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Biological Impact Assessment of an Introduced Exotic Parasitoid *Diadegma semiclausum* (Hellen) on *Plutella xylostella* (Linnaeus) and Its Local Natural Enemies in Kenya.

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DECLARATION BY CANDIDATE.

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

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DEDICATION.

To all my family members and friends.

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LIST OF ABBREVIATIONS

AVRDC Asian Vegetable Research and Development Centre

DBM Diamondback moth

DNA Deoxyribonucleic acid

DRIP Dissertation Research Internship Program

GPS Geographic Position System

ICIPE International Centre of Insect Physiology and Ecology

ITS Internal transcribed spacer

JKUAT Jomo Kenyatta University of Agriculture and Technology

PCR Polymerase Chain Reaction

RFLP Restriction Fragment Length Polymorphism

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ABSTRACT.

An exotic parasitoid *Diadegma semiclausum* (Hellen) was imported to Kenya from Taiwan as a biological control agent of *Plutella xylostella* (Linnaeus) in 2001. The performance of this parasitoid elsewhere in the world has been encouraging especially in Europe, America and Asia. It has been known to significantly contribute to the mortality and thus management of the Diamondback moth. However surveys done in Kenya reveal that parasitism by a local parasitoid of the same genus, *Diadegma molliplla* (Holmgren), account for less than 15%.

Cabbage was grown during the two cabbage growing seasons at Wundanyi Division, Taita Taveta District and Limuru Division, Kiambu District on ¼ an hectare of land. The treatments consisted of every fortnight application of a biological insecticide (Thuricide®), a synthetic pesticide (Karate®), a botanical insecticide (Neemroc®), and control plots where only water was applied. Sampling was done before pesticide application through random scouting of ten plants in every plot and 3 to 5 L4 of prepupal stage sampled for further monitoring in the laboratory. An exclusion experiment was carried out in both sites and seasons to assess the impact of *D. semiclausum* on the survival/mortality of *P. xylostella* and a field simulated screen house study also conducted in ICIPE to assess the effect of parasitoid density on DBM survival.

Introduction of D. *semiclausum* in Kenya has resulted in rise of parasitism rates up to 60%, accompanied with a concomitant reduction in parasitism by local parasitoids. *D. semiclausum* now contributes almost 100% of total parasitism as an indication of

competitive displacement of the local parasitoids. The introduced parasitoid established and contributed to the mortality of *P. xylostella* faster at Wundanyi Division, Taita Taveta District, than it did at Limuru Division, Kiambu District due to locality and climatic variations between sites.

The killing power of *D. semiclausum* was seen to be much higher than can be measured by parasitism rates only. As the parasitoid numbers increased in the field so did the unexplained mortality, signifying that more DBM larvae die due to parasitoid disturbance than can be measured by those utilized by the parasitoid for breeding in the field. The survival of *P. xylostella* was reduced by more than 60% within the first year of release of *D. semiclausum* as an indication that this form of biological control will drastically reduce DBM numbers in the field once established.

Polymerase Chain Reaction - Restriction Fragment Length Polymorphism (PCR-RFLP) on the sampled materials (recovered parasitoids), using the already established molecular markers, indicated a clear molecular distinction between *D. molliplla* and *D. semiclausum* with no indication of any interbreeding *in situ*.

Karate®, Lambda-cyhalothrin, a commonly used pyrethroid in the management of *P. xylostella* in Kenya, had a negative effect on the establishment and performance of the introduced parasitoid whereas Thuricide®, a *B.t* extract, seemed to be more compatible with this form of classical biological control, resulting in significantly higher levels of parasitism in both sites and seasons.

DBM populations in Karate - treated plots were significantly higher than in other treatment probably as an indication of apparent build-up in resistance to this commonly used pesticide. This could also be as a result of the lethal effects it has on the DBM natural enemy complex in the field. As a result Karate treated plots had significantly higher cabbage-damage scores based on the scale of 1 to 5. Thuricide treated plots, on the other hand, had significantly lower DBM numbers and cabbage-damage index (P=0.0001, df=1110).

Key words: Parasitoid, *Diadegma semiclausum, Diadegma molliplla, Plutella xylostella* and PCR- RFLP

CHAPTER 1

1.0 GENERAL INTRODUCTION AND LITERATURE REVIEW

1.1 Introduction

The main cruciferous species grown in East Africa are rape (*Brassica carinata* L. and *B. napus* L.), Kales (*B. oleracea* L.var.*acephala*), Chinese cabbage (*B. chinensis* L.) and cauliflower (*B. oleracea* L.var. *botrytis*). These vegetables are grown both for home consumption and domestic market. They are an important source of income for small-scale farmers and they are eaten as accompaniment to a basic starchy dish such a cassava or maize, often in lieu of meat (Seif and Löhr, 1998).

Diamondback moth (DBM), (*Plutella xylostella* (L.)) has been described as the most important and most difficult vegetable pest to control worldwide (Lim, 1986). It has become the most destructive pest of crucifer plants throughout the world with annual cost of management being estimated at one billion US Dollars (Talekar, 1992; Adane - Kassa and Abate, 1995; Nyambo and Pekke, 1995; Oduor *et al.*, 1996; Seif and Löhr, 1998).

It has been reported that DBM easily develops resistance to a wide range of synthetic insecticides, including biopesticides (Lim, 1986; Shelton *et al.*, 1996; Verkerk and Wright, 1997; Zheng *et al.*, 1997). For this reason many countries such as New Zealand, Indonesia, Taiwan, Malaysia and Philippines have developed and implemented bio-control based IPM approaches which have proved successful (Ooi, 1990; Talekar *et al.*, 1990; Poelking, 1992; Biever, 1996; Eusebio and Morallo-Rejesus, 1996; Iga, 1997).

High damage levels by DBM on cabbage crop have been observed in Kenya even though it had been sprayed with pyrethroids (Permethrin 10 EC and Dimethoate 40 EC) regularly. Local farmers have perceived a gradual decrease in the effectiveness of the chemical pesticides indicating an apparent build- up of resistance in local populations of the moth to synthetic pesticides applied regularly in the past (Kibata, 1996). This observation underscores the need to develop a biorational system of improved DBM management in Kenya.

1.2 Use of Pesticides.

A wide variety of chemical insecticides including organophosphates, carbamates, synthetic pyrethroids, botanical and biological pesticides have been used to control the DBM, with resistance being observed to almost all pesticides including the microbial pesticide such as *Bacillus thuringiensis* Berl (Kirsch and Schmutterer, 1988; Talekar *et al.*, 1990; Hama, 1992; Shelton and Wyman, 1992).

Apart from pesticide resistance development by DBM, pesticides have other problems including creation of secondary pests, resurgence of primary pests and environmental and human health hazards. One of the key goals of any integrated pest management program is to minimize the use of pesticides, thereby preserve the naturally occurring biological control agents in addition to reducing the cost of production (Van Driesche, 1983).

Neem, *Azadractin indica* A. Juss insecticide formulation products have been screened and found to be effective in the control of DBM (Kirsch, 1987; Schmutterer, 1992;

Javaid *et al.*, 2000). It is believed that their complex chemistry will make it difficult for the DBM to develop resistance (Vollinger, 1995). Neem insecticides have been known to affect insect pests in a number of ways including repellence, phago-deterrence, inhibited oogenesis and disrupted development (Schmutterer, 1990, 1995). Moreover, they have not been known to have any side effects on the longevity and foraging behavior of the parasitoids (Akol *et al.*, 2002).

1.3 Biological control

Biological control can be defined as the regulation of an organism's population density by natural enemies to levels lower than would prevail in their absence (DeBach and Huffacker, 1976). Natural control agents like diseases, predators and parasitoids have been known to play a major role in suppressing DBM population growth (Lim, 1986; Talekar N. S. 1992).

Three groups of entomopathogenic agents such as viruses, fungi and bacteria have been isolated and have been known to control DBM populations *in situ*. According to Grzywacz *et al.*, (2002), fourteen genetic isolates of *P. xylostella* (L.) granuloviruses have been reported in Kenya though in small number of infected larvae. Of these fourteen isolates, only two granuloviruses have been commercialized to date, namely, *Cydia pomonella* GV. and *Adoxophyes orana* GV. (Grzywacz *et al.*, 2002). These granuloviruses are potential biopesticides for controlling DBM and are known to have no effect on other Lepidopteran pests and other beneficial natural enemies such as Syrphid and spiders in the field (Odour *et al.*, 1996; Grzywacz *et al.*, 2002; Ogutu *et al.*, 2002).

The role of predators in the mortality of Diamondback moth has not been adequately studied. Various predators have been reported to contribute to DBM mortality, including Spiders of family Linyphiidae, Theridiidae and Tetragnathidae, Syrphids, Coccinellids, Nabids, Chrysopids, Staphylinids, Wasps, and Anthocorids (Oatman and Platner, 1969). However, their contribution to the regulation of the DBM population has not been quantified and methods to study them have not been fully developed. This is partly due to the feeding behavior of most predators, which often kill and eat the entire prey.

The most important and abundant natural enemies of DBM are hymenopterous parasitoids especially from genus *Diadegma* (Ichneumonidae). The earliest parasitoid introduction against DBM was made in New Zealand where *Diadegma semiclausum* (Hellen) and *Diadromus collaris* (Gravenhorst) were introduced from England (Hardy, 1938; Thomas and Ferguson, 1989). *D. semiclausum* was introduced in Indonesia in the 1950s and because of overuse of insecticides, its beneficial effect was not realized until 1980s (Sastrosiswojo, 1987). Success was only achieved after synthetic chemical pesticides were substituted with *B. thuringiensis*. in the early 1980s (Saatrosiswoyo and Sastrodihardjo, 1986).

