGGTGCTATTACTATATTATTAACTGA
TCGTAATATAAATACAAGATTTTTTGATCCATCAGGTGGTGGAGATCCAATTTTATATCAACATTTATTTT
>Kt7
AATATTATATTTTATATTTGGATTATGATCAGGAATATTAGGATTTTCAATAAGTTTAATTATTCGTTTAGAATTAGGAATAC
CTGGGAGTTTAATTGGTAATGATCAAATTTATAATAGAATTGTAACTTCTCATGCATTTATTATAATTTTTTTTATAGTTATAC
CAGTAATAATTGGTGGATTTGGAAATTGATTAATTCCTTTAATATTAGGTTCTCCAGATATATCTTTTCCTCGAATAAATAAT
ATAAGTTTTTGATTATTAATTCCTTCTTTATTATTATTAATTTTAAGTAGATTTATTAATGTTGGTGTGGGAACAGGTTGAACA

GTTTATCCTCCATTATCATTAATTTTAGGTCATGGTGGTATATCTGTTGATTTAGGTATTTTTTCTTTACATTTGGCTGGTGCTT
CTTCAATTATAGGTGCTGTAAATTTTATTACTACAATTATAATTATACGTTCTAATTTATTTAATATAGATAAAATATCTTTAT
TTTCTTGATCAGTATTTATTACAGCAATTTTATTATTATTATCTTTGCCTGTTTTAGCTGGTGCTATTACTATATTATTAACTGA
TCGTAATATAAATACAAGATTTTTTGATCCCCCAGGTGGTGGAGAGCCAATTTTATATCACATT
>Kt8
GGAATATTATATTTTATATTTGGATTATGATCAGGAATATTAGGATTTTCAATAAGTTTAATTATTCGTTTAGAATTAGGAAT
ACCTGGGAGTTTAATTGGTAATGATCAAATTTATAATAGAATTGTAACTTCTCATGCATTTATTATAATTTTTTTTATAGTTAT
ACCAGTAATAATTGGTGGATTTGGAAATTGATTAATTCCTTTAATATTAGGTTCTCCAGATATATCTTTTCCTCGAATAAATA
ATATAAGTTTTTGATTATTAATTCCTTCTTTATTATTATTAATTTTAAGTAGATTTATTAATGTTGGTGTGGGAACAGGTTGAA
CAGTTTATCCTCCATTATCATTAATTTTAGGTCATGGTGGTATATCTGTTGATTTAGGTATTTTTTCTTTACATTTGGCTGGTGC
TTCTTCAATTATAGGTGCTGTAAATTTTATTACTACAATTATAAATATACGTTCTAATTTATTTAATATAGATAAAATATCTTT
ATTTTCTTGATCAGTATTTATTACAGCAATTTTATTATTATTATCTTTGCCTGTTTTAGCTGGTGCTATTACTTTATTATTAACT
GATCGTAATATAAATACAAGATTTTTTGATCCATACAGGTGGTGGAGATCCAATTTTATATCAACAT
>Kt9
AATATTATATTTTATATTTGGATTATGATCAGGAATATTAGGATTTTCAATAAGTTTAATTATTCGTTTAGAATTAGGAATAC
CTGGGAGTTTAATTGGTAATGATCAAATTTATAATAGAATTGTAACTTCTCATGCATTTATTATAATTTTTTTTATAGTTATAC
CAGTAATAATTGGTGGATTTGGAAATTGATTAATTCCTTTAATATTAGGTTCTCCAGATATATCTTTTCCTCGAATAAATAAT
ATAAGTTTTTGATTATTAATTCCTTCTTTATTATTATTAATTTTAAGTAGATTTATTAATGTTGGTGTGGGAACAGGTTGAACA
GTTTATCCTCCATTATCATTAATTTTAGGTCATGGTGGTATATCTGTTGATTTAGGTATTTTTTCTTTACATTTGGCTGGTGCTT
CTTCAATTATAGGTGCTGTAAATTTTATTACTACAATTATAAATATACGTTCTAATTTATTTAATATAGATAAAATATCTTTAT
TTTCTTGATCAGTATTTATTACAGCAATTTTATTATTATTATCTTTGCCTGTTTTAGCTGGTGCTATTACTTTATTATTAACTGA
TCGTAATATAAATACAAGATTTTTTGATCCATCAGGTGGTGGAGATCCAATTTTATATCAACATTTATTTT
>Kt10
AATATTATATTTTATATTTGGATTATGATCAGGAATATTAGGATTTTCAATAAGTTTAATTATTCGTTTAGAATTAGGAATAC
CTGGGAGTTTAATTGGTAATGATCAAATTTATAATAGAATTGTAACTTCTCATGCATTTATTATAATTTTTTTTATAGTTATAC
CAGTAATAATTGGTGGATTTGGAAATTGATTAATTCCTTTAATATTAGGTTCTCCAGATATATCTTTTCCTCGAATAAATAAT
ATAAGTTTTTGATTATTAATTCCTTCTTTATTATTATTAATTTTAAGTAGATTTATTAATGTTGGTGTGGGAACAGGTTGAACA
GTTTATCCTCCATTATCATTAATTTTAGGTCATGGTGGTATATCTGTTGATTTAGGTATTTTTTCTTTACATTTGGCTGGTGCTT
CTTCAATTATAGGTGCTGTAAATTTTATTACTACAATTATAAATATACGTTCTAATTTATTTAATATAGATAAAATATCTTTAT
TTTCTTGATCAGTATTTATTACAGCAATTTTATTATTATTATCTTTGCCTGTTTTAGCTGGTGCTATTACTATATTATTAACTGA
TCGTAATATAAATACAAGATTTTTTGATCCATCAGGTGGTGGAGATCCAATTTTATATCAACATTTATTTT
>Kt26
AATATTATATTTTATATTTGGATTATGATCGGGAATATTAGGATTTTCAATAAGTTTAATTATTCGTTTAGAATTAGGAATAC
CTGGGAGTTTAATTGGTAATGATCAAATTTATAATAGAATTGTAACTTCTCATGCATTTATTATAATTTTTTTTATAGTTATAC
CAGTAATAATTGGTGGATTTGGAAATTGATTAATTCCTTTAATATTAGGTTCTCCAGATATATCTTTTCCTCGAATAAATAAT
ATAAGTTTTTGATTATTAATTCCTTCTTTATTATTATTAATTTTAAGTAGATTTATTAATGTTGGTGTGGGAACAGGTTGAACA
