Chapter 10
Establishment of an Exotic Parasitoid *Cotesia vestalis* in Coastal Areas of Kenya as Biological Control Agent of *Plutella xylostella*

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Abstract The aims of this study were to follow up on the establishment of *Cotesia vestalis* in the coastal regions of Kenya, assessing its contribution in the management of the diamondback moth (*Plutella xylostella*), parasitism rates, and cultural practices affecting its establishment. Post-release surveys were carried out in five counties in Kenya, Kitui, Kajiado, Kwale, Machakos and Makueni, between 2015 and 2016. The results showed that the overall parasitism rate of *C. vestalis* in 2015 ranged between 0% and 37.86% while that in 2016 ranged from 0% to 32.19% in the different counties. Farmers carry out routine sprays, either weekly or fortnightly, with different synthetic insecticides. Pyrethroids (60.56%) constituted most of the insecticides used, while only 3.18% of the products used were plant or microorganism-based. Farmers did most of their cabbage production during the rainy season, with

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production being greatly reduced during the dry seasons. The sampled diamondback moths from Kajiado, Kitui, Makueni, Kwale and Malawi had ≥98% similarity to *Cotesia vestalis*, as shown by bioinformatics analyses using PCR amplified products of 700 bp, obtained for the mitochondrial COI gene. Alignment showed highly conserved regions, and the phylogenetic analysis revealed two close lineages corresponding to *Cotesia vestalis* (Genbank accession: FJ154897) and *Cotesia* spp. (acc. HM430398). Data provide a clear indication that the parasitoid became established in its release sites in Kenya, although parasitism rates are still low probably due to heavy pesticide use and climatic factors.

Keywords *Plutella xylostella* · *Cotesia vestalis* · Biological control · DNA barcoding · PCR

10.1 Introduction

Cruciferous vegetables, such as kales and cabbages, are widely grown in Africa for subsistence purposes, as well as for income generation (Ayalew et al. 2002; Lohr and Kfir 2004; Macharia et al. 2005; Grzywacz et al. 2010). The annual global production is estimated at 29 tonnes/ha, while productions in Malawi and Kenya reach approximately 20.7 and 30.9 tonnes/ha, respectively (FAOSTAT 2014).

Crucifer production, worldwide, is often hindered by insect pests which include the diamondback moth (*Plutella xylostella* L.), cabbage aphids (*Brevicoryne brassicae* L.), leaf miners (*Liriomyza brassicae* L.), thrips (*Thrips tabaci* L.), and cabbageworms (*Pieris rapae* L.) (Hines and Hutchison 2001; Bjorksten et al. 2005; Munthali 2009). Among these insect pests, *P. xylostella* is the most destructive one in Eastern Africa (Nyambo and Pekke 1995; Badenes-Perez and Shelton 2006). In instances where no management strategies are put in place, the small larvae of *P. xylostella* can cause losses between 90% and 98% (Sandur 2004; Macharia et al. 2005). Worldwide losses of crucifer vegetables by *P. xylostella* are estimated at US$ 1.4 billion (Zalucki et al. 2012), incurring US$ 4–5 billion in management costs (Furlong et al. 2013). In Kenya, annual losses of US$ 7.9 million, due to *P. xylostella* infestations, have been reported (Macharia et al. 2005).

Management of *P. xylostella* in Kenya has mainly focused on the use of pesticides, with reported cases of overuse resulting in increases in production costs, health hazards, development of resistance, and destruction of natural enemies (Badenes-Perez and Shelton 2006; Cooper 2009; Macharia et al. 2013). Biological control has thus been promoted as an alternative (Rowell et al. 2005; Kahuthia-Gathu 2012). The parasitoids of *P. xylostella* mainly found in the East Africa region are *Diadegma mollipla* Holmgren and *Oomyzus sokolowskii* Kurdjumov. However, their parasitism rates have been reported to be below 15% in Kenya, Tanzania,

Malawi and Ethiopia (Lohr and Kfir 2004). One of the most effective parasitoids used in the management of *P. xylostella* is *Cotesia vestalis* Haliday, with reports of 78–88% parasitism rates (Smith and Villet 2001; Rowell et al. 2005). Among all the *C. vestalis* biotypes, the South African biotype is the most effective, owing to its predominance in both low and high altitudes (Talekar and Shelton 1993; Kfir 1997; Verkerk and Wright 1997; Mosiane et al. 2003), high thermal tolerance (Talekar and Yang 1991), and high parasitism rates (Waladde et al. 2001; Smith 2002).