In Taiwan, DBM has been a serious problem since the mid 1960s (Chen and Su, 1986). *D. semiclausum* was imported from Indonesia and was reported to cause more than 70% parasitisation in highland areas (AVRDC, 1988). The species now occurs throughout the highland areas of central Taiwan and provides substantial savings in DBM control (Talekar *et al.*, 1990; Talekar, 1992). Temperatures in the highlands (15-25°C) are

suitable for its establishment whereas temperatures in the lowlands (20-30°C) are too high (Talekar, 1992). In the highlands of northern Philippines, a single release of *D. semiclausum* in 1989 at the beginning of the season resulted in 64% parasitism of DBM at harvest (Poelking, 1992).

The genus *Diadegma* comprises one of the most efficient and widespread Diamondback moth parasitoids worldwide. One species, *Diadegma mollipla* (Holmgren) has also been recorded in Kenya, Tanzania, Uganda and South Africa (Kfir, 1997; Seif and Löhr, 1998; Löhr and Kfir, 2002). Data collected in recent field surveys in Kenya, Malawi and Tanzania indicate that the parasitism rate by this species is generally below 15% (Seif and Löhr, 1998).

Much of the success in Biocontrol programs in Asia involving *D. semiclausum* are confined to the temperate highlands where crucifers are widely grown throughout the year and in these regions introduction of this larval parasitoid alone has resulted in dramatic drop in pesticide use (Ooi, 1990; Talekar *et al.*, 1990; Poelking, 1992; Biever, 1996; Eusebio and Morallo-Rejesus, 1996; Iga, 1997).

Owing to the fact that crucifer production in East Africa is predominantly confined to the cool highlands, *D. semiclausum* is therefore likely to be the best candidate for classical biological control. In October 2001, *D. semiclausum* was introduced by ICIPE from AVRDC Taiwan to Kenya. The first field release was made in July 2002 in a pilot site in

Taita Hills and after only two weeks, the first field recoveries of this parasitoid were made (Löhr and Gathu, personal comm.).

Within the East and Southern Africa regions, there is some information on the pest status on DBM (Nyambo and Pekke, 1995; Kibata, 1996; Seif and Löhr, 1998). Until recently little was known about the indigenous natural enemies' fauna in the region (Adane-Kassa and Abate, 1995; Nyambo and Pekke, 1995; Oduor *et al.*, 1996; Seif and Löhr, 1998). Surveys conducted by the ongoing biological control project have contributed much to the knowledge of indigenous natural enemies within the East African region. Among the most common local parasitoids that have been recorded include parasitic Hymenopterans such as *D. mollipla* (Hymenoptera: Ichneumonidae), a larval parasitoid, *Oomyzus sokolowskii* Holmgren (Hymenoptera: Eulophidae), *Apanteles spp.* (Hymenoptera: Braconidae), *Itoplectis spp.* (Hymenoptera: Ichneumonidae), which are larval-pupal parasitoids, and *Brachymeria spp.* (Hymenoptera: Chalcididae) which is a pupal parasitoid (Kibata, 1996; Oduor *et al.*, 1996; Ayalew *et al.*, 2002; Löhr and Kfir, 2002).

1.4 Biology of *Diadegma semiclausum*

Diadegma semiclausum is a solitary koinobiont endoparasitoid of DBM, black in color and 5-7 mm long. Females live up to 37 days when fed on 10% sugar solution and 73 days when fed on dilute Honey and can lay eggs for a period of 28 days after emergence. Males can survive for a period of 40 days when fed on either sugar solution or dilute honey (Ooi, 1980; Yang *et al.*, 1993).

DBM has four larval stages and all of them are attacked by *D. semiclausum* with preference to the second and third larval instars (Velasco, 1982; Talekar and Yang, 1991; Konig *et al.*, 1993). *D. semiclausum* oviposition in the first three larval instars of the DBM results in more male offspring, whereas the forth instar gives rise to more female progeny (Konig *et al.*, 1993). The hatching and development of the parasitoid eggs depends on temperature. A temperature in the range 15°C-25°C results in the sex ratio of about 1:1 and 25 ° C is considered as the optimum temperature for parasitoid development (Yang *et al.*, 1993).

After pupation of the host larva, the parasitoid larva completes eating up the host and thereafter forms its own cocoon. An adult parasitoid emerges in about five days after cocoon formation (at 25°C) and the adult parasitoid feed on flower nectar, mate and start laying eggs after emergence (Fitton and Walker, 1992).

Parasitism rates of this parasitoid are host-density-dependent and super parasitism is known to result in production of more female than male progeny (Koning *et al.*, 1993). When the parasitoid is allowed to choose between parasitized and unparasitized DBM

larvae, it is well able to distinguish between parasitized and unparasitized host larvae, showing preference for the unparasitized larvae (Yang *et al.*, 1993).

1.5 Null hypotheses

i. DBM does not cause serious losses in cabbage production in Kenya.

ii. Indigenous parasitoids are efficient in controlling DBM populations.

iii. *Diadegma semiclausum* will not become the most important parasitoid and will not contribute, to a great extent, to the control of DBM populations after establishment.

1.6 Alternative hypotheses

i. DBM cause serious losses in cabbage production in Kenya.

ii. Indigenous parasitoids are not efficient in controlling DBM populations.

iii. *Diadegma semiclausum* will become the most important parasitoid and contribute to a great extent to the control of DBM populations after establishment.

1.7 Objectives

1.7.1 General objective

- The aim of this study was to evaluate the biological impact of the release of *D. semiclausum* on DBM and its local natural enemies in Kenya.

1.7.2 Specific objectives

i. To determine the DBM population dynamics at Limuru and Wundanyi in Kenya.

ii. To assess the contribution of indigenous parasitoids to the overall mortality of DBM in field populations.

iii. To evaluate the impact of *D. semiclausum* on DBM population dynamics.

iv. To evaluate the impact of *D. semiclausum* on population dynamics of the indigenous parasitoids of DBM.

1.8 Justification

Absence of effective natural enemies, especially parasitoids, has been cited as a major cause of Diamondback moth's high pest status in most parts of the world (Lim, 1992). In 1953 Diamondback moth became the first crop pest in the world to develop resistance to DDT (Ankersmith, 1953) and in many countries this pest has become resistant to every synthetic insecticide used against it in the field (Talekar *et al.*, 1990).

In addition, Diamondback moth has been reported to be the first insect to develop resistance to bacterial insecticide *B. thuringiensis* in the field (Kirsch and Schmutterer, 1988; Hama, 1992; Shelton and Wyman, 1992). These observations underscore the need to develop a rational system of improved DBM management. *D. semiclausum* has been known to effectively control DBM elsewhere (Ooi, 1990; Talekar *et al.*, 1990; Poelking, 1992; Biever, 1996; Eusebio and Morallo-Rejesus, 1996; Iga, 1997) and it is important that its success be evaluated after its introduction in Kenya.

It is very important to assess the effectiveness of an introduced exotic parasitoid in relation to the local natural enemies in order to develop an effective biocontrol strategy. It has been observed from other findings that an imported parasitoid can competitively displace the indigenous parasitoids from its host (Bess *et al.*, 1961; Greenberg and Szyska, 1984; Hu *et al.*, 1998; Shu *et al.*, 2000) thus need to assess the impact of *D. semiclausum* not only on the population dynamics of the pest in question (DBM) but also on the indigenous parasitoids.

CHAPTER 2

2.0 POPULATION DYNAMICS OF THE DIAMONDBACK MOTH

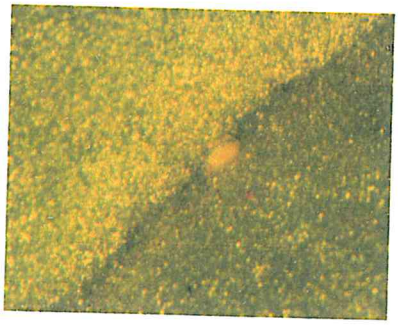
2.1 INTRODUCTION

The Diamondback moth (Lepidoptera: Yponomeutidae) is a key pest affecting vegetable production in Kenya (Kibata, 1996; Oduor *et al.*, 1996). Its life cycle takes two to three weeks, depending on temperature, availability and suitability of food (Hsu and Wang, 1971; Bhala *et al.*, 1986; Lu *et al.*, 1988; Sarnthoy *et al.*, 1989).

The moth is a small, grayish brown, with diamond-shaped markings on its wings. The moths are active at night, hiding by day in leaf litter at the base of the plants. They lay small yellow eggs in clusters or singly along the ribs and lower parts of the leaves. A female DBM can lay up to 188 eggs in a lifetime (Harcourt, 1954). The first larval instar mines into the spongy mesophyll tissues whilst subsequent stages (second, third and fourth instars) are surface feeders with characteristic "window feeding" where they consume all tissues except the upper epidermis (Harcourt, 1957).

The last stage larva (fourth instar) is a voracious feeder that causes more injury than the first three larval instars. When the fourth instar larva has completed its feeding, it spins a loose, transparent silken cocoon on either side of the leaf surface where it pupates and remains until the adult emerges. The duration required for completion of the four larval instars and the pupal period depends on temperature (Hsu and Wang, 1971; Bhala *et al.*, 1986; Lu *et al.*, 1988; Sarnthoy *et al.*, 1989).

Adult, longevity
About 2 weeks



Egg stage, 3-4 days

Overall life cycle
About 16-24 days

Pupal stage, 4-6 days

Damaging stage (larval)
7-11 days larval stage

Plate 2.1: The life cycle of Diamondback moth at 20-25°C.