GTTTATCCTCCATTATCATTAATTTTAGGTCATGGTGGTATATCTGTTGATTTAGGTATTTTTTCTTTACATTTGGCTGGTGCTT
CTTCAATTATAGGTGCTGTAAATTTTATTACTACAATTATAAATATACGTTCTAATTTATTTAATATAGATAAAATATCTTTAT
TTTCTTGATCAGTATTTATTACAGCAATTTTATTATTATTATCTTTGCCTGTTTTAGCTGGTGCTATCACTATATTATTAACTGA
TCGTAATATAAATACAAGATTTTTTGATCCATCAGGTGGTGGGAGATCCAATTTTATATCAACATTTATTTT
>Kt27
AATATTATATTTTATATTTGGATTATGATCAGGAATATTAGGATTTTCAATAAGTTTAATTATTCGTTTAGAATTAGGAATAC
CTGGGAGTTTAATTGGTAATGATCAAATTTATAATAGAATTGTAACTTCTCATGCATTTATTATAATTTTTTTTATAGTTATAC
CAGTAATAATTGGTGGATTTGGAAATTGATTAATTCCTTTAATATTAGGTTCTCCAGATATATCTTTTCCTCGAATAAATAAT
ATAAGTTTTTGATTATTAATTCCTTCTTTATTATTATTAATTTTAAGTAGATTTATTAATGTTGGTGTGGGAACAGGTTGAACA
GTTTATCCTCCATTATCATTAATTTTAGGTCATGGTGGTATATCTGTTGATTTAGGTATTTTTTCTTTACATTTGGCTGGTGCTT
CTTCAATTATAGGTGCTGTAAATTTTATTACTACAATTATAAATATACGTTCTAATTTATTTAATATAGATAAAATATCTTTAT

TTTCTTGATCAGTATTTATTACAGCAATTTTATTATTATTATCTTTGCCTGTTTTAGCTGGTGCTATTACTATATTATTAACTGA
TCGTAATATAAATACAAGATTTTTTGATCCATCAGGTGGTGGAGATCCAATTTTATATCAACATTTATTTT
>Kt28
AATATTATATTTTATATTTGGATTATGATCGGGAATATTAGGATTTTCAATAAGTTTAATTATTCGTTTAGAATTAGGAATAC
CTGGGAGTTTAATTGGTAATGATCAAATTTATAATAGAATTGTAACTTCTCATGCATTTATTATAATTTTTTTTATAGTTATAC
CAGTAATAATTGGTGGATTTGGAAATTGATTAATTCCTTTAATATTAGGTTCTCCAGATATATCTTTTCCTCGAATAAATAAT
ATAAGTTTTTGATTATTAATTCCTTCTTTATTATTATTAATTTTAAGTAGATTTATTAATGTTGGTGTGGGAACAGGTTGAACA
GTTTATCCTCCATTATCATTAATTTTAGGTCATGGTGGTATATCTGTTGATTTAGGTATTTTTTCTTTACATTTGGCTGGTGCTT
CTTCAATTATAGGTGCTGTAAATTTTATTACTACAATTATAAATATACGTTCTAATTTATTTAATATAGATAAAATATCTTTAT
TTTCTTGATCAGTATTTATTACAGCAATTTTATTATTATTATCTTTGCCTGTTTTAGCTGGTGCTATCACTATATTATTAACTGA
TCGTAATATAAATACAAGATTTTTTGATCCATCAGGTGGTGGAGATCCAATTTTATATCAACATTTATTTTTATTTTTTGGTCA
CCCTAAAATA
>Kt29
AATATTATATTTTATATTTGGATTATGATCGGGAATATTAGGATTTTCAATAAGTTTAATTATTCGTTTAGAATTAGGAATAC
CTGGGAGTTTAATTGGTAATGATCAAATTTATAATAGAATTGTAACTTCTCATGCATTTATTATAATTTTTTTTATAGTTATAC
CAGTAATAATTGGTGGATTTGGAAATTGATTAATTCCTTTAATATTAGGTTCTCCAGATATATCTTTTCCTCGAATAAATAAT
ATAAGTTTTTGATTATTAATTCCTTCTTTATTATTATTAATTTTAAGTAGATTTATTAATGTTGGTGTGGGAACAGGTTGAACA
GTTTATCCTCCATTATCATTAATTTTAGGTCATGGTGGTATATCTGTTGATTTAGGTATTTTTTCTTTACATTTGGCTGGTGCTT
CTTCAATTATAGGTGCTGTAAATTTTATTACTACAATTATAAATATACGTTCTAATTTATTTAATATAGATAAAATATCTTTAT
TTTCTTGATCAGTATTTATTACAGCAATTTTATTATTATTATCTTTGCCTGTTTTAGCTGGTGCTATCACTATATTATTAACTGA
TCGTAATATAAATACAAGATTTTTTGATCCATCAGGTGGTGGAGATCCAATTTTATATCAACATTTATTTT
>Kt30
AATATTATATTTTATATTTGGATTATGATCGGGAATATTAGGATTTTCAATAAGTTTAATTATTCGTTTAGAATTAGGAATAC
CTGGGAGTTTAATTGGTAATGATCAAATTTATAATAGAATTGTAACTTCTCATGCATTTATTATAATTTTTTTTATAGTTATAC
CAGTAATAATTGGTGGATTTGGAAATTGATTAATTCCTTTAATATTAGGTTCTCCAGATATATCTTTTCCTCGAATAAATAAT
ATAAGTTTTTGATTATTAATTCCTTCTTTATTATTATTAATTTTAAGTAGATTTATTAATGTTGGTGTGGGAACAGGTTGAACA
GTTTATCCTCCATTATCATTAATTTTAGGTCATGGTGGTATATCTGTTGATTTAGGTATTTTTTCTTTACATTTGGCTGGTGCTT
CTTCAATTATAGGTGCTGTAAATTTTATTACTACAATTATAAATATACGTTCTAATTTATTTAATATAGATAAAATATCTTTAT
TTTCTTGATCAGTATTTATTACAGCAATTTTATTATTATTATCTTTGCCTGTTTTAGCTGGTGCTATCACTATATTATTAACTGA
TCGTAATATAAATACAAGATTTTTTGATCCATCAGGTGGTGGAGATCCAATTTTATATCAACATTTATTTT
>Kw7
AATATTATATTTTATATTTGGTCTATGATCAGGAATATTAGGATTTTCAATAAGATTGTTAATTCGTTTAGAATTAGGAATAC