Once introduced into Uganda, *C. vestalis* became established (ICIPE, unpublished data). It has been detected on the Kenyan side of Lake Victoria. Additional releases of *C. vestalis* were carried out in Kajiado, Machakos, Kitui and Makueni counties (Nyambo et al. 2008). However, repeated releases in the eastern region of Kenya resulted in very low parasitism rates (0.5–26.9%) (Nyambo et al. 2008; Kahuthia-Gathu 2012). Although the establishment of *C. vestalis* at the release sites was confirmed by other studies, most of them relied on morphological identification. A recent release of *C. vestalis* in Kwale County in 2013 resulted in a very low parasitism rate (unpublished data). This prompted the need to investigate the cultural practices that preclude the successful establishment of the parasitoid in Kenya, particularly in the coastal area. This study also aimed at confirming the molecular identity of the specimens that prevail in those counties and comparing them with those of Southern Africa biotypes, to ascertain their virulence and parasitism rates, in the various regions.

10.2 Materials and Methods

10.2.1 Study Sites

Surveys for the *P. xylostella* and its parasitoid, *C. vestalis*, were carried out in five counties: Kwale (Matuga, Diani and Lungalunga), Kajiado, Kitui, Makueni and Machakos (Fig. 10.1). Kajiado, Kitui, Makueni and Machakos counties represented *C. vestalis* post-release sites in the arid regions at mid-altitude (882–1918 m asl) of Kenya, while Kwale County represented the humid lowlands (3–416 m asl).

The surveys for *P. xylostella* and *C. vestalis* in Kwale County were conducted in the Lungalunga, Matuga and Diani regions. Kwale County experiences a bi-modal rainfall distribution, with long rains expected from March to June, and short rains from October to December. The annual rainfall range is 400–1680 mm per year. The soils are generally sandy loam, while some parts are richer in claye and fertile. On the other hand, Kitui, Kajiado, Makueni and Machakos counties are semi-arid areas, which are generally hot and dry. The rainfall distribution is bimodal: long rains are expected between March and May, while short rains are usually expected between October and December. The annual average rainfall ranges between 500 and

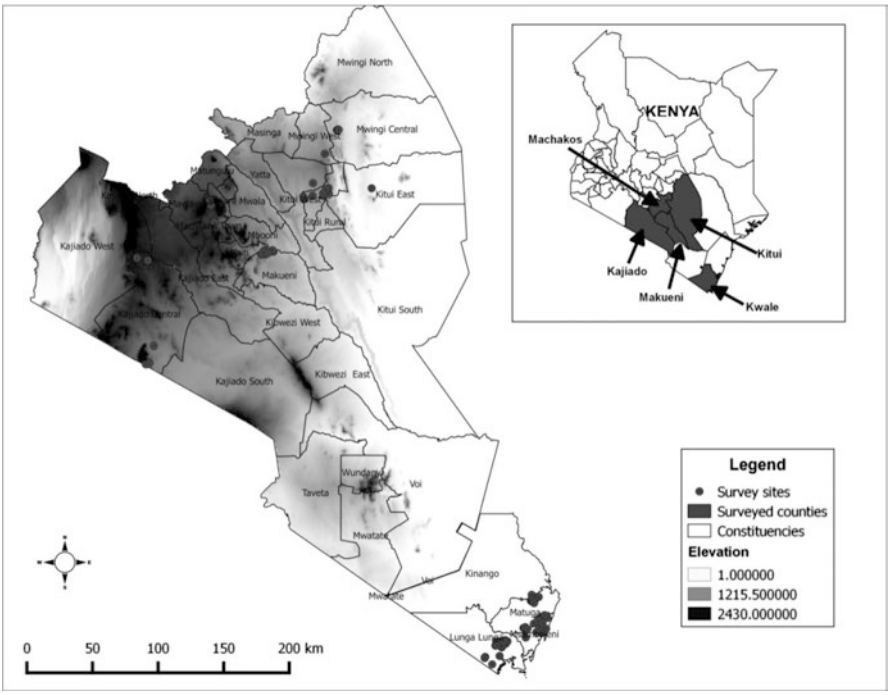

Fig. 10.1 Locations of the survey sites in selected counties of Kenya for recovery of *Cotesia vestalis*

1300 mm, and soils are generally sandy with low fertility. Cabbage and kales are the main cruciferous vegetables produced for subsistence use, and for commercial production to a smaller extent.

10.2.2 Sampling for Plutella xylostella *and Parasitoids on Cultivated Crucifers*

Two surveys were carried out in Kwale County's during the rainy (June 2015, August 2015) and dry season (January 2016). Two surveys were also carried out in the eastern region, during the rainy (November 2015) and dry season (March 2016). The farms sampled were those on which cabbage is produced, and were at least 1 km apart. Ten (10) farms per region in each county were surveyed during each visit, and the same fields were visited on every survey. In cases where a field was not under crucifer crop production, it was replaced by another farm nearby. However, fewer farms in some regions of Kwale County were surveyed, due to a huge decline

in the number of farms on crucifer production because of dry weather conditions in January 2016, since farmers without access to irrigation could not produce vegetables during that period. Ten (10) randomly selected cabbage plants per farm were sampled and thoroughly inspected for *P. xylostella* larvae, pupa and parasitoid cocoons on the leaves. The samples collected were put into plastic containers fitted with a cloth mesh at the top to allow for ventilation, which were lined with a paper towel at the bottom to prevent condensation. The containers were labelled with the field numbers. The types and numbers of samples collected, other pests found on the crops, field numbers, host plants, and collection dates were recorded and the samples taken to the laboratories. The farms sampled were geo-referenced using a Global Positioning System (GPS, model Magellan® Triton™ 400).