Host plants on which Diamondback moth feeds include Cabbage (Brassica *oleracea* L. var. *capitata*), kale (B. *oleracea* L. var. *alboglabra*), cauliflower (B. *oleracea* L. var. *botrytis*), broccoli (*B. oleracea* L. var. *italica*), radish (*Raphanus sativas* L.), and turnip (B. *rapa* L. *pekinensis*). DBM also feeds on a number of cruciferous plants, most of which are considered weeds *viz Arabis graba* (L.) Bernh., *Armoracia lapathifolia* Gilib., *Barbarea stricta* Andrz., *Barbarea vulgaris* R. Br., *Basela alba* L., *Beta vulgaris* L. *Brassica caulorapha* Pasq., *Brassica kaber* (D. C.) L. C. Wheeler, *Brassica napobrassica* Mill., *Bunias orientalis* L., *Capsella bursa-pastoris* (L.) Medic., *Cardamine amara* L., *Cardamine cordifolia* L., *Cardiamine pratensis* L., *Cheiranthus cheiri* L., *Conringa orientalis* (L) (Ghesquire, 1939; Harcourt, 1957; Rai and Tripathi, 1985; Crafford and Chown, 1987).

The host range of DBM is limited to Crucifers that contain Mustard oils (Gupta and Thorsteinson, 1960; Nayar and Thorsteinson, 1963; Thygesen, 1968; Hillyer and Thorsteinson, 1971) such as sinigrin, sinalbin, and glucocheirolin, which act as specific feeding stimulants. Non-host plants may contain these stimulants but also contain feeding inhibitors or toxins (Gupta and Thorsteinson, 1960). Certain chemicals such as sulfur-containing glucosinolates or its metabolites act as oviposition stimulants (Gupta and Thorsteinson, 1960; Reed *et al.*, 1989). Thus, sulfur-deficient plants are not attractive to DBM for oviposition.

The numbers of DBM in the field vary from time to time depending on a number of factors including agronomic practices, seasonality and environmental conditions.

The objectives of this study were to investigate the population trends of *P. xylostella* under different commonly employed cabbage-crop protection practices against this pest and to investigate their effects on the establishment and performance of *D. semiclausum*.

2.2 MATERIALS AND METHODS

2.2.1 Site description

This study was based at Limuru (01° 08' 12'' S; 036° 37' 51'' E) and Wundanyi (03° 22' 29'' S; 038° 20' 16'' E), GPS readings, with altitudes of 1,600 m and 1,900 m above the sea level respectively. These two sites are separated by a distance of about 500 km and are renowned for vegetable production all round the year. They are main suppliers of kale and cabbage to the two main Kenyan cities *viz* Nairobi and Mombasa respectively. Limuru has a mean rainfall of 450 – 700 mm while Wundanyi has a mean rainfall of 750 – 1200 mm per year and mean temperature of 18°C for both sites.

2.2.2 Experimental design

Two preferred cabbage varieties, Gloria F1 and Copenhagen market were planted during the two crop seasons, January to April and April to July, 2003. The experimental design adopted was the Latin square design, whereby the field was divided into sixteen equal plots of size 5.4 x 3.2 m. Four-weeks old cabbage seedlings from the green house were transplanted at a spacing of 40cm within the row and 60cm between rows, resulting in a total of 63 plants per plot. A border row and column on all the four sides was maintained to act as a buffer for any chemical that might spill over from the neighboring plots, leaving 35 plants that were sampled.

Four treatments (Table 2.1) comprising of a biological insecticide (Thuricide®), a synthetic pyrethroid (Karate®), a botanical insecticide (Neemroc®) and control plots were applied.

Table 2.1 A list of insecticides used in the control of Diamondback moth during the two cabbage growing seasons at Limuru Division, Central Province and Wundanyi Division, Coast Province of Kenya.

Insecticide	Common name	Concentration	Pesticide type
Lambda-cyhalothrin	Karate®	3.2ml/L.	Pyrethroid
Bacillus thuringiensis	Thuricide®	0.75g/L.	Biological
Neemroc 0.03%	Neemroc EC®	5ml/L	Botanical
Control	Tap water	-	Control

Formulations were done as per the manufacturer's prescription. Applications of test insecticides were done using a knapsack sprayer with a cone nozzle calibrated to deliver 400 liters of spray per hectare. Sampling was done after every fourth night to monitor the DBM numbers. In each of the pilot site, ten plants were randomly selected from every plot and their damage levels established based on the scale of one to five (shown below) and all the DBM larvae, pupae and adults on each plant counted and recorded. A maximum of 5 big larvae (3rd or 4^{th} instars) pre-pupae or pupae were collected from every sampled plant with all samples from same sampling plot placed in the same plastic containers. The containers had perforated and meshed lids and an absorbent paper at the bottom to absorb moisture. Each container was clearly labeled indicating the collection date, treatment and site. Larvae were then provided with cabbage leaf portions and the samples placed in a cool box until further sorting in the laboratory.

Diamondback moth field damage score (scale 1-5):

1= No damage

2= Few isolated holes on outer leaves

3= Most of the leaf area of the outer/lower leaves destroyed, some damage in the inner leaves

4= Outer/lower leaves completely destroyed, moderate attack of inner /upper leaves

5= Inner/upper leaves with severe attack, heads/leaves unmarketable

In the laboratory the field samples were put individually in ventilated cylindrical plastic vials (2.2 cm diameter and 7.5 cm height) and the larvae provided with sections of cabbage leaves for food until pupation. These were checked daily for the emergence of adult moths and parasitoids after which all parasitoids were identified and parasitation rates calculated. Parasitism rates were calculated by dividing the number of emerged parasitoids together with those that died at the larval stage due to parasitism over the total number of samples collected then multiplied by 100 to make it a percentage.

Fluctuations of the maximum and minimum temperatures, relative humidity and rainfall were recorded using a Data logger and rain gauge to establish a correlation between moth or parasitoid count and environmental parameters. Analysis of variance (ANOVA) was used to test differences in DBM survival and parasitoid performance between treatments and seasons and means separated using Student Newman-Keuls test (SNK).

2.2.3 Egg mortality

Twenty five newly emerged pairs (male and female each) of *P. xylostella* were placed in an oviposition cage made of Perspex measuring 50cm x50cm x80cm where they were fed with 10% sugar solution and allowed to oviposit overnight on three full-grown cabbage plants with six fully-extended leaves which were numbered. These plants were removed from the cage the following day and eggs were marked by putting a dot next to each egg using a marker pen. Plants were thereafter transported to a cabbage field where they were positioned randomly in untreated/control plots. Holes were dug and the potted plants placed therein and covered by soil up to the stem base level just like other field plants.

After a period of 48 hours, these plants were taken back to the laboratory where eggs were counted and those that were missing recorded. 25 eggs were randomly selected from those recovered in each plant, incubated in individual vials with a section of cabbage leaf till emergence to determine parasitism, viability and subsequent larval/ pupal mortality. Chi-square was used to analyze the difference in hatchability between eggs collected in second, sixth and tenth day.

2.3. RESULTS

2.3.1 DBM population fluctuations

Diamondback moth (DBM) counts in the field varied between sites, seasons, and treatments with DBM population means showing significant differences between treatments ($P<0.05$).

a) Wundanyi

In Wundanyi first season saw DBM populations rise from an average of 1.1 DBM per plant during the second week to reach its peak on the sixth week after transplanting. Karate-treated plots had the highest DBM counts with a maximum average of 18.7 DBM per plant. Control plots came second with a mean of 10.8 followed by Thuricide-treated plots with 8.6, and then Neemroc-treated plots with an average of 7.6 DBM per plant. From the sixth week onwards, DBM population decreased in all treatments until the end of the season with Karate-treated plots still recording the highest DBM numbers even in the tenth week with an average of 2.2, while the rest were tying at 1.4 DBM per plant (Fig. 2.1).

During the first season at Wundanyi, Karate-treated plots had significantly higher DBM numbers ($P=0.0001$, $df=950$) compared to all other treatments with a mean of 7.3 DBM larvae/pupae per plant while thuricide-treated plots had only 3.7 DBM larvae/pupae per plant (Table 2.2). Variation in DBM numbers among different treatments contributed to significant differences ($P=0.0001$, $df=950$) in damage levels with Karate-treated plots

suffering the highest mean damage level of 2.4 while Thuricide-treated plots had the lowest mean damage level of 2.0 (Table 2.3).

Low DBM populations were registered during the second season at Wundanyi with a mean of less than one DBM larvae/pupae per plant until the 8th week in all treatments (Fig. 2.1). However, towards the end of the season DBM numbers rose with Control plots recording the highest average score of 4.75 DBM per plant followed by Karate and Neemroc-treated plots with 2.8 and 2.1 respectively. Thuricide treated plots recorded the lowest average number of DBM of 1.0 DBM per plant.

In Wundanyi, the second season witnessed low DBM infestations (Table 2.2) with Control and Karate-treated plots having an average of 1.0 and 0.9 DBM per plant respectively while thuricide-treated plots had a significantly lower mean DBM numbers per plant of 0.2 (P=0.0001, df =950). Due to low DBM numbers registered during the second season at Wundanyi, damage levels were almost similar in all treatments except Control plots in which case they were significantly higher (P=0.0001, df =950)(Table 2.3).

b) Limuru

In Limuru, the first season registered relatively high DBM numbers per plant. Commencing on the second week after transplanting, all treatments had a mean score of about 1.3 DBM per plant followed by a sharp rise from the fourth week, reaching the peak in the sixth week in all treatments. Karate-treated plots had a mean of 18.8 DBM per

plant, control and Neemroc treated plots ranking second with 15.0 DBM per plant each while Thuricide-treated plots recorded the lowest population mean of 9.4 DBM per plant (Fig. 2.2). This was followed by a sharp drop in DBM populations among all the treatments in the sixth week followed by a slight rise until the tenth week. Even at the end of the season, Karate-treated plots still had the highest DBM infestation with an average of 7.7 DBM per plant followed by the Control with 3.1, then Neemroc with 2.3 and Thuricide-treated plots had the lowest DBM density of 0.9 DBM per plant.