CAGGAAGATTAATTGGTAATGATCAAATTTATAATAGTATTGTAACTTCTCATGCTTTTATTATAATTTTTTTTATAGTTATAC
CTGTAATAATTGGTGGTTTTGGGAATTGATTAATTCCTTTAATATTAGGATCTCCTGATATATCTTTTCCTCGAATAAATAATA
TAAGTTTTTGATTATTAATTCCTTCTTTATTATTATTAATTATAAGAAGATTTATTAATGTGGGAGTTGGTACTGGTTGAACTG
TATATCCACCTTTATCATTAATTTTAGGTCATGGTGGTATATCTGTTGATTTAGGTATTTTTTCTTTACATTTAGCTGGTGCATC
TTCAATTATAGGTGCTGTAAATTTTATTACTACTATTATAAATATACGTTCAAATTTATTTAATATAGATAAAATATCTTTATT
TTCTTGATCTGTATTTATTACTGCTATTTTATTATTATTATCTTTACCTGTTTTAGCTGGTGCTATTACTATATTATTAACTGAT
CGTAATATAAATACAAGATTTTTTGATCCTTCTGGTGGTGGTGATCCAATTCTTTATCAACATTTATTTTATTTTTTGGCCCCC
AAAAA
>Kw8
AATATTATATTTTATATTTGGATTATGATCAGGAATATTAGGATTTTCAATAAGTTTAATTATTCGTTTAGAATTAGGAATAC
CTGGGAGTTTAATTGGTAATGATCAAATTTATAATAGAATTGTAACTTCTCATGCATTTATTATAATTTTTTTTATAGTTATAC
CAGTAATAATTGGTGGATTTGGAAATTGATTAATTCCTTTAATATTAGGTTCTCCAGATATATCTTTTCCTCGAATAAATAAT
ATAAGTTTTTGATTATTAATTCCTTCTTTATTATTATTAATTTTAAGTAGATTTATTAATGTTGGTGTGGGAACAGGTTGAACA
GTTTATCCTCCATTATCATTAATTTTAGGTCATGGTGGTATATCTGTTGATTTAGGTATTTTTTCTTTACATTTGGCTGGTGCTT
CTTCAATTATAGGTGCTGTAAATTTTATTACTACAATTATAAATATACGTTCTAATTTATTTAATATAGATAAAATATCTTTAT

TTTCTTGATCAGTATTTATTACAGCAATTTTATTATTATTATCTTTGCCTGTTTTAGCTGGTGCTATTACTATATTATTAACTGA
TCGTAATATAAATACAAGATTTTTTGATCCATCAGGTGGTGGAGATCCAATTTTATATCAACATTTATTTT
>Kw9
AATATTATATTTTATATTTGGATTATGATCAGGAATATTAGGATTTTCAATAAGTTTAATTATTCGTTTAGAATTAGGAATAC
CTGGGAGTTTAATTGGTAATGATCAAATTTATAATAGAATTGTAACTTCTCATGCATTTATTATAATTTTTTTTATAGTTATAC
CAGTAATAATTGGTGGATTTGGAAATTGATTAATTCCTTTAATATTAGGTTCTCCAGATATATCTTTTCCTCGAATAAATAAT
ATAAGTTTTTGATTATTAATTCCTTCTTTATTATTATTAATTTTAAGTAGATTTATTAATGTTGGTGTGGGAACAGGTTGAACA
GTTTATCCTCCATTATCATTAATTTTAGGTCATGGTGGTATATCTGTTGATTTAGGTATTTTTTCTTTACATTTGGCTGGTGCTT
CTTCAATTATAGGTGCTGTAAATTTTATTACTACAATTATAAATATACGTTCTAATTTATTTAATATAGATAAAATATCTTTAT
TTTCTTGATCAGTATTTATTACAGCAATTTTATTATTATTATCTTTGCCTGTTTTAGCTGGTGCTATTACTATATTATTAACTGA
TCGTAATATAAATACAAGATTTTTTGATCCATCAGGTGGTGGAGATCCAATTTTATATCAACATTTATTTT
>Kw10
AATATTATATTTTATATTTGGATTATGATCAGGAATATTAGGATTTTCAATAAGTTTAATTATTCGTTTAGAATTAGGAATAC
CTGGGAGTTTAATTGGTAATGATCAAATTTATAATAGAATTGTAACTTCTCATGCATTTATTATAATTTTTTTTATAGTTATAC
CAGTAATAATTGGTGGATTTGGAAATTGATTAATTCCTTTAATATTAGGTTCTCCAGATATATCTTTTCCTCGAATAAATAAT
ATAAGTTTTTGATTATTAATTCCTTCTTTATTATTATTAATTTTAAGTAGATTTATTAATGTTGGTGTGGGAACAGGTTGAACA
GTTTATCCTCCATTATCATTAATTTTAGGTCATGGTGGTATATCTGTTGATTTAGGTATTTTTTCTTTACATTTGGCTGGTGCTT
CTTCAATTATAGGTGCTGTAAATTTTATTACTACAATTATAAATATACGTTCTAATTTATTTAATATAGATAAAATATCTTTAT
TTTCTTGATCAGTATTTATTACAGCAATTTTAATATTATTATCTTTGCCTGTTTCACCTGGTGCCATTACTATATTATTAACTGA
TCGTAATATAAATACAA
>Kw22
AATATTATATTTTATATTTGGATTATGATCAGGAATATTAGGATTTTCAATAAGTTTAATTATTCGTTTAGAATTAGGAATAC
CTGGGAGTTTAATTGGTAATGATCAAATTTATAATAGAATTGTAACTTCTCATGCATTTATTATAATTTTTTTTATAGTTATAC
CAGTAATAATTGGTGGATTTGGAAATTGATTAATTCCTTTAATATTAGGTTCTCCAGATATATCTTTTCCTCGAATAAATAAT
ATAAGTTTTTGATTATTAATTCCTTCTTTATTATTATTAATTTTAAGTAGATTTATTAATGTTGGTGTGGGAACAGGTTGAACA
GTTTATCCTCCATTATCATTAATTTTAGGTCATGGTGGTATATCTGTTGATTTAGGTATTTTTTCTTTACATTTGGCTGGTGCTT
CTTCAATTATAGGTGCTGTAAATTTTATTACTACAATTATAAATATACGTTCTAATTTATTTAATATAGATAAAATATCTTTAT
TTTCTTGATCAGTATTTATTACAGCAATTTTATTATTATTATCTTTGCCTGTTTTAGCTGGTGCTATTACTATATTATTAACTGA