Sample processing was conducted at the International Centre of Insect Physiology and Ecology (ICIPE), Duduville Campus, Kasarani, Nairobi. The samples collected were kept at room temperature (23 ± 2 °C), 50–70% relative humidity, and at a photoperiod of 12:12 h (Light: Darkness). Fresh cabbage leaves were provided daily to the *P. xylostella* larvae. The emergence of either *P. xylostella* or parasitoid adults was checked daily until no further emergence was observed. All the *P. xylostella* and different parasitoid samples that emerged were sexed, identified, and recorded. Identification and sexing of the parasitoids collected was carried out using a Leica EZ4D microscope at a magnification of 10× to enable viewing of the parasitoid genitalia. Moreover, the presence or absence of detailed features that identify *C. vestalis* were used, based on IntKey (Dallwitz et al. 1999). Parasitoids from each county were reared in Perspex cages measuring 20 × 20 × 20 cm (external dimensions) in the laboratory to obtain a larger number for molecular work. Rearing of *C. vestalis* was carried out by exposing them to 2nd and 3rd instar larvae of *P. xylostella* on cabbages for 24 h, removal of the plants and putting the larvae into lunch boxes (11.5 cm diam., 6 cm high), and daily feeding on cabbages until emergence. The *C. vestalis* were fed daily on honey droplets on a paper strip.

10.2.3 Survey on Pest Management Practices

Household interviews were conducted to collect information on cultural practices on cabbage farming using structured questionnaires (See Appendix A below). The information collected included: the type of cruciferous crop cultivated, variety grown, planting time, harvesting intervals, intercropped farms, soil type, type of manure and fertiliser applied and their application period, irrigation type and rainfed farms, the pest management strategies applied by farmers, reason for pesticide application, frequency of their application, last application dates, and the change in the frequency on use of the management strategies over time, in addition to the farmers' knowledge on use of natural enemies in management of *P. xylostella*.

10.2.4 Molecular Identification of Cotesia vestalis

The *Cotesia* spp. samples were used for molecular work. Additional samples similar to those released in East Africa (South African biotype) were obtained from the Department of Agricultural Research Services (DARS) in Malawi. All the samples were preserved in 95% ethanol and stored at −20 °C while awaiting genomic DNA extraction. Prior to molecular characterisation, adults were morphologically identified according to the various features used for the identification of *Cotesia* species of economic importance, as described by IntKey (Dallwitz et al. 1999). Lateral, ventral and dorsal images of the sample specimens were taken with a Leica EZ4D microscope, using LAS EZ software ver. 3.0.0. This was followed by surface sterilisation in 3% sodium hypochlorite, rinsing thrice in distilled water, and placing the samples in labelled 1.5 mL tubes. DNA extraction was performed using ISOLATE II Genomic DNA Kit (Bioline, UK) following the manufacturer's protocol. Amplification of the target COI gene was done using universal barcode primers; LCO-1490 (5′ GGTCAACAAATCATAAAGATATTG G 3′) and HCO-2198 (5′ TAAACTTCAGGGTGACCAAAAAATA 3′) (Folmer et al. 1994). The PCR was carried out in a total reaction volume of 20 µL containing 5× My *Taq* Reaction Buffer (5 mM dNTPs, 15 mM $MgCl_2$, stabilisers and enhancers), 10 µmole of each primer, 0.5 mM $MgCl_2$, 0.25 µL My *Taq* DNA polymerase (Bioline, UK) and 15 ng/µL of DNA template. The reaction was set up in the Nexus Mastercycler gradient (Eppendorf). The following cycling conditions were used: initial denaturation for 2 min at 95 °C, followed by 40 cycles of 30 s at 95 °C, 40 s annealing at 50.6 °C, and 1 min at 72 °C, then a final elongation step of 10 min at 72 °C.

The amplified PCR products were resolved through a 1.2% agarose gel stained with 10 mg/mL ethidium bromide. DNA bands on the gel were analysed and documented using a KETA GL imaging system trans-illuminator (Wealtec Corp). Successively amplified products were excised and purified using Isolate II PCR and Gel Kit (Bioline, UK) following the manufacturer's instructions. The purified samples were shipped to Macrogen Inc. Europe Laboratory, the Netherlands, for bidirectional sequencing.

10.2.5 Sequence Analysis

The chromatograms were examined with Chromas ver. 2.5.1 (Hall 1999), and when ambiguous sites were found, they were corrected to produce two alternative sequences, corresponding to high and low peaks, respectively, and a sequence was created. The consensus sequences generated from both strands were compared to those available at GenBank, using the built-in BLAST utility (http://blast.ncbi.nlm.nih.gov/Blast.cgi). Multiple alignment was done with ClustalX version 2.1 (Thompson et al. 1997). jModeltest 2.1.7 program was used to provide an estimate

of the best-fit model selection (Darriba et al. 2012). The sequences were then run in RAxML v8.2.0 (Stamatakis 2014) to get the best-scoring Maximum likelihood tree, with a bootstrap test of 1000 replications. The tree was constructed using Fig Tree v. 1.4 (Rambaut 2012).