Karate-treated plots had significantly higher seasonal DBM numbers than all other treatments (P=0.0001, df =1110) with a mean of 7.5 DBM per plant, which was thrice as high as thuricide-treated plots which had an average of 2.5 DBM per plant (Table 2.2). Variation in the numbers of DBM per plant in this area resulted in variation in the damage levels among all treatments (Table 2.3). Karate-treated plots, as was the case at Wundanyi, registered the highest damage level of 2.2 while Thuricide-treated plots had an average damage score of 1.7 (Table 2.3).

The second season at Limuru also registered very low DBM numbers. Until the 8th week, all treatments had similar DBM population numbers with a mean of about 0.4 DBM per plant (Fig. 2.2) which rose slightly from the eighth week till harvesting. During harvesting, Karate-treated and control plots had an average of 6.0 and 5.5 DBM per plant respectively, Neemroc with 3.2, while Thuricide-treated plots registered 1.8 DBM per plant.

Though there was a low DBM infestation per plant during the second season at Limuru, Karate-treated and control plots had significantly higher seasonal DBM numbers per plant (P=0.0001, df =1110) with a mean of 1.8 and 1.7 DBM per plant respectively, while thuricide-treated plots had 0.7 DBM per plant (Table 2.2). Low DBM infestations during this season led to similarly low damage levels which tied at an average of 1.5 DBM per plant.

The association between the numbers of *P. xylostella* larvae and pupae collected in the field and temperature is shown in Fig. 2.3. As temperature decreased progressively from the first season towards the second, there was a corresponding decrease in DBM numbers in both sites.

2.3.2 Field parasitism rates

There was a steady rise in parasitism levels from the start of the first season at Wundanyi, with peak levels being attained towards the end of the season. Parasitism rates in Thuricide-treated plots rose steadily from 25% during the second week to 45.2% during the tenth week. Parasitism in Karate-treated plots rose from 16.7% during the 2nd week to 38.5% in the 8th week, while in Neemroc-treated plots parasitism of 16.7% was recorded during the 2nd week rising to 41.7% in the 8th week, with a further rise to 50% by the twelfth week. Control plots recorded parasitism of 20% during the 2nd week rising to 47.1% by the 10th week (Fig. 2.4).

Thuricide-treated plots had significantly higher seasonal percentage parasitism (P=0.05, df =64) of 38.8% while Karate-treated plots, on the other hand, recorded the lowest seasonal percentage parasitism rate of 24.5% during the first season at Wundanyi (Table 2.4). Neemroc-treated and Control plots had seasonal percentage parasitism rates of 35.1% and 32.9% respectively.

During the second season at Wundanyi a further rise in parasitism rates was witnessed, though DBM populations were very low, and consequently sampling to determine parasitism was only possible from the 8th week. Thuricide-treated plots had 40% of its DBM population parasitized by *D. semiclausum* by the 8th week and 52.6% during the twelfth week. Karate-treated plots had 35% and 45% of their larvae/pupae parasitized in the eighth and tenth weeks respectively, whereas Neemroc-treated and control plots had 37.5% and 38.9% of their larvae/pupae parasitized during the second week and 52.7% and 52% by the tenth week respectively (Fig. 2.5).

High seasonal percentage parasitism rates were registered during the second season at Wundanyi (Table 2.4). Though there were no significant differences in percentage parasitism rates among different treatments during this season, Karate-treated plots still recorded the lowest parasitism levels.

Percentage parasitism rates in Limuru were dominated by a local parasitoid, *O. sokolowskii* during the first cabbage- planting season. This parasitoid attained the highest parasitism rate of 37.6% in the 8th week after transplanting in control plots. As was the

case at Wundanyi, parasitism levels rose from the 2nd week reaching the peak in the 8th week in all treatments. Thuricide-treated and control plots had relatively higher parasitism rates rising from 12.5% and 16.8% in the second week to 31% and 37.6% in the 8th week respectively (Fig. 2.6). Karate-treated plots had the lowest levels of parasitism with a maximum of 18.8% during the 6th week, which decreased further to 3.5% during the fourteenth week after transplanting.

Diadegma semiclausum recorded low parasitism levels during the first season at Limuru in all treatments, with no significant differences between them (Table 2.4). Thuricide-treated and control plots had seasonal percentage parasitism of 3.9% each, Neemroc-treated plots 2.8% and Karate-treated plots attaining the lowest parasitism level of 2.3%.

Percentage parasitism levels by *D. semiclausum* rose over time in all treatments during the second season at Wundanyi, with Thuricide-treated and control plots recording highest mean rates of parasitism of 16.7 % and 15.8% during the tenth week which rose to 25.5% and 25% during the twelfth week after transplanting respectively. Neemroc-treated plots had 14.3% of its larvae or pupae parasitized in the 8th week and 23.8% in the twelfth week with Karate recording parasitism of 12% in the eighth week and 21% during the twelfth week (Fig. 2.7).

Thuricide-treated plots recorded the highest mean seasonal percentage parasitism of 21.4% closely followed by Control plots with 21.1%. Neemroc-treated plots had a

seasonal percentage parasitism of 20.4% while Karate-treated plots recorded the lowest seasonal percentage parasitism of 16.7% (Table 2.4).

2.3.3 Egg mortality

There wasn't any egg parasitism recorded in the field, a likely indication of absence or scarcity of *Trichogramma* spp. in these two study sites. More than 40% of the eggs marked and exposed in the field could not be recovered after 48 hours of exposure, most probably as a result of predation. Among those recovered, about 15% did not hatch, meaning that less than half of the eggs do successively hatch and are thereafter subjected to other larval, pupal or adult mortality (Fig. 2.8 a).

There were no significant differences in hatchability between the eggs laid by a 2, 6 and 10 days old adult moths (χ^2=1.32, DF=2). However the number of eggs that successfully developed to adult moths in each treatment were significantly different, decreasing from 84%, 69.6% and 53.8% (χ^2 =6.6, Df=2, P=0.04) (Fig. 2.8 b).

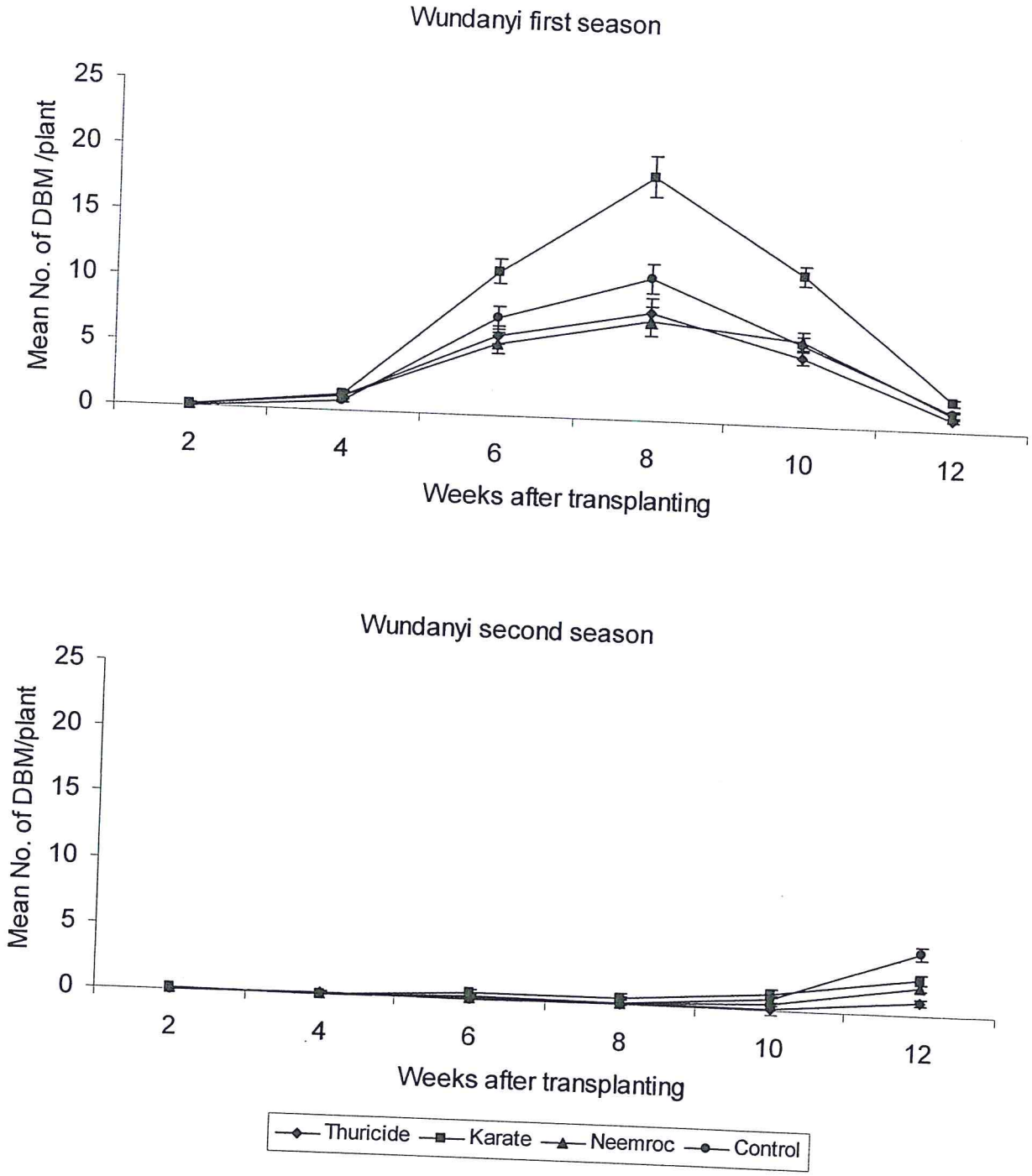


Fig 2.1 Effects of pesticide treatment on DBM infestation during two cabbage growing seasons (January- April and April-July, 2003) at Wundanyi Division, Coast Province of Kenya.