TCGTAATATAAATACAAGATTTTTTGATCCATCAGGTGGTGGAGATCCAATTTTATATCAACATTTATTTT
>Kw23
AATATTATATTTTATATTTGGATTATGATCAGGAATATTAGGATTTTCAATAAGTTTAATTATTCGTTTAGAATTAGGAATAC
CTGGGAGTTTAATTGGTAATGATCAAATTTATAATAGAATTGTAACTTCTCATGCATTTATTATAATTTTTTTTATAGTTATAC
CAGTAATAATTGGTGGATTTGGAAATTGATTAATTCCTTTAATATTAGGTTCTCCAGATATATCTTTTCCTCGAATAAATAAT
ATAAGTTTTTGATTATTAATTCCTTCTTTATTATTATTAATTTTAAGTAGATTTATTAATGTTGGTGTGGGAACAGGTTGAACA
GTTTATCCTCCATTATCATTAATTTTAGGTCATGGTGGTATATCTGTTGATTTAGGTATTTTTTCTTTACATTTGGCTGGTGCTT
CTTCAATTATAGGTGCTGTAAATTTTATTACTACAATTATAAATATACGTTCTAATTTATTTAATATAGATAAAATATCTTTAT
TTTCTTGATCAGTATTTATTACAGCAATTTTATTATTATTATCTTTGCCTGTTTTAGCTGGTGCTATTACTATATTATTAACTGA
TCGTAATATAAATACAAGATTTTTTGATCCATCAGGTGGTGGAGATCCAATTTTATATCAACATTTATTTT
>Kw24
AATATTATATTTTATATTTGGATTATGATCAGGAATATTAGGATTTTCAATAAGTTTAATTATTCGTTTAGAATTAGGAATAC
CTGGGAGTTTAATTGGTAATGATCAAATTTATAATAGAATTGTAACTTCTCATGCATTTATTATAATTTTTTTTATAGTTATAC
CAGTAATAATTGGTGGATTTGGAAATTGATTAATTCCTTTAATATTAGGTTCTCCAGATATATCTTTTCCTCGAATAAATAAT
ATAAGTTTTTGATTATTAATTCCTTCTTTATTATTATTAATTTTAAGTAGATTTATTAATGTTGGTGTGGGAACAGGTTGAACA
GTTTATCCTCCATTATCATTAATTTTAGGTCATGGTGGTATATCTGTTGATTTAGGTATTTTTTCTTTACATTTGGCTGGTGCTT
CTTCAATTATAGGTGCTGTAAATTTTATTACTACAATTATAAATATACGTTCTAATTTATTTAATATAGATAAAATATCTTTAT
TTTCTTGATCAGTATTTATTACAGCAATTTTATTATTATTATCTTTGCCTGTTTTAGCTGGTGCTATTACTATATTATTAACTGA
TCGTAATATAAATACAAGATTTTTTGATCCATCAGGTGGTGGAGATCCAATTTTATATCAACATTTATTTT

>Kw25
AATATTATATTTTATATTTGGATTATGATCAGGAATATTAGGATTTTCAATAAGTTTAATTATTCGTTTAGAATTAGGAATAC
CTGGGAGTTTAATTGGTAATGATCAAATTTATAATAGAATTGTAACTTCTCATGCATTTATTATAATTTTTTTTATAGTTATAC
CAGTAATAATTGGTGGATTTGGAAATTGATTAATTCCTTTAATATTAGGTTCTCCAGATATATCTTTTCCTCGAATAAATAAT
ATAAGTTTTTGATTATTAATTCCTTCTTTATTATTATTAATTTTAAGTAGATTTATTAATGTTGGTGTGGGAACAGGTTGAACA
GTTTATCCTCCATTATCATTAATTTTAGGTCATGGTGGTATATCTGTTGATTTAGGTATTTTTTCTTTACATTTGGCTGGTGCTT
CTTCAATTATAGGTGCTGTAAATTTTATTACTACAATTATAAATATACGTTCTAATTTATTTAATATAGATAAAATATCTTTAT
TTTCTTGATCAGTATTTATTACAGCAATTTTATTATTATTATCTTTGCCTGTTTTAGCTGGTGCTATTACTATATTATTAACTGA
TCGTAATATAAATACAAGATTTTTTGATCCATCAGGTGGTGGAGATCCAATTTTATATCAACATTTATTTT
>Kw26
AATATTATATTTTATATTTGGATTATGATCAGGAATATTAGGATTTTCAATAAGTTTAATTATTCGTTTAGAATTAGGAATAC
CTGGGAGTTTAATTGGTAATGATCAAATTTATAATAGAATTGTAACTTCTCATGCATTTATTATAATTTTTTTTATAGTTATAC
CAGTAATAATTGGTGGATTTGGAAATTGATTAATTCCTTTAATATTAGGTTCTCCAGATATATCTTTTCCTCGAATAAATAAT
ATAAGTTTTTGATTATTAATTCCTTCTTTATTATTATTAATTTTAAGTAGATTTATTAATGTTGGTGTGGGAACAGGTTGAACA
GTTTATCCTCCATTATCATTAATTTTAGGTCATGGTGGTATATCTGTTGATTTAGGTATTTTTTCTTTACATTTGGCTGGTGCTT
CTTCAATTATAGGTGCTGTAAATTTTATTACTACAATTATAAATATACGTTCTAATTTATTTAATATAGATAAAATATCTTTAT
TTTCTTGATCAGTATTTATTACAGCAATTTTATTATTATTATCTTTGCCTGTTTTAGCTGGTGCTATTACTATATTATTAACTGA
TCGTAATATAAATACAAGATTTTTTGATCCATCAGGTGGTGGAGATCCAATTTTATATCAACATTTATTTT
>Mk1
AATATTATATTTTATATTTGGATTATGATCAGGAATATTAGGATTTTCAATAAGTTTAATTATTCGTTTAGAATTAGGAATAC
CTGGGAGTTTAATTGGTAATGATCAAATTTATAATAGAATTGTAACTTCTCATGCATTTATTATAATTTTTTTTATAGTTATAC
CAGTAATAATTGGTGGATTTGGAAATTGATTAATTCCTTTAATATTAGGTTCTCCAGATATATCTTTTCCTCGAATAAATAAT
ATAAGTTTTTGATTATTAATTCCTTCTTTATTATTATTAATTTTAAGTAGATTTATTAATGTTGGTGTGGGAACAGGTTGAACA
GTTTATCCTCCATTATCATTAATTTTAGGTCATGGTGGTATATCTGTTGATTTAGGTATTTTTTCTTTACATTTGGCTGGTGCTT