The sequences obtained were submitted to the Barcode of Life Database (BOLD) and deposited in GenBank (Accession numbers: ABZ6416 and ADL5635). DNA voucher specimens were stored at ICIPE Molecular Pathology Lab., Arthropod Pathology Unit (APU).

10.2.6 Data Analysis

The parasitism rate of *P. xylostella* by the solitary parasitoid, *C. vestalis* was calculated as follows:

$$\%\,parasitism = \frac{\text{Sum of } \textit{Cotesia vestalis}}{\text{total adults}\left(\text{parasitoids} + \text{DBM}\right)} * 100$$

Calculations of parasitism excluded samples that died before emergence (Nofemela and Kfir 2005). The data on the *P. xylostella* and *C. vestalis* densities were checked for normality using the Shapiro-Wilk test, and log-transformed to correct for overdispersion, while proportional data were arcsine transformed. The data for each survey period were then subjected to Analysis of Variance (ANOVA). Means were separated using Tukey's Honest Significant Difference (HSD) test at 5% level of significance. R Studio software (ver. 2.15.1) was used for all analyses (http://www.rstudio.org/).

10.3 Results

10.3.1 Incidence of Plutella xylostella *and Parasitism Rates by* Cotesia vestalis *in Kwale County in the Coastal Region*

The P. *xylostella* densities for the June 2015 survey (12.22 ± 1.88 per farm, mean ± SD) was over ten times the densities in Jan 2016 (1.18 ± 0.67 per farm) (Fig. 10.2). On the other hand, the *C. vestalis* densities for Jan 2016 (0.65 ± 0.37 per farm) were more than twice the densities in June 2015 (0.31 ± 0.11 per farm) (Fig. 10.2). Parasitism rates by *C. vestalis* in Jan 2016 (7.35 ± 3.523%) were over twice the parasitism in June (2.83 ± 1.02%) (data not shown). The comparison of the *P. xylostella* densities between the two survey periods showed significant differences ($F = 43.11$; $df = 1,74$; $P < 0.05$), while the *C. vestalis* densities ($F = 0.982$; $df = 1,74$; $P = 0.325$) were not significantly different.

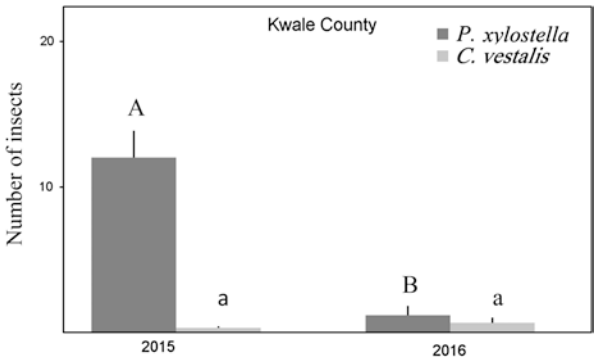

Fig. 10.2 The number of *Plutella xylostella* per farm and its parasitoid *C. vestalis* recorded on cabbage in Kwale County in 2015 and 2016

10.3.2 Incidence of Plutella xylostella *and Parasitism Rates by* Cotesia vestalis *in the Eastern Region*

During the November 2015 survey, the density of *P. xylostella* in Kitui was fivefold (1 ± 0.39 per farm), compared with that recorded in Makueni (0.2 ± 0.13 per farm). In the same survey, the densities of *C. vestalis* in Makueni were 2.25-fold (1.8 ± 0.80 per farm) higher than in Kitui (0.8 ± 0.33a per farm) (Fig. 10.3). There were no significant differences observed between *P. xylostella* (F = 3.358; df = 1, 18; p = 0.0835) and *C. vestalis* densities (F = 0.548; df = 1,18; p = 0.469) in the two counties. The parasitism rates in Makueni (37.86 ± 1 3.88%) were almost twice those in Kitui (20.9 ± 0.92%).

In March 2016, the *P. xylostella* density in Kitui (2.30 ± 1.76 per farm) was over three times higher than in Makueni (0.6 ± 0.50 per farm) (Fig. 10.3 below). 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. The parasitism rate by C. *vestalis* in Kitui (32.19 ± 12.64%) was more than twice the rate recorded in Kitui (15 ± 10.67%). There was no significant difference between *P. xylostella* (F = 0.228; df = 1,38; p = 0.636) and *C. vestalis* (F = 2.896; df = 1,38; p = 0.0969) densities during the 2015 and 2016 surveys, in the Eastern Region.