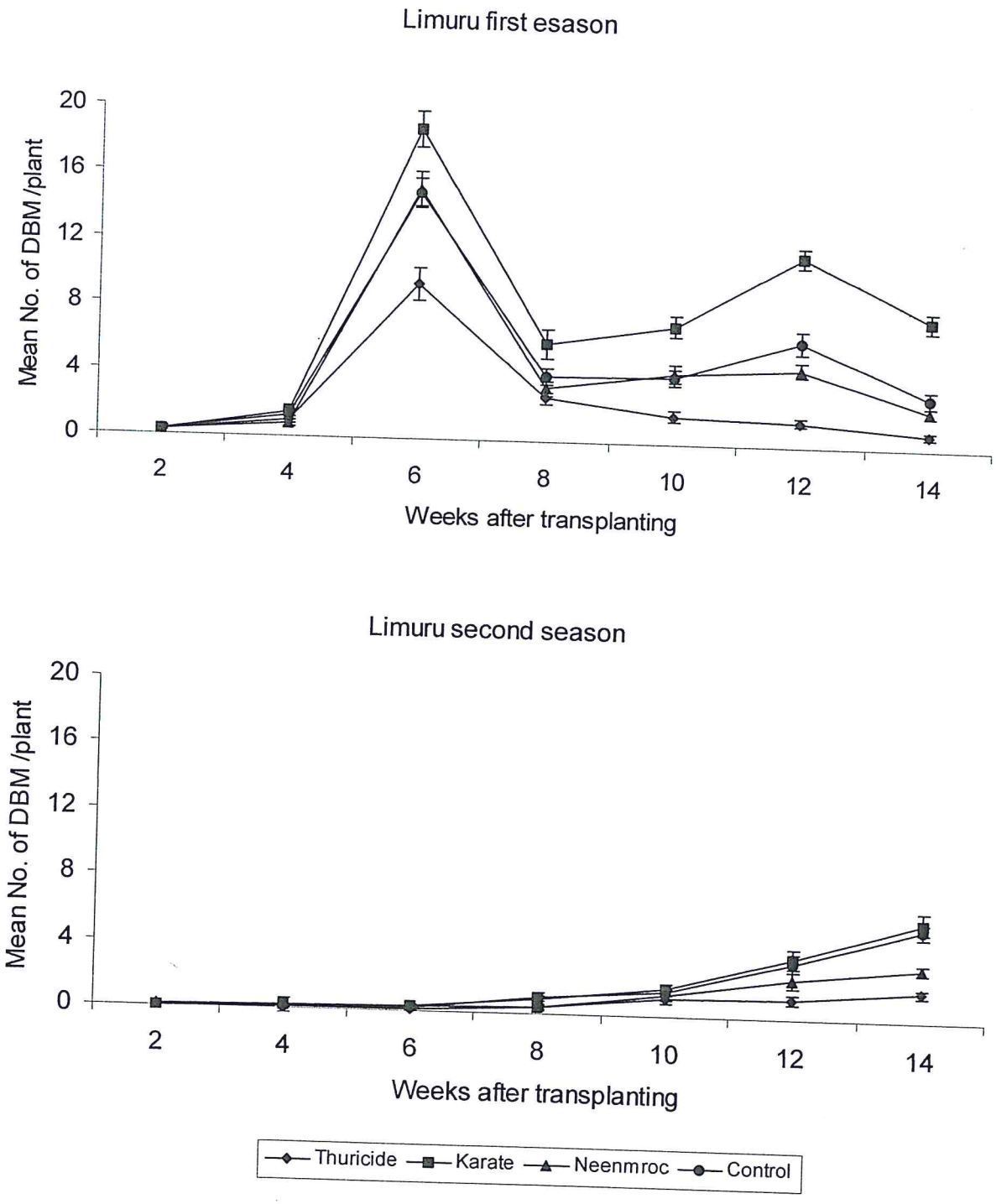


Fig 2.2 Effects of pesticide treatments on DBM infestation during the two cabbage growing seasons (January- April and April-July, 2003) at Limuru Division, Central Province of Kenya.

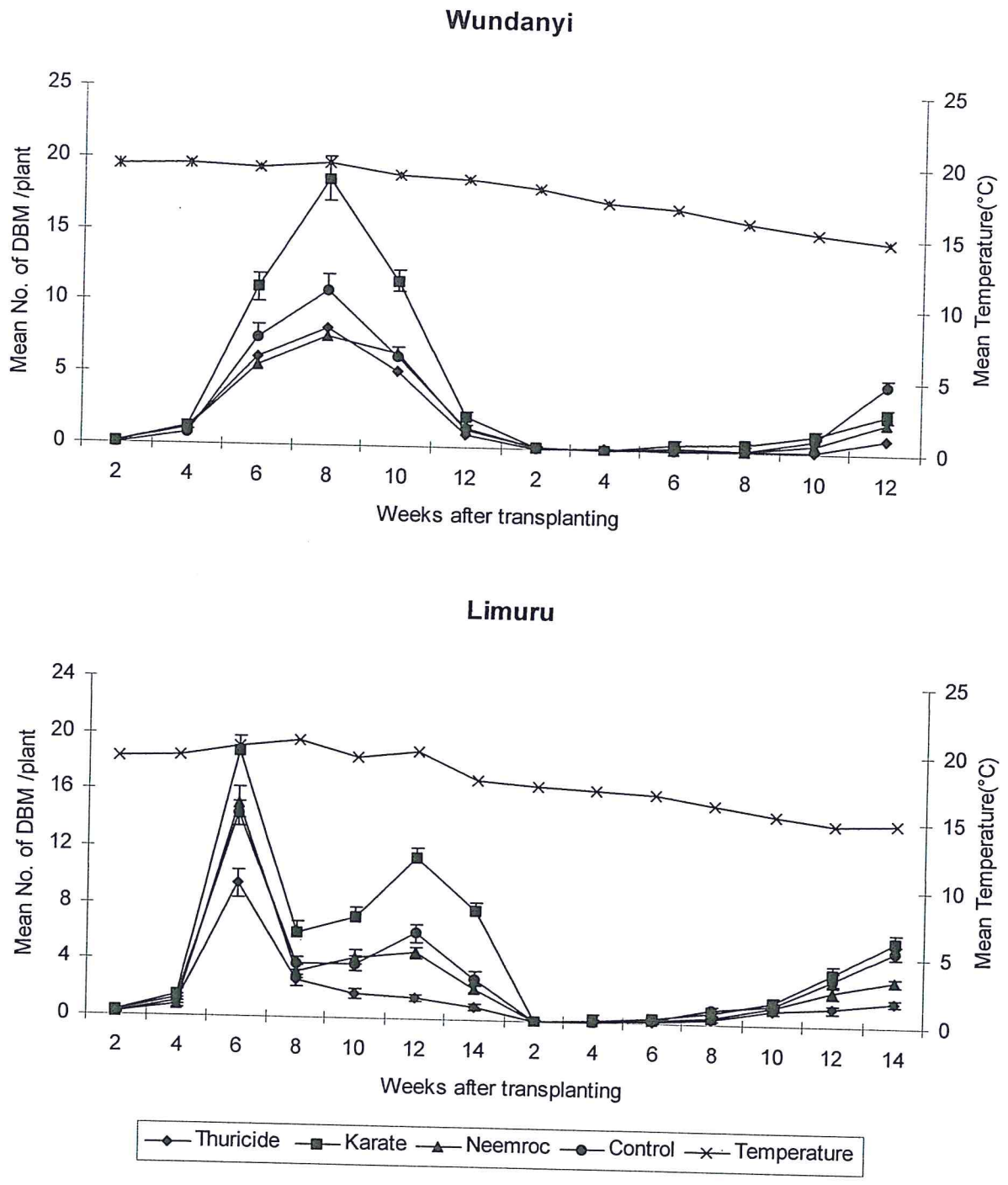


Fig 2.3 Effects of Temperature on DBM population in Wundanyi Division, Coast Province and Limuru Division, Central Province of Kenya across the two cabbage growing seasons (January- April and April-July, 2003).

Table 2.2 Mean±SE numbers of DBM per plant during the two cabbage growing seasons (January- April and April-July, 2003) on different insecticide treatments at Wundanyi Division, Coast Province and Limuru Division, Central Province of Kenya.

	Average number of DBM per plant			
	First season		Second season	
Treatment	**Wundanyi**	**Limuru**	**Wundanyi**	**Limuru**
Thuricide®	3.7±0.3 b	2.5±0.3 c	0.2±0.1 c	0.7±0.1 c
Karate®	7.3±0.6 a	7.5±0.4 a	0.9±0.1 a	1.8±0.2 a
Neemroc®	3.9±0.3 b	4.4±0.3 b	0.5±0.1 b	1.1±0.1 b
Control	4.5±0.4 b	4.9±0.3 b	1.0±0.2 a	1.7±0.2 a
df	950	1110	950	1110
Pr>F	0.0001	0.0001	0.0001	0.0001

Means within the same column followed by the same letter are not significantly different (P>0.05), mean separation by Student Newman-Keuls test.