CTTCAATTATAGGTGCTGTAAATTTTATTACTACAATTATAAATATACGTTCTAATTTATTTAATATAGATAAAATATCTTTAT
TTTCTTGATCAGTATTTATTACAGCAATTTTATTATTATTATCTTTGCCTGCTTC
>Mk2
AATATTATATTTTATATTTGGATTATGATCAGGAATATTAGGATTTTCAATAAGTTTAATTATTCGTTTAGAATTAGGAATAC
CTGGGAGTTTAATTGGTAATGATCAAATTTATAATAGAATTGTAACTTCTCATGCATTTATTATAATTTTTTTTATAGTTATAC
CAGTAATAATTGGTGGATTTGGAAATTGATTAATTCCTTTAATATTAGGTTCTCCAGATATATCTTTTCCTCGAATAAATAAT
ATAAGTTTTTGATTATTAATTCCTTCTTTATTATTATTAATTTTAAGTAGATTTATTAATGTTGGTGTGGGAACAGGTTGAACA
GTTTATCCTCCATTATCATTAATTTTAGGTCATGGTGGTATATCTGTTGATTTAGGTATTTTTTCTTTACATTTGGCTGGTGCTT
CTTCAATTATAGGTGCTGTAAATTTTATTACTACAATTATAAATATACGTTCTAGTTTATTTAATATAGATAAAATCCCTTTAT
TTTCTTGATCACCATTTATTACCCCAAGCCATTTTATTATTATTATCTTTGCCCGTTTCCCCTGGCGCTATTACTATATTATTAA
CTGATCG
>Mk3
AATATTATATTTTATATTTGGATTATGATCGGGAATATTAGGATTTTCAATAAGTTTAATTATTCGTTTAGAATTAGGAATAC
CTGGGAGTTTAATTGGTAATGATCAAATTTATAATAGAATTGTAACTTCTCATGCATTTATTATAATTTTTTTTATAGTTATAC
CAGTAATAATTGGTGGATTTGGAAATTGATTAATTCCTTTAATATTAGGTTCTCCAGATATATCTTTTCCTCGAATAAATAAT
ATAAGTTTTTGATTATTAATTCCTTCTTTATTATTATTAATTTTAAGTAGATTTATTAATGTTGGTGTGGGAACAGGTTGAACA
GTTTATCCTCCATTATCATTAATTTTAGGTCATGGTGGTATATCTGTTGATTTAGGTATTTTTTCTTTACATTTGGCTGGTGCTT
CTTCAATTATAGGTGCTGTAAATTTTATTACTACAATTATAAATATACGTTCTAATTTATTTAATATAGATAAAATATCTTTAT
TTTCTTGATCAGTATTTATTACAGCAATTTTATTATTATTATCCTTGCCCGTTTTCCCTGGACCTATCACTATGTTAAAAACTG
ATCGTAATATAAATACCA
>Mk4
AAGATATTGGAATATTATATTTTATATTTGGATTATGATCAGGAATATTAGGATTTTCAATAAGTTTAATTATTCGTTTAGAA
TTAGGAATACCTGGGAGTTTAATTGGTAATGATCAAATTTATAATAGAATTGTAACTTCTCATGCATTTATTATAATTTTTTTT

ATAGTTATACCAGTAATAATTGGTGGATTTGGAAATTGATTAATTCCTTTAATATTAGGTTCTCCAGATATATCTTTTCCTCGA
ATAAATAATATAAGTTTTTGATTATTAATTCCTTCTTTATTATTATTAATTTTAAGTAGATTTATTAATGTTGGTGTGGGAACA
GGTTGAACAGTTTATCCTCCATTATCATTAATTTTAGGTCATGGTGGTATATCTGTTGATTTAGGTATTTTTTTTTTACATTTGG
CTGGTGCTTCTTCAATTATAGGTGCTGTAAATTTTATTACTACAATTATAAATATACGTTTTAATTTATTTAATATAGATAAAA
TATCTTTATTTTCTTGATCAGTATTTATTACAAAAATTTTATTATTATCATCTTTGCCTGTTTTAGC
>Mk5
AATATTATATTTTATATTTGGATTATGATCAGGAATATTAGGATTTTCAATAAGTTTAATTATTCGTTTAGAATTAGGAATAC
CTGGGAGTTTAATTGGTAATGATCAAATTTATAATAGAATTGTAACTTCTCATGCATTTATTATAATTTTTTTTATAGTTATAC
CAGTAATAATTGGTGGATTTGGAAATTGATTAATTCCTTTAATATTAGGTTCTCCAGATATATCTTTTCCTCGAATAAATAAT
ATAAGTTTTTGATTATTAATTCCTTCTTTATTATTATTAATTTTAAGTAGATTTATTAATGTTGGTGTGGGAACAGGTTGAACA
GTTTATCCTCCATTATCATTAATTTTAGGTCATGGTGGTATATCTGTTGATTTAGGTATTTTTTCTTTACATTTGGCTGGTGCTT
CTTCAATTATAGGTGCTGTAAATTTTATTACTACAATTATAAATATACGTTCTAATTTATTTAATATAGATAAAATATCTTTAT
TTTCTTGATCAGTATTTATTACAGCAATTTTATTATTATTATCTTTGCCTGTTTTAGCTGGTGCTATTACTATATTATTAACTGA
TCGTAATATAAATACAAGATTTTTTGATCCATCAGGTGGTGGAGATCCAATTTTATATCAACATTTATTTT
>Mk6
AATATTATATTTTATATTTGGATTATGATCAGGAATATTAGGATTTTCAATAAGTTTAATTATTCGTTTAGAATTAGGAATAC
CTGGGAGTTTAATTGGTAATGATCAAATTTATAATAGAATTGTAACTTCTCATGCATTTATTATAATTTTTTTTATAGTTATAC
CAGTAATAATTGGTGGATTTGGAAATTGATTAATTCCTTTAATATTAGGTTCTCCAGATATATCTTTTCCTCGAATAAATAAT
ATAAGTTTTTGATTATTAATTCCTTCTTTATTATTATTAATTTTAAGTAGATTTATTAATGTTGGTGTGGGAACAGGTTGAACA
GTTTATCCTCCATTATCATTAATTTTAGGTCATGGTGGTATATCTGTTGATTTAGGTATTTTTTCTTTACATTTGGCTGGTGCTT
CTTCAATTATAGGTGCTGTAAATTTTATTACTACAATTATAAATATACGTTCTAATTTATTTAATATAGATAAAATATCTTTAT