10.3.3 Survey on Pest Management Practices

A baseline survey of pest management practices indicated that 89.25% of the farm households interviewed at the coastal region were using insecticides. The farmers used a total of 18 different active ingredients of synthetic insecticides under differ-

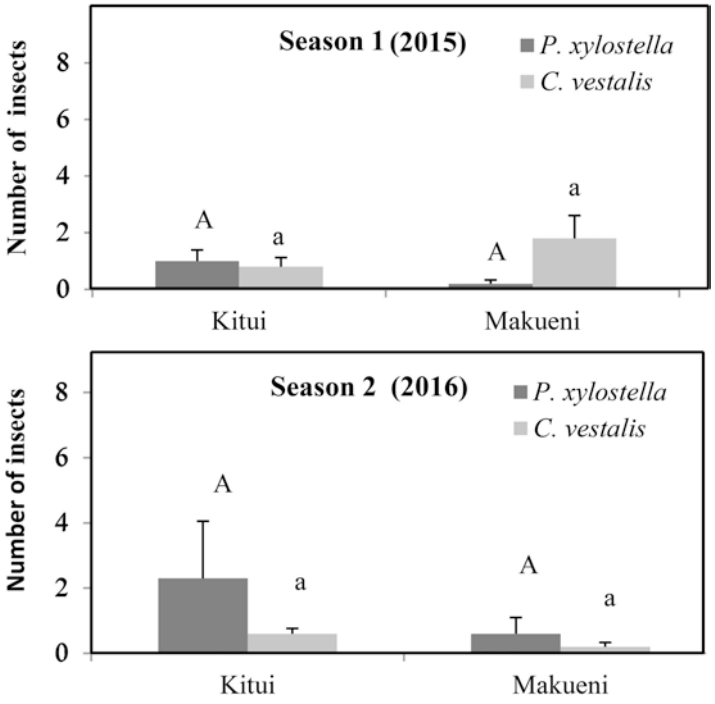

Fig. 10.3 The densities of *P. xylostella* and *C. vestalis* for the different survey periods in Eastern region counties of Kenya

ent application regimes. The majority of the insecticides used in the Coastal region were pyrethroids, constituting 75.47% of the total pesticides, followed by neonicotinoids (8.49%) and carbamates (5.66%) (Table 10.1).

Based on the household data collected in Kitui, Makueni and Machakos counties, 78.57% of the farmers were spraying their vegetables with insecticides. The most commonly used insecticides were pyrethroids (46.16%), with a usage threefold higher than that of organophosphates (15.55%). Additionally, only 5.5% of the products used were micro-organism derived, while 1.1% were plant-derived products (Table 10.1).

The proportion of farmers that practised intercropping in the Coastal region ranged between 9.09% and 51.16%, compared to 0–2.5% in the Eastern region, depending on the growing period. On the other hand, the proportion of farms under irrigation in the coastal region was 78.75%, whereas it was 73.12% in the Eastern region (Table 10.2).

The common pests, other than *P. xylostella,* based on pest densities and frequency of occurrence were cabbage aphids, bollworms, whiteflies, thrips, cabbage loopers, cutworms and leafminers. Bollworms were the most abundant in the Coastal region in both 2015 (65.05%) and 2016 (41.18%). In the Eastern region,

Table 10.1 Use of different insecticide active ingredients in crucifer crops at the Coastal and Eastern semi-arid region of Kenya

Active ingredient	Substance group	Usage frequency (%)	
		Coast	Eastern Region
Lambda-cyhalothrin	Pyrethroid	41.51	16.67
Alphacypermethrin	Pyrethroid	24.53	13.33
Deltamethrin	Pyrethroid	3.77	1.11
Beta-Cyfluthrin	Pyrethroid	3.77	3.33
Cypermethrin	Pyrethroid	1.89	12.22
Mancozeb	Carbamate	2.83	1.11
Carbosulfan	Carbamate	0.94	0
Methomyl	Carbamate	0.94	5.56
Propamocarb	Carbamate	0.94	2.22
Chlorpyrifos	Organophosphate	0	12.22
Dimethoate	Organophosphate	0	1.11
Diazinon	Organophosphate	0	2.22
Thiamethoxam	Neonicotinoid	3.77	4.44
Imidacloprid	Neonicotinoid	2.83	3.33
Acetamiprid	Neonicotinoid	1.89	2.22
Hydrochloride	Neonicotinoids	0	2.22
Lufenuron	Benzoylurea	3.77	3.33
Metalaxyl	Phenylamide	2.83	0
Fluopicolide	Benzamide	0.94	0
Carbendazim	Benzimidazole	0.94	0
Pyridaben	Pyridazinone	0.94	0
Sulphur	Fluoride	0.94	0
Hexaconazole	Triazole	0	2.22
Flubendiamide	Benzenedicarboxamide	0	2.22
Azoxystrobin	Strobilurin	0	1.11
Diafenthiuron	Thiourea	0	1.11
Emamectin benzoate	Micro-organism derived	0	1.11
Abamectin	Micro-organism derived	0	3.33
Azadirachtin	Plant derived	0	1.11
Bt	Microbial pesticide	0	1.11