Table 2.3 Mean±SE damage score during two cabbage growing seasons (January- April and April-July, 2003) on different insecticide treatments at Wundanyi Division, Coast province and Limuru Division, Central Province of Kenya.

	Average damage score per plant			
	First season		Second season	
Treatment	**Wundanyi**	**Limuru**	**Wundanyi**	**Limuru**
Thuricide®	2.0±0.0 d	1.7±0.0 d	1.4±0.0 a	1.5±0.0 a
Karate®	2.4±0.1 a	2.2±0.1 a	1.4±0.0 a	1.5±0.0 a
Neemroc®	2.1±0.0 c	1.9±0.0 c	1.4±0.0 a	1.5±0.0 a
Control	2.3±0.0 b	2.0±0.1 b	1.5±0.0 a	1.5±0.0 a
df	950	1110	950	1110
Pr>F	0.0001	0.0001	0.2886	0.7097

Means within the same column followed by the same letter are not significantly different (P>0.05), mean separation by Student Newman-Keuls test.

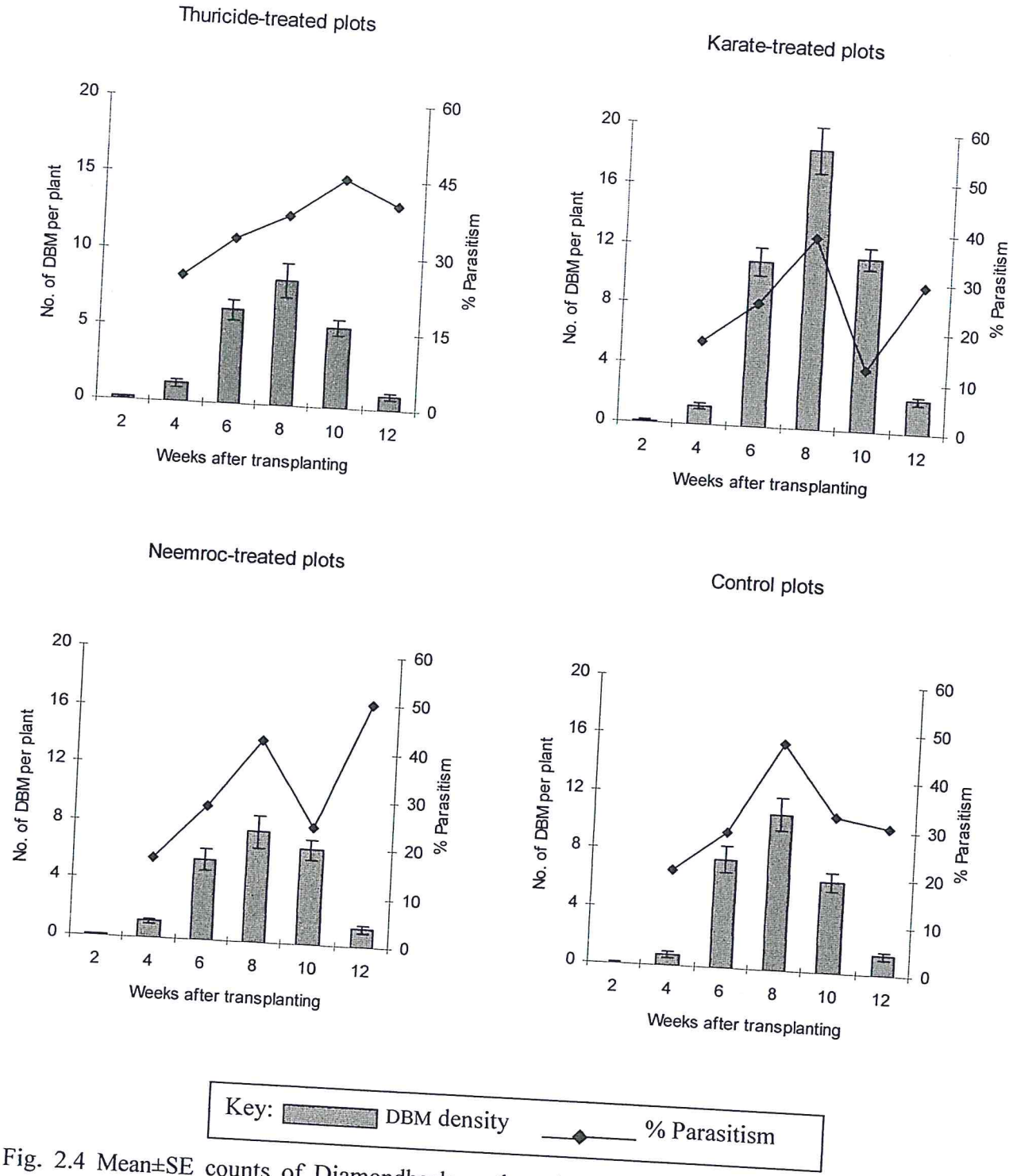


Fig. 2.4 Mean±SE counts of Diamondback moth and parasitism levels by *Diadegma semiclausum* in different pesticide treatments at Wundanyi, Coast Province of Kenya during the first cabbage growing season, January- April, 2003.

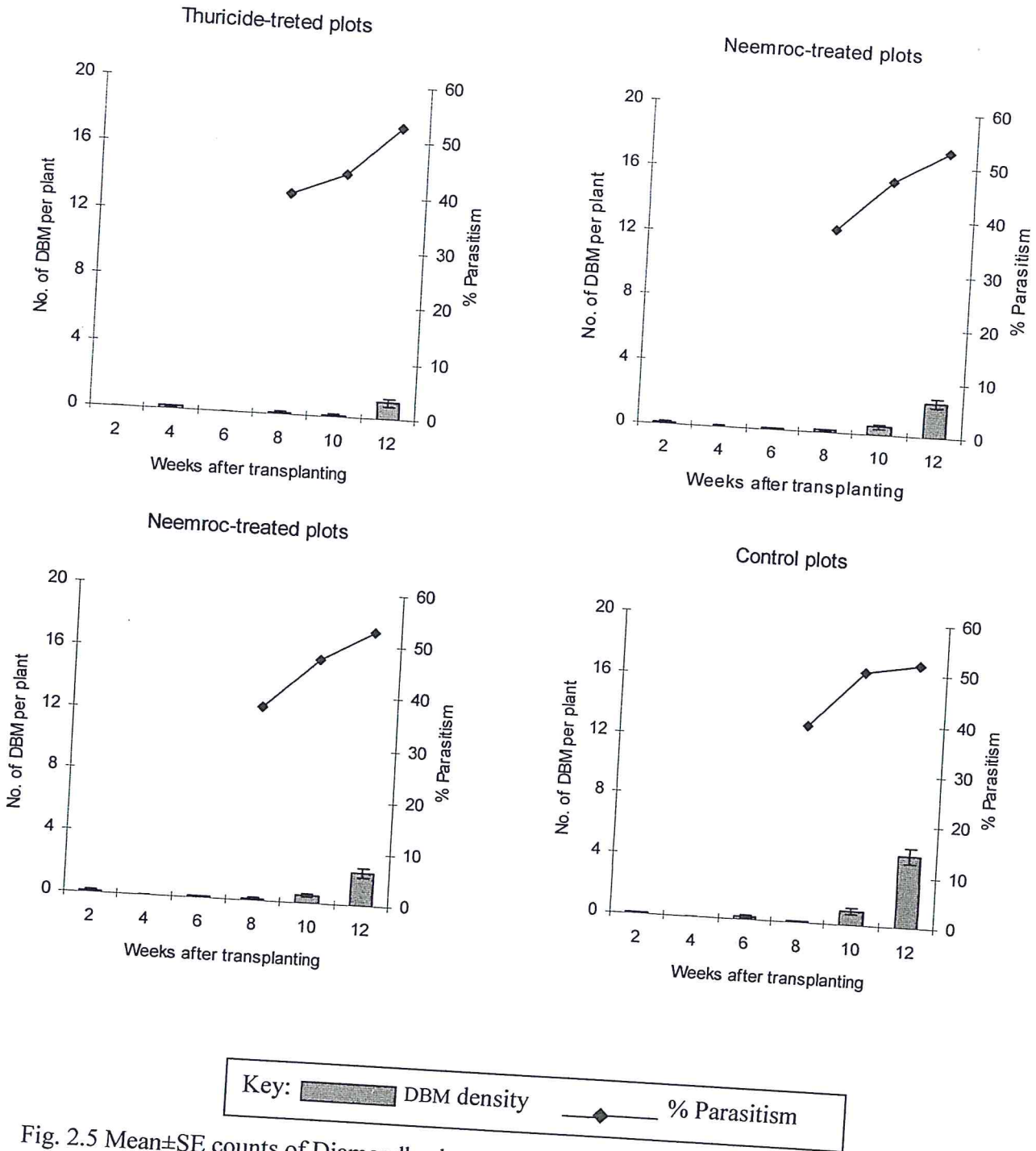


Fig. 2.5 Mean±SE counts of Diamondback moth and parasitism levels by *Diadegma semiclausum* in different pesticide treatments at Wundanyi, Coast Province of Kenya during the second cabbage growing season, April-July, 2003.