TTTCTTGATCAGTATTTATTACAGCAATTTTATTATTATTATCTTTGCCTGTTTTAGCTGGTGCTATTACTATATTATTAACTGA
TCGTAATATAAATACAAGATTTTTTGATCCATCAGGTGGTGGAGATCCAATTTTATATCAACATTTATTTT
>Mk7
AATATTATATTTTATATTTGGATTATGATCAGGAATATTAGGATTTTCAATAAGTTTAATTATTCGTTTAGAATTAGGAATAC
CTGGGAGTTTAATTGGTAATGATCAAATTTATAATAGAATTGTAACTTCTCATGCATTTATTATAATTTTTTTTATAGTTATAC
CAGTAATAATTGGTGGATTTGGAAATTGATTAATTCCTTTAATATTAGGTTCTCCAGATATATCTTTTCCTCGAATAAATAAT
ATAAGTTTTTGATTATTAATTCCTTCTTTATTATTATTAATTTTAAGTAGATTTATTAATGTTGGTGTGGGAACAGGTTGAACA
GTTTATCCTCCATTATCATTAATTTTAGGTCATGGTGGTATATCTGTTGATTTAGGTATTTTTTCTTTACATTTGGCTGGTGCTT
CTTCAATTATAGGTGCTGTAAATTTTATTACTACAATTATAAATATACGTTCTAATTTATTTAATATAGATAAAATATCTTTAT
TTTCTTGATCAGTATTTATTACAGCAATTTTATTATTATTATCTTTGCCTGTTTTAGCTGGTGCTATTACTATATTATTAACTGA
GCGTAATATAAATACAAGATTTTTTGATCCATCAGGTGGTGGAGATCCAATTTTATATCAACATTTATTTT
>Mk8
AATATTATATTTTATATTTGGATTATGATCAGGAATATTAGGATTTTCAATAAGTTTAATTATTCGTTTAGAATTAGGAATAC
CTGGGAGTTTAATTGGTAATGATCAAATTTATAATAGAATTGTAACTTCTCATGCATTTATTATAATTTTTTTTATAGTTATAC
CAGTAATAATTGGTGGATTTGGAAATTGATTAATTCCTTTAATATTAGGTTCTCCAGATATATCTTTTCCTCGAATAAATAAT
ATAAGTTTTTGATTATTAATTCCTTCTTTATTATTATTAATTTTAAGTAGATTTATTAATGTTGGTGTGGGAACAGGTTGAACA
GTTTATCCTCCATTATCATTAATTTTAGGTCATGGTGGTATATCTGTTGATTTAGGTATTTTTTCTTTACATTTGGCTGGTGCTT
CTTCAATTATAGGTGCTGTAAATTTTATTACTACAATTATAAATATACGTTCTAATTTATTTAATATAGATAAAATATCTTTAT
TTTCTTGATCAGTATTTATTACAGCAATTTTATTATTATTATCTTTGCCTGTTTTAGCTGGTGCTATTACTATATTATTAACTGA
TCGTAATATAAATACAAGATTTTTTGATCCATCAGGTGGTGGAGATCCAATTTTATATCAACATTTATTTT
>Mk9
AATATTATATTTTATATTTGGATTATGATCAGGAATATTAGGATTTTCAATAAGTTTAATTATTCGTTTAGAATTAGGAATAC
CTGGGAGTTTAATTGGTAATGATCAAATTTATAATAGAATTGTAACTTCTCATGCATTTATTATAATTTTTTTTATAGTTATAC
CAGTAATAATTGGTGGATTTGGAAATTGATTAATTCCTTTAATATTAGGTTCTCCAGATATATCTTTTCCTCGAATAAATAAT
ATAAGTTTTTGATTATTAATTCCTTCTTTATTATTATTAATTTTAAGTAGATTTATTAATGTTGGTGTGGGAACAGGTTGAACA
GTTTATCCTCCATTATCATTAATTTTAGGTCATGGTGGTATATCTGTTGATTTAGGTATTTTTTCTTTACATTTGGCTGGTGCTT

CTTCAATTATAGGTGCTGTAAATTTTATTACTACAATTATAAATATACGTTCTAATTTATTTAATATAGATAAAATATCTTTAT
TTTCTTGATCAGTATTTATTACAGCAATTTTATTATTATTATCTTTGCCTGTTTTAGCTGGTGCTATTACTATATTATTAACTGA
TCGTAATATAAATACAAGATTTTTTGATCCATCAGGTGGTGGAGATCCAATTTTATATCAACATTTATTTT
>Mk10
AAGATATTGGAATATTATATTTTATATTTGGATTATGATCAGGAATATTAGGATTTTCAATAAGTTTAATTATTCGTTTAGAA
TTAGGAATACCTGGGAGTTTAATTGGTAATGATCAAATTTATAATAGAATTGTAACTTCTCATGCATTTATTATAATTTTTTTT
ATAGTTATACCAGTAATAATTGGTGGATTTGGAAATTGATTAATTCCTTTAATATTAGGTTCTCCAGATATATCTTTTCCTCGA
ATAAATAATATAAGTTTTTGATTATTAATTCCTTCTTTATTATTATTAATTTTAAGTAGATTTATTAATGTTGGTGTGGGAACA
GGTTGAACAGTTTACCCCCCATTATCATTAATTTTAGGTCATGGTGGTATATCTGTTGATTTAGGTATTTTTTCTTTACATTTG
GCTGGTGGTTCTTCAATTATAGGTGCTGTAAATTTTATTACTACAATTATAAATATACGTTCTAATTTATTTTATATAGGTAAA
ATATCTTTATTTTCTTGATCAGTATTTATTACAGCACTTTTATTA
>Mw11
TATTGGAATATTATATTTTATATTTGGATTATGATCGGGAATATTAGGATTTTCAATAAGTTTAATTATTCGTTTAGAATTAGG
AATACCTGGGAGTTTAATTGGTAATGATCAAATTTATAATAGAATTGTAACTTCTCATGCATTTATTATAATTTTTTTTATAGT
TATACCAGTAATAATTGGTGGATTTGGAAATTGATTAATTCCTTTAATATTAGGTTCTCCAGATATATCTTTTCCTCGAATAA
ATAATATAAGTTTTTGATTATTAATTCCTTCTTTATTATTATTAATTTTAAGTAGATTTATTAATGTTGGTGTGGGAACAGGTT
GAACAGTTTATCCTCCATTATCATTAATTTTAGGTCATGGTGGTATATCTGTTGATTTAGGTATTTTTTCTTTACATTTGGCTG