Table 10.2 Farming practices adopted by farmers in the regions surveyed

Farming practices						
	Coast region			Eastern region		
Survey period	2015	2016	Mean	2015	2016	Mean
Intercropping (%)	30.125	29.41	29.76	2.5	0	1.25
Irrigation (%)	65.855	88.24	77.04	50	97.5	73.75

Table 10.3 Ranking of other pests attacking crucifers in the surveyed regions

	Coastal region		Eastern region	
Pests	2015	2016	2015	2016
Bollworms	65.05%	41.18%	6.67%	15%
Whiteflies	2.17%	23.53%	26.67%	75.0%
Aphids	12.58%	–	43.33%	67.5%
Cabbage loopers	3.03%	11.76%	3.33%	5%
Thrips	–	–	6.67%	35%
Leafminers	–	–	6.67%	5%
Cutworms	–	–	3.33%	–
Other caterpillars	1.515	5.88%	6.67%	35%

Fig. 10.4 1% Agarose gel electrophoretic image of the *C. vestalis* PCR products amplified using the HCO/LCO primers. Lanes and samples are: 1–10 (Kajiado), 10–19 (Kitui), 20–28 (Kwale), 29–38 (Makueni), 39–48 (Malawi), L = 100 bp DNA ladder (Bioline)

cabbage aphids (43.33%) were ranked as the most common pest in 2015, while whiteflies (75.0%) were the most abundant in 2016 (Table 10.3). In regard to farmers' knowledge on parasitoids, only 41.8% of the farmers in the Coastal region and 30% in Eastern region were well-informed about the use of parasitoids for management of *P. xylostella.*

10.3.4 Quantification of DNA and PCR Amplification

The purity of the DNA, based on the ratio of absorbance at $260/280_{nm}$ for all samples, ranged from 1.92 to 2.72, while the nucleic acid concentration of the samples ranged between 30.8 and 146.3 ng/μL. A total of 48 samples (from Kitui, Kwale, Makueni, Kajiado and Malawi) were amplified by PCR, with a band size of approximately 700 base pairs (Fig. 10.4).

10.3.5 Bioinformatics Analysis

BLAST results for the 48 samples from Kajiado, Kitui, Kwale, Makueni and Malawi indicated the complete mitochondrion genome as best hit (E-Value 0.0), with ≥98% similarity to *Cotesia vestalis* (Genbank accession: FJ154897). The only exception was Kw7 from Kwale that recorded the cytochrome oxidase subunit 1 (COI) gene as best hit (E-value 0.0), with 98% similarity to a *Cotesia* spp. (Genbank accession: HM430398). All the COI-aligned sequences showed a high degree of conserved residues among the 48 samples. One sample from each group was used to represent samples from the five regions (Fig. 10.5).

10.3.6 Phylogenetic Analysis

The *C. vestalis* sequences grouped into two clusters. The first one had only one sample (Kw7) collected from Kajiado County, which corresponded to *Cotesia* spp. The second cluster included the remaining samples, which corresponded to *Cotesia vestalis*. The second cluster was further separated into several groups. The cluster with the highest bootstrap value (99%) had two samples (Mw11 and Mw23) from Malawi. Most of the samples were supported by low bootstrap values.

Fig. 10.5 Multiple sequence alignment of *C. vestalis* sequences produced by ClustalX. Conserved regions are marked with asterisks above the sequences. The sequences were sampled from five regions denoted as follows: *Kj* = Kajiado, *Kt* = Kitui, *Kw* = Kwale, *Mw* = Malawi, *Mk* = Makueni (see Table 10.4 for samples details)

Table 10.4 Details of the sequenced samples and their corresponding Genbank accession numbers

Species name	Sample ID	Location	Country	Genbank Accession No.
Cotesia vestalis	Kj6-Kj10, Kj21, Kj22, Kj24, Kj25	Kajiado	Kenya	ABZ6416
Cotesia vestalis	Kt6-Kt10, Kt26-Kt30	Kitui	Kenya	ABZ6416
Cotesia vestalis	Kw8-Kw10, Kw22-Kw25	Kwale	Kenya	ABZ6416
Cotesia vestalis	Kw7			ADL5635
Cotesia vestalis	Mk1-Mk10	Makueni	Kenya	ABZ6416
Cotesia vestalis	Mw11-Mw15, Mw21-Mw25	Malawi	Malawi	ABZ6416

Table 10.5 Estimates of evolutionary divergence over sequence pairs between groups (generated by Mega 6)

Sample[a]	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	–

[a]*Kt* Kitui, *Mw* Malawi, *Kj* Kajiado, *Mk* Makueni, *Kw* Kwale

10.3.7 Genetic Distances and Distance Summary

The divergent distances between the five groups of sequences were estimated by pairwise genetic distances estimated from COI sequences, based on a Kimura-two-parameter algorithm (Tamura et al. 2013). The highest nucleotide distance was between Kw and Mk, with a value of 0.018 (Table 10.5). The distance summary, shown in bold, indicated a within-species mean distance of 23.51%, with maximum distance as 75%, and a minimum distance as 0%. The alignment option used was the Kimura-2-parameter.