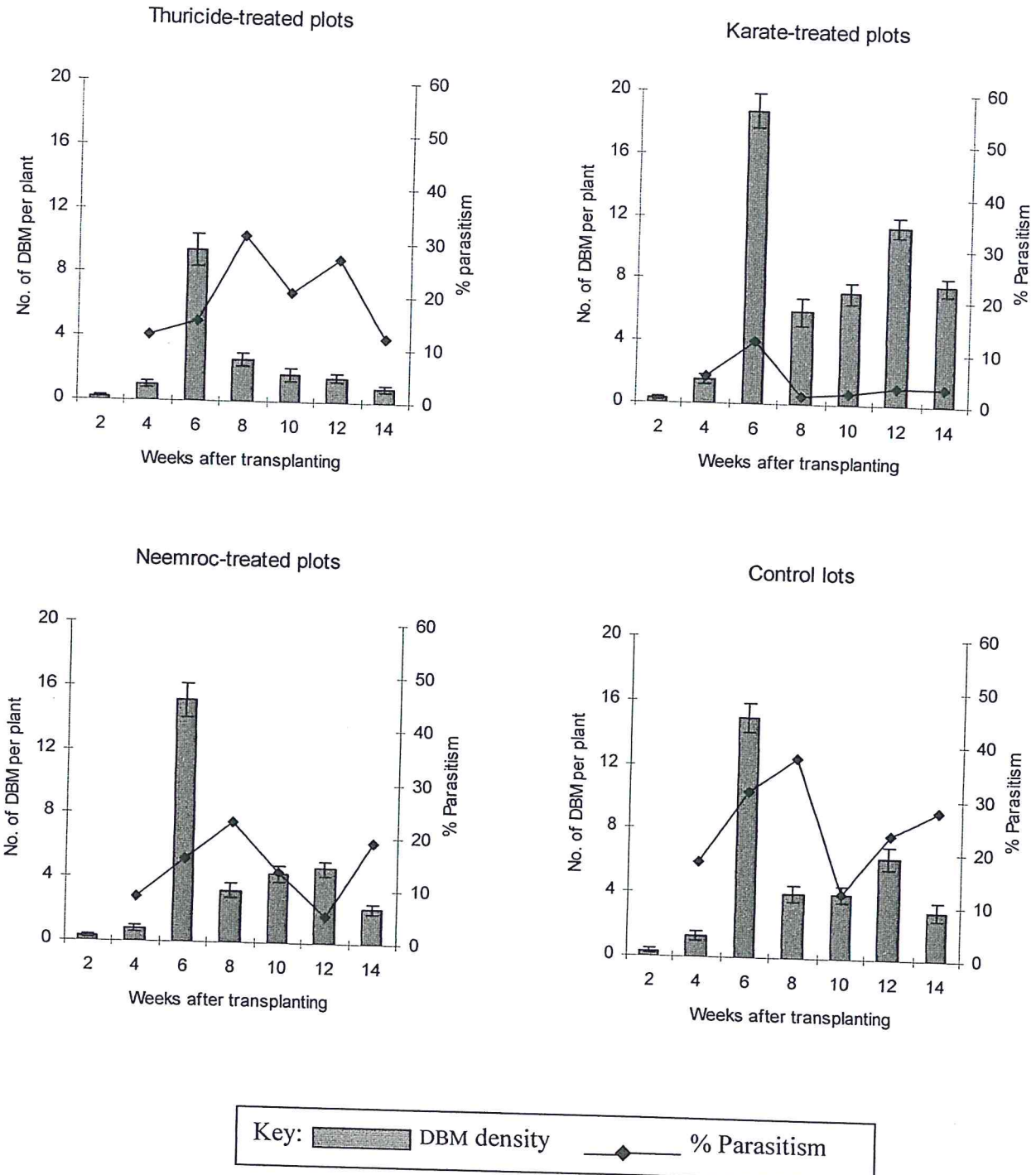


Fig. 2.6 Mean±SE counts of Diamondback moth and parasitism levels by *Oomyzus sokolowskii* in different pesticide treatments at Limuru Division, Central Province of Kenya during the first cabbage growing season, January- April, 2003.

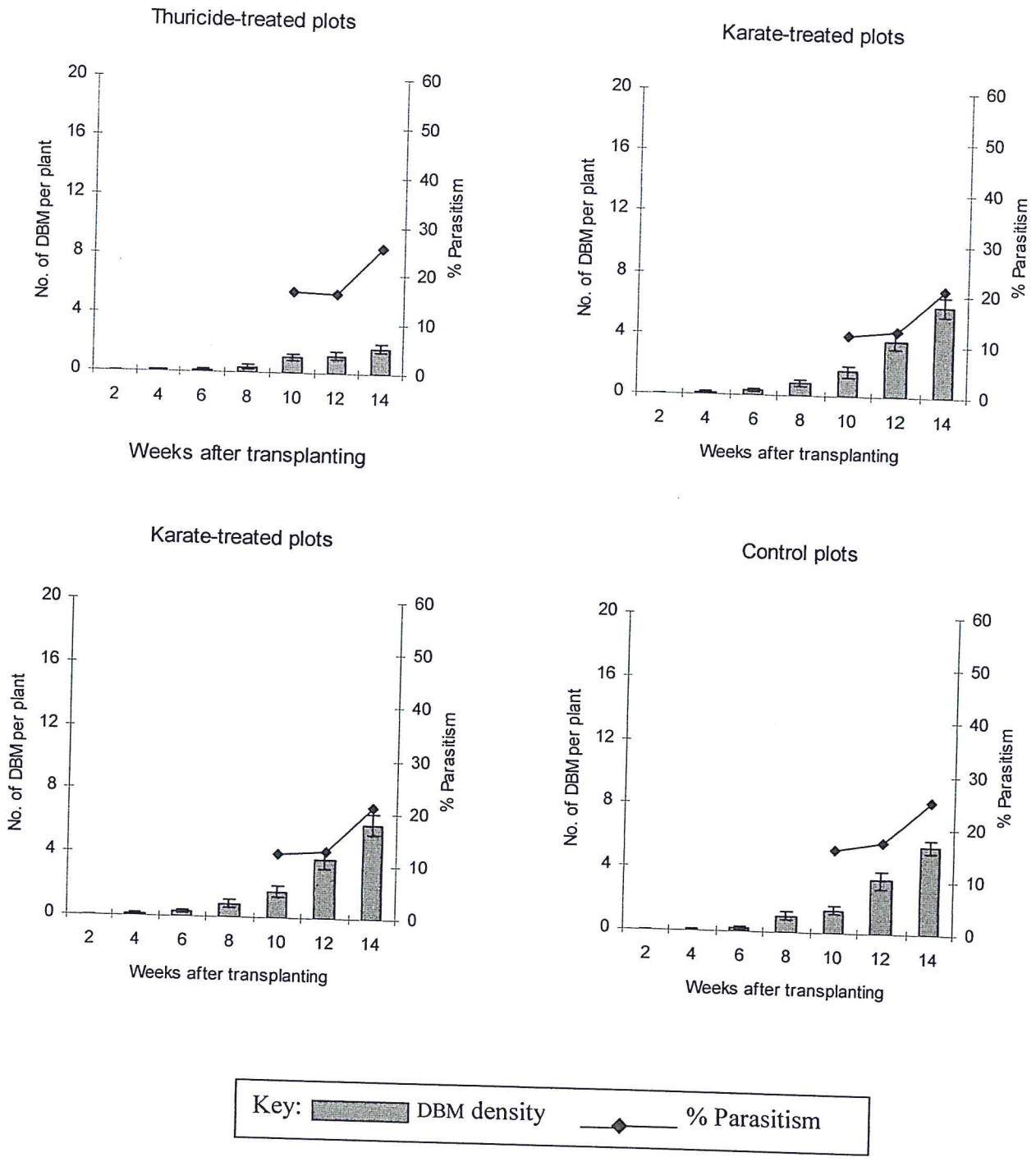


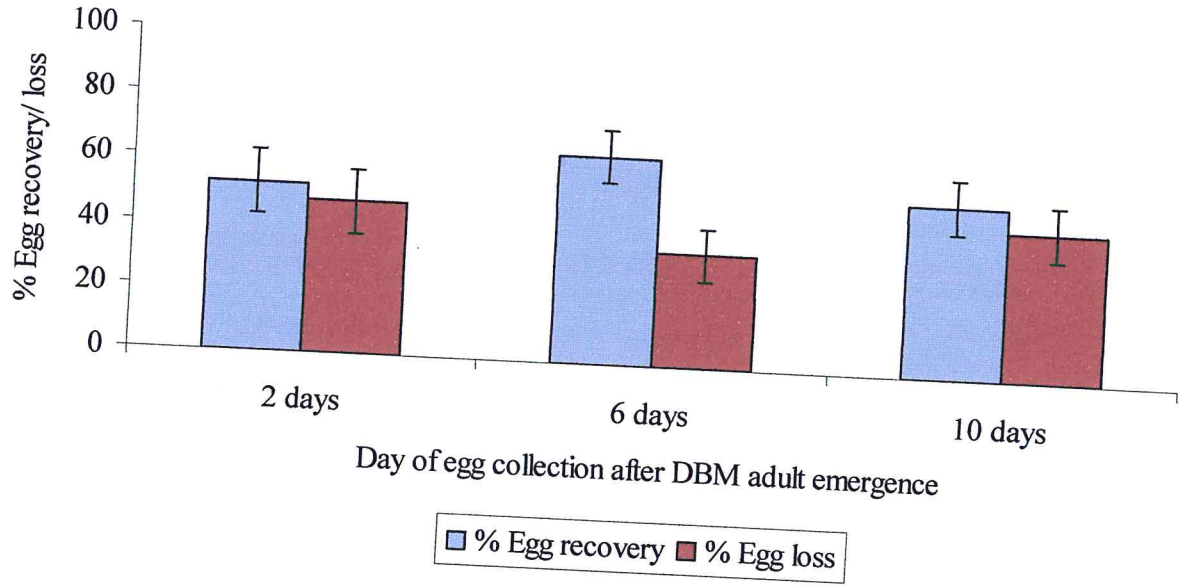
Fig. 2.7 Mean±SE counts of Diamondback moth and parasitism levels by *Diadegma semiclausum* in different pesticide treatments at Limuru Division, Central Province of Kenya during the second cabbage growing season, April-July, 2003.

Table 2.4 Effect of different insecticide treatments on the performance of *Diadegma semiclausum* at Wundanyi Division, Coast Province and Limuru Division, Central Province of Kenya during the two cabbage growing seasons (January- April and April-July), 2003.

	Mean seasonal parasitism			
	First season		Second season	
Treatment	**Wundanyi**	**Limuru**	**Wundanyi**	**Limuru**
Thuricide®	38.8±4.1 a	3.9±1.2 a	45.5±3.7 a	21.4±3.7 a
Karate®	24.5±4.1 b	2.3±0.7 a	39.5±2.9 a	16.7±3.1 a
Neemroc®	35.1±3.1 ba	2.8±1.3 a	45.7±4.4 a	20.4±3.6 a
Control	32.9±2. 8 ba	3.9±1.2 a	46.9±4.1 a	21.1±3.5 a
df	64	80	8	12
Pr>F	0.0526	0.6917	0.5454	0.7649

Means within the same column followed by the same letter are not significantly different (P>0.05), mean separation by Student Newman-Keuls test.

a)Mean percentage *Plutella xylostella* field egg recovery after two days exposure in a cabbage field.



b) Effect of age of an ovipositing DBM on the survival of DBM to adulthood.

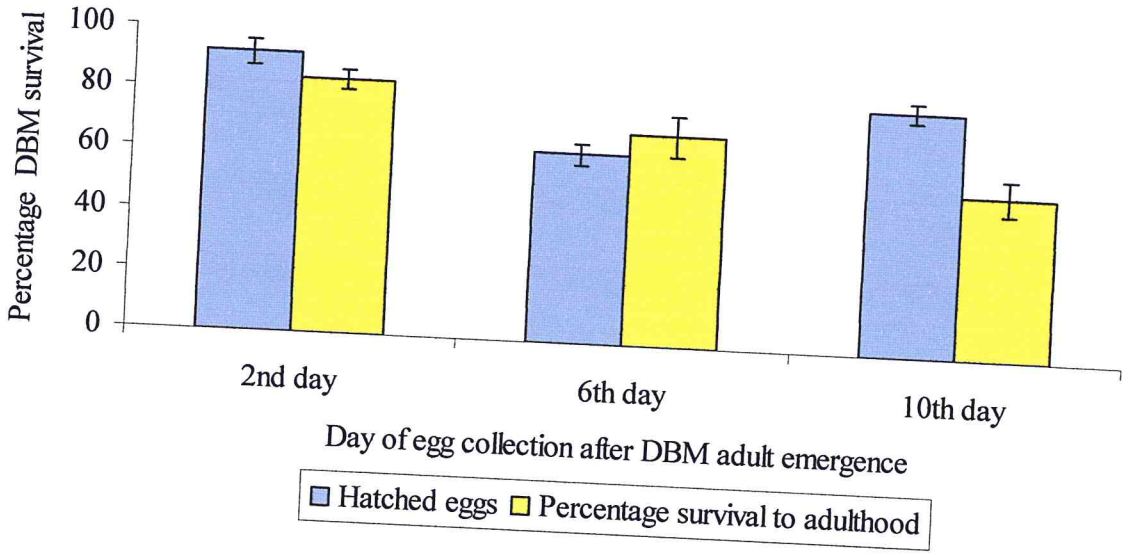


Fig 2.8 Mean percentage DBM egg recovery two days after exposure in a cabbage field and effect of age of an ovipositing adult on the survival of DBM to adulthood at Wundanyi Division, Coast Province of Kenya.

2.4 DISCUSION

2.4.1. Establishment and impact of *D. semiclausum* on DBM population Limuru Division, Central Province and Wundanyi Division, Coast Province of Kenya.

The population of *P. xylostella* was monitored at Wundanyi, coast province and Limuru, central province of Kenya for a period of seven months and population trends traced. On the basis of this study, exponential growth phase was observed between the second and the eighth weeks after transplanting followed by a gradual population decline till harvest. One hypothesis may explain this sigmoid growth of *P. xylostella* that at the beginning of the first season DBM had optimum conditions such as enough quality food (cabbage vegetative material), suitable climatic conditions, low parasitoid numbers /natural enemies within the field and therefore could multiply at their maximum potential. After a period of six to eight weeks, the quality of food started to decline due to defoliation and the parasitoid population increased thus perturbing the moth population. In addition, climatic factors could have contributed to the decline of the *P. xylostella* and eventually stabilizing towards the end of the season (Wakisaka *et al.*, 1992; Goulloux *et al.*, 2003).

Parasitism rates on DBM, irrespective of the crop protection practices employed, were significantly higher in Wundanyi than Limuru. The number of *D. semiclausum* recovered from the field rose steadily from the 2nd week after transplanting to nearly 50% between the 8th and 10th weeks in Wundanyi. Limuru area registered low levels of parasitism by *D. semiclausum,* with *O. sokolowskii*, a local parasitoid being the dominant parasitoid during the first season. As was the case with *D. semiclausum, O. sokolowskii* equally

performed better in the Control and Thuricide-treated plots, getting to 37.6% during the eighth week after transplanting.

Host-parasitoid interactions have been discussed by several authors and have been appreciated as potential contributors to population stability through a density dependence manner (Hassell and Varley, 1969; Hassell, 1970), through inverse density dependence (Hassell, 1984; Hassell *et al.*, 1985) or through random (density-independent) factors (Hassell, 1985, 1987). Parasitism levels in this study rose with increase in DBM numbers in the field (Fig. 2.4 to Fig. 2.7) after which the moth population decreased as parasitism increased. This suggests that *D. semiclausum* contributed to DBM population stabilization in a density dependent manner. This finding is in agreement with other findings (Goodwin, 1979; Shu *et al.*, 2000; Smith and Villet, 2002).

Low DBM counts during the second cabbage-planting season could be as a result of a combination between climatic factors and increased activities of natural enemies (Wakisaka *et al.*, 1992; Odour *et al.*, 1996). High parasitism rates attained towards the end of the first season in Wundanyi area reduced DBM adults available for oviposition in subsequent generations, thereby keeping DBM populations at equilibrium till the last few weeks before harvesting when DBM population increased slightly (Fig. 2.1).

Owing to the fact that this is the first year of release this could be an indication that the introduced parasitoid would establish and control DBM to manageable level within a span of a few years. Results obtained from this study indicate that a sustainable and cost-

effective integrated pest management strategy may likely be achieved in the near future and possibly pesticide application minimized, as is the case in Cape Verde Island, South Africa (Waage and Cherry, 1992).

2.4.2 Effects of various crop protection measures on DBM population, and parasitoid performance

Karate-treated plots had significantly higher DBM numbers which translated to a significantly higher damage levels during the first cabbage growing season in both sites due to high DBM infestation (Tables 2.2 and 2.3). These results are consistent with other findings (Guan-Son *et al.*, 1986), and could be an indication that Karate® is no longer providing the desired level of DBM control. Parasitism among treatments ranged from 5.8% to 52.7% (Fig 2.4-2.7). Such variation in parasitism rates among different treatments point out to the adverse effect of this broad spectrum pyrethroid (Karate®) on the establishment and performance of *D. semiclausum*. This broad-spectrum pesticide has been known to reduce natural enemy complex associated with DBM in the field causing DBM upsurge or resurgence (Mukenfuss *et al.*, 1992). This explains why there were high DBM populations observed in Karate-treated plots and heralds a warning of a possible DBM resistance development to this broadly used pesticide.

Thuricide, a *Bt* formulation is a selective biopesticide on the other hand and was quite efficient in DBM control. Due to its selective nature (Mori, 1992, Ho, *et al.*, 2002) this pesticide did not affect natural enemies which in turn kept DBM numbers low. It is therefore recommend that applications of Thuricide be made incase pest population rise

above the threshold levels in the field as part of the Integrated Pest Management strategy to augment *D. semiclausum.* Application of this biopesticide should be promoted among farmers to replace the broad-spectrum pesticides such as Karate® which are no longer effective in the control of DBM, in addition to being hazardous to the environment (Oduor *et al.*, 1996). This will mean a shift from broad spectrum pesticide to a more specific and environmentally friendly one that can favor parasitoid establishment.

Neemroc-treated plots always had the second fewest DBM numbers to Thuricide-treated plots. The above finding coupled with the fact that relatively high parasitism levels were attained in Neemroc-treated plots points to the fact that this insecticide is compatible with this form of biological control. Schmutterer (1992) suggested that the delayed action of Neem insecticides on DBM larvae does allow parasitoids to develop within Neem insecticide-treated hosts and that this pesticide does not affect host acceptance and survival of *D. mollipla* (Akol *et al.*, 2002). However, it may result in undesirable effects on the efficiency of parasitoid foraging or may render DBM larvae unsuitable hosts for propagation of parasitoid hosts (Akol *et al.*, 2003).

Higher parasitism levels attained in the control plots comparative to Karate-