GTGCTTCTTCAATTATAGGTGCTGTAAATTTTATTACTACAATTATAAATATACGTTCTAATTTATTTAATATAGATAAAATAT
CTTTATTTTCTTGATCAGTATTTATTACAGCAATTTTATTATTATTATCTTTGCCTGTTTTAGCTGGTGCTATCACTATATTATT
AACTGATCGTAATATAAATACAAGATTTTTTGATCCATCAGGTGGTGGAGATCCAATTTTATATCAACATTTATTTT
>Mw12
AATATTATATTTTATATTTGGATTATGATCGGGAATATTAGGATTTTCAATAAGTTTAATTATTCGTTTAGAATTAGGAATAC
CTGGGAGTTTAATTGGTAATGATCAAATTTATAATAGAATTGTAACTTCTCATGCATTTATTATAATTTTTTTTATAGTTATAC
CAGTAATAATTGGTGGATTTGGAAATTGATTAATTCCTTTAATATTAGGTTCTCCAGATATATCTTTTCCTCGAATAAATAAT
ATAAGTTTTTGATTATTAATTCCTTCTTTATTATTATTAATTTTAAGTAGATTTATTAATGTTGGTGTGGGAACAGGTTGAACA
GTTTATCCTCCATTATCATTAATTTTAGGTCATGGTGGTATATCTGTTGATTTAGGTATTTTTTCTTTACATTTGGCTGGTGCTT
CTTCAATTATAGGTGCTGTAAATTTTATTACTACAATTATAAATATACGTTCTAATTTATTTAATATAGATAAAATATCTTTAT
TTTCTTGATCAGTATTTATTACAGCAATTTTATTATTATTATCTTTGCCTGTTTTAGCTGGTGCTATCACTATATTATTAACTGA
TCGTAATATAAATACAAGATTTTTTGATCCATCAGGTGGTGGAGATCCAATTTTATATCAACATTTATTTT
>Mw13
AATATTATATTTTATATTTGGATTATGATCGGGAATATTAGGATTTTCAATAAGTTTAATTATTCGTTTAGAATTAGGAATAC
CTGGGAGTTTAATTGGTAATGATCAAATTTATAATAGAATTGTAACTTCTCATGCATTTATTATAATTTTTTTTATAGTTATAC
CAGTAATAATTGGTGGATTTGGAAATTGATTAATTCCTTTAATATTAGGTTCTCCAGATATATCTTTTCCTCGAATAAATAAT
ATAAGTTTTTGATTATTAATTCCTTCTTTATTATTATTAATTTTAAGTAGATTTATTAATGTTGGTGTGGGAACAGGTTGAACA
GTTTATCCTCCATTATCATTAATTTTAGGTCATGGTGGTATATCTGTTGATTTAGGTATTTTTTCTTTACATTTGGCTGGTGCTT
CTTCAATTATAGGTGCTGTAAATTTTATTACTACAATTATAAATATACGTTCTAATTTATTTAATATAGATAAAATATCTTTAT
TTTCTTGATCAGTATTTATTACAGCAATTTTATTATTATTATCTTTGCCTGTTTTAGCTGGTGCTATCACTATATTATTAACTGA
TCGTAATATAAATACAAGATTTTTTGATCCATCAGGTGGTGGAGATCCAATTTTATATCAACATTTATTTT
>Mw14
AATATTATATTTTATATTTGGATTATGATCGGGAATATTAGGATTTTCAATAAGTTTAATTATTCGTTTAGAATTAGGAATAC
CTGGGAGTTTAATTGGTAATGATCAAATTTATAATAGAATTGTAACTTCTCATGCATTTATTATAATTTTTTTTATAGTTATAC
CAGTAATAATTGGTGGATTTGGAAATTGATTAATTCCTTTAATATTAGGTTCTCCAGATATATCTTTTCCTCGAATAAATAAT
ATAAGTTTTTGATTATTAATTCCTTCTTTATTATTATTAATTTTAAGTAGATTTATTAATGTTGGTGTGGGAACAGGTTGAACA
GTTTATCCTCCATTATCATTAATTTTAGGTCATGGTGGTATATCTGTTGATTTAGGTATTTTTTCTTTACATTTGGCTGGTGCTT
CTTCAATTATAGGTGCTGTAAATTTTATTACTACAATTATAAATATACGTTCTAATTTATTTAATATAGATAAAATATCTTTAT
TTTCTTGATCAGTATTTATTACAGCAATTTTATTATTATTATCTTTGCCTGTTTTAGCTGGTGCTATCACTATATTATTAACTGA
TCGTAATATAAATACAAGATTTTTTGATCCATCAGGTGGTGGAGATCCAATTTTATATCAACATTTATTTT

>Mw15
AATATTATATTTTATATTTGGATTATGATCGGGAATATTAGGATTTTCAATAAGTTTAATTATTCGTTTAGAATTAGGAATAC
CTGGGAGTTTAATTGGTAATGATCAAATTTATAATAGAATTGTAACTTCTCATGCATTTATTATAATTTTTTTTATAGTTATAC
CAGTAATAATTGGTGGATTTGGAAATTGATTAATTCCTTTAATATTAGGTTCTCCAGATATATCTTTTCCTCGAATAAATAAT
ATAAGTTTTTGATTATTAATTCCTTCTTTATTATTATTAATTTTAAGTAGATTTATTAATGTTGGTGTGGGAACAGGTTGAACA
GTTTATCCTCCATTATCATTAATTTTAGGTCATGGTGGTATATCTGTTGATTTAGGTATTTTTTCTTTACATTTGGCTGGTGCTT
CTTCAATTATAGGTGCTGTAAATTTTATTACTACAATTATAAATATACGTTCTAATTTATTTAATATAGATAAAATATCTTTAT
TTTCTTGATCAGTATTTATTACAGCAATTTTATTATTATTATCTTTGCCTGTTTTAGCTGGTGCTATCACTATATTATTAACTGA
TCGTAATATAAATACAAGATTTTTTGATCCATCAGGTGGTGGAGATCCAATTTTATATCAACATTTATTTT
>Mw21
AATATTATATTTTATATTTGGATTATGATCGGGAATATTAGGATTTTCAATAAGTTTAATTATTCGTTTAGAATTAGGAATAC
CTGGGAGTTTAATTGGTAATGATCAAATTTATAATAGAATTGTAACTTCTCATGCATTTATTATAATTTTTTTTATAGTTATAC
CAGTAATAATTGGTGGATTTGGAAATTGATTAATTCCTTTAATATTAGGTTCTCCAGATATATCTTTTCCTCGAATAAATAAT
ATAAGTTTTTGATTATTAATTCCTTCTTTATTATTATTAATTTTAAGTAGATTTATTAATGTTGGTGTGGGAACAGGTTGAACA
GTTTATCCCCCCTTATCATTAATTTTAGGTCATGGTGGTATATCTGTTGATTTAGGTATTTTTTCTTTACATTTGGCTGGTGCTT
CTTCAATTATAGGTGCTGTAAATTTTATTACTACAATTATAAATATACGTTTTAATTTATTTTATATAGATAAAATATCTTTAT
TTTTTTGATCAGTATTTATTACAGCAATTTTATTATTATTATCTTTGCCTGTTTTAGCTGGTGCTATCACTATATTATTAACTGA
TCGTAATATAAATACATC
>Mw22
AATATTATATTTTATATTTGGATTATGATCGGGAATATTAGGATTTTCAATAAGTTTAATTATTCGTTTAGAATTAGGAATAC
CTGGGAGTTTAATTGGTAATGATCAAATTTATAATAGAATTGTAACTTCTCATGCATTTATTATAATTTTTTTTATAGTTATAC
CAGTAATAATTGGTGGATTTGGAAATTGATTAATTCCTTTAATATTAGGTTCTCCAGATATATCTTTTCCTCGAATAAATAAT
ATAAGTTTTTGATTATTAATTCCTTCTTTATTATTATTAATTTTAAGTAGATTTATTAATGTTGGTGTGGGAACAGGTTGAACA
GTTTATCCTCCATTATCATTAATTTTAGGTCATGGTGGTATATCTGTTGATTTAGGTATTTTTTCTTTACATTTGGCTGGTGCTT
CTTCAATTATAGGTGCTGTAAATTTTATTACTACAATTATAAATATACGTTCTAATTTATTTAATATAGATAAAATATCTTTAT
TTTCTTGATCAGTATTTATTACAGCAATTTTATTATTATTATCTTTGCCTGTTTTAGCTGGTGCTATCACTATATTATTAACTGA
TCGTAATATAAATACAAGATTTTTTGATCCATCAGGTGGTGGAGATCCAATTTTATATCAACATTTATTTT
>Mw23
GATATTGGAATATTATATTTTATATTTGGATTATGATCGGGAATATTAGGATTTTCAATAAGTTTAATTATTCGTTTAGAATTA
GGAATACCTGGGAGTTTAATTGGTAATGATCAAATTTATAATAGAATTGTAACTTCTCATGCATTTATTATAATTTTTTTTATA
GTTATACCAGTAATAATTGGTGGATTTGGAAATTGATTAATTCCTTTAATATTAGGTTCTCCAGATATATCTTTTCCTCGAATA
AATAATATAAGTTTTTGATTATTAATTCCTTCTTTATTATTATTAATTTTAAGTAGATTTATTAATGTTGGTGTGGGAACAGGT
TGAACAGTTTATCCTCCATTATCATTAATTTTAGGTCATGGTGGTATATCTGTTGATTTAGGTATTTTTTCTTTACATTTGGCTG
GTGCTTCTTCAATTATAGGTGCTGTAAATTTTATTACTACAATTATAAATATACGTTCTAATTTATTTAATATAGATAAAATAT
CTTTATTTTCTTGATCAGTATTTATTACAGCAATTTTATTATTATTATCTTTGCCTGTTTTAGCTGGTGCTATCACTATATTATT
AACTGATCGTAATATAAATACAAGATTTTTTGATCCATCAGGTGGTGGAGATCCAATTTTATATCAACATTTATTTT
>Mw24
AATATTATATTTTATATTTGGATTATGATCGGGAATATTAGGATTTTCAATAAGTTTAATTATTCGTTTAGAATTAGGAATAC
CTGGGAGTTTAATTGGTAATGATCAAATTTATAATAGAATTGTAACTTCTCATGCATTTATTATAATTTTTTTTATAGTTATAC
CAGTAATAATTGGTGGATTTGGAAATTGATTAATTCCTTTAATATTAGGTTCTCCAGATATATCTTTTCCTCGAATAAATAAT
ATAAGTTTTTGATTATTAATTCCTTCTTTATTATTATTAATTTTAAGTAGATTTATTAATGTTGGTGTGGGAACAGGTTGAACA
GTTTATCCTCCATTATCATTAATTTTAGGTCATGGTGGTATATCTGTTGATTTAGGTATTTTTTCTTTACATTTGGCTGGTGCTT
CTTCAATTATAGGTGCTGTAAATTTTATTACTACAATTATAAATATACGTTCTAATTTATTTAATATAGATAAAATATCTTTAT
TTTCTTGATCAGTATTTATTACAGCAATTTTATTATTATTATCTTTGCCTGTTTTAGCTGGTGCTATCACTATATTATTAACTGA
TCGTAATATAAATACAAGATTTTTTGATCCATCAGGTGGTGGAGTATCCAATTTTATATCAACATTTATTTT
>Mw25
AATATTATATTTTATATTTGGATTATGATCGGGAATATTAGGATTTTCAATAAGTTTAATTATTCGTTTAGAATTAGGAATAC

CTGGGAGTTTAATTGGTAATGATCAAATTTATAATAGAATTGTAACTTCTCATGCATTTATTATAATTTTTTTTATAGTTATAC
CAGTAATAATTGGTGGATTTGGAAATTGATTAATTCCTTTAATATTAGGTTCTCCAGATATATCTTTTCCTCGAATAAATAAT
ATAAGTTTTTGATTATTAATTCCTTCTTTATTATTATTAATTTTAAGTAGATTTATTAATGTTGGTGTGGGAACAGGTTGAACA
GTTTATCCTCCATTATCATTAATTTTAGGTCATGGTGGTATATCTGTTGATTTAGGTATTTTTTCTTTACATTTGGCTGGTGCTT
CTTCAATTATAGGTGCTGTAAATTTTATTACTACAATTATAAATATACGTTCTAATTTATTTAATATAGATAAAATATCTTTAT
TTTCTTGATCAGTATTTATTACAGCAATTTTATTATTATTATCTTTGCCTGTTTTAGCTGGTGCTATCACTATATTATTAACTGA
TCGTAATATAAATACAAGATTTTTTGATCCATCAGGTGGTGGAGATCCAATTTTATATCAACATTTATTTT