10.4 Discussion

The study of *P. xylostella* dynamics in the semi-arid and coastal areas of Kenya showed much of variability with the planting period in both coastal and eastern regions. Despite several releases, *C. vestalis* parasitism rates in the coastal region and eastern regions were below 14% and 38%, respectively. Misuse of pesticides could be one of the factors underlying the poor establishment of *C. vestalis* in both regions. Moreover, farmers continue spraying on a calendar basis, using one or more broad-spectrum synthetic insecticides either weekly or fortnightly, not only to manage *P. xylostella*, but also other pests including aphids, whiteflies and bollworms. There are reports that the fields with minimum pesticide usage or the

organically managed ones have higher densities of parasitoids than those that are frequently sprayed (Ayalew et al. 2002; Kfir 2004; Rowell et al. 2005). Moreover, some products used by farmers, such as spinosad, have detrimental effects on *C. vestalis* (Oliveira et al. 2011), thus slowing down their increase and reducing the parasitoid efficacy. Overreliance on rainfall for vegetable production necessitated broken and discontinuous productions, and could have been a factor behind the poor establishment of *C. vestalis* in the release regions. According to the farmers, their frequency of pesticide usage was on the rise, compared with the previous years. They attributed this to the reduced efficacy of the pesticides that they were using. Several studies have shown that *P. xylostella* developed resistance to abamectin, lufenuron, methomyl and emamectin (Pu et al. 2010; Santos et al. 2011), yet these pesticides are still widely used by farmers.

In the Philippines lowlands, an IPM technology which involved augmentative parasitoid releases and judicious spraying using selective insecticides with strong support from extension efforts, resulted in the successful establishment of *C. vestalis*, reduced *P. xylostella* densities, increased yields and reduced production costs (Morallo-Rejesus et al. 1997; Rowell et al. 2005; Jankowski et al. 2007). The same strategy can be adopted in Kenya to increase its efficacy of as a biological control agent. Moreover, stronger links between research and extension would be very helpful in informing the farmers on the use of safe insect management measures against pests and would go a long way in creating farmer awareness of parasitoids. Furthermore, the adapting of IPM programmes that help in management of other common pests such as aphids, bollworms and whiteflies would greatly reduce spraying, since farmers currently continue spraying, even in absence of *P. xylostella*, and this eventually affect its parasitoids. In a bid to achieve better pest management within the existing cropping system, smallholder farmers need to have access to information on effective IPM technologies through participatory technology transfer approaches. Molecular analysis of the samples collected from Kajiado, Kitui, Kwale, Makueni and Malawi showed that the specimens from the surveyed areas had ≥98% similarity to Cotesia vestalis. The DNA quantification and amplification confirmed that the target gene was present in all samples. Furthermore, bioinformatics showed that all samples had the complete mitochondrion genome as best hit, except one. Some of the samples from different regions clustered together, an indicator that they shared ancestry, while other samples from the same location fell under different clusters, which is an indicator of the occurrence of divergence among the species, over time. The results suggest that there is very close similarity in all the samples, regardless of where they were collected from. This shows that the South African biotype of C. vestalis that was released in the previous years was active in the surveyed areas. The COI gene is highly conserved, as revealed by multiple sequence alignment, and it can be used as a viable marker in confirming Cotesia establishment upon release in the study sites. Similar studies have reported successful use of COI gene in determining the origin of Cotesia flavipes in Ethiopia (Assefa et al. 2008).

In conclusion, this study and the molecular results conclusively show that *C. vestalis* has established in and around the different release sites in Kenya. However, its contribution in management of *P. xylostella* is still low, and this could mainly be attributed to the excessive use of broad-spectrum insecticides. Moreover, the COI

gene greatly improved the identification of the *C. vestalis* without much dependence on morphological characters.

Appendix

Farmer questionnaire used for *Cotesia vestalis* post-release Surveys (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/intercrop_____
Farmer's knowledge on natural enemies
_____
_____
_____

References

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. *Journal of Economic Entomology, 101*, 686–691.

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

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. *The International Journal of Pest Management, 52*, 303–315.

Bjorksten, T. A., Robinson, M., & La Salle, J. (2005). Species composition and population dynamics of leafmining flies and their parasitoids in Victoria. *Australian Journal of Entomology, 44*, 186–191.

Cooper, J. (2009). *Pest management in horticultural crops; an integrated approach to vegetable pest management with the aim of reducing reliance on pesticides in Kenya*. Final technical report.

Dallwitz, M. J., Paine, T. A., & Zurcher, E. J. (1999). User's guide to the DELTA Editor. https://www.delta-intkey.com/

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

de Oliveira, A. C., de Siqueira, H. Á. A., de Oliveira, J. V., da Silva, J. E., & Michereff, F. M. (2011). Resistance of Brazilian diamondback moth populations to insecticides. *Science in Agriculture, 68*, 154–159.

FAOSTAT. (2014). Food and agriculture organisation, United Nations. http://www.fao.org/faostat.

Folmer, O., Black, M., Hoeh, W., Lutz, R., & Vrijenhoek, R. (1994). DNA primers for amplification of mitochondrial cytochrome C oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology, 3*, 294–299.

Furlong, M. J., Wright, D. J., & Dosdall, L. M. (2013). Diamondback moth ecology and management: Problems, progress, and prospects. *Annual Review of Entomology, 58*, 517–541.

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 Protection, 27*, 906–914.

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 Protection, 29*, 68–79.

Hall, T. A. (1999). BioEdit: A user-friendly biological sequence alignment editor and analysis program for windows 95/98/NT. In *Nucleic acids symposium series* (pp. 95–98).

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*, 190–196.

Jankowski, A., Mithöfer, D., Löhr, B., & Weibel, H. (2007). Economics of biological control in cabbage production in two countries in East Africa. In *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.

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 Bioscience, 46*, 3103–3112.

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

Kfir, R. (2004). Effect of parasitoid elimination on populations of diamondback moth in cabbage. In *The management of diamondback moth and other crucifer pests: Proceedings of the 4th international workshop* (pp. 197–205). Gosford: The Regional Institute Ltd.

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

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. *Bulletin of Entomological Research, 97*, 337–350.

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 Protection, 24*, 981–989.

Macharia, I., Mithöfer, D., Waibel, H., et al. (2013, September 22–25). Health effects of pesticide use among vegetable farmer in Kenya. In *AAAE fourth international conference*, Hammamet, Tunisia. African Association of Agricultural Economists (AAAE).

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. In *The Management of Diamondback Moth and other crucifer pests*. Proceedings of the Third International Workshop (pp. 267–275).

Mosiane, S. M., Kfir, R., & Villet, M. H. (2003). Seasonal phenology of the diamondback moth, *Plutella xylostella* (L.),(Lepidoptera: Plutellidae), and its parasitoids on canola, *Brassica napus* (L.), in Gauteng province, South Africa. *African Entomology: Journal of the Entomological Society of Southern Africa, 11*, 277–285.

Munthali, D. C. (2009). Evaluation of cabbage varieties for resistance to the cabbage aphid. *African Entomology: Journal of the Entomological Society of Southern Africa, 17*, 1–7.

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. *African Entomology: Journal of the Entomological Society of Southern Africa, 13*, 71–83.

Nyambo, B., & Pekke, A. (1995). *Brassica planning workshop for East and Southern Africa region, Lilongwe-Malawi*. GTZ-IPM Horticulture Nairobi Kenya.

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. In *Proceedings of the XXII international congress of entomology*, pp. 6–12.

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

Rambaut, A. (2012). FigTree v1. 4 2006–2012. Institute of Evolutionary Biology, The University of Edinburgh, Edinburgh, UK.

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

Sandur, S. (2004). *Implications of Diamondback moth control for Indian cabbage farmers*. A report produced for the CIMBAA. http://www.cimbaa.org/downloads/cimbaa_indiandbm_reportfinal-%20apr05.pdf. Last Accessed 17 september 2008.

Santos, V. C., de Siqueira, H. A. A., da Silva, J. E., & de Farias, M. (2011). Insecticide resistance in populations of the diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), from the state of Pernambuco, Brazil. *Neotropical Entomology, 40*, 264–270.

Shi, Z., Guo, S., Lin, W., & Liu, S. (2004). 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 Management Science, 60*, 1213–1219.

Smith, T.J. (2002). *The diamondback moth, Plutella xylostella (L.), (Lepidoptera: Plutellidae) and its biological control in the eastern cape province, South Africa.*

Smith, T.J., & Villet, M.H. (2001, November 26–29). 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, p. 249.

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

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

Talekar, N. S., & Yang, J. C. (1991). Characteristics of parasitism of diamondback moth by two larval parasites [*Apanteles plutellae*, *Diadegma eucerophaga*]. *Entomophaga, 36*, 95.

Tamura, K., Stecher, G., Peterson, D., Filipski, A., & Kumar, S. (2013). MEGA6: Molecular evolutionary genetics analysis version 6.0. *Molecular Biology and Evolution, 30*, 2725–2729.

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 Research, 25*, 4876–4882.

Verkerk, R. H. J., & Wright, D. J. (1997). Field-based studies with the diamondback moth tritrophic system in Cameron highlands of Malaysia: Implications for pest management. *International Journal of Pest Management, 43*, 27–33.

Vrijenhoek, R. (1994). DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology, 3*, 294–299.

Waladde, S. M., Leutle, M. F., & Villet, M. H. (2001). Parasitism of *Plutella xylostella* (Lepidoptera, Plutellidae): Field and laboratory observations. *South African Journal of Plant and Soil, 18*, 32–37.

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? *Journal of Economic Entomology, 105*, 1115–1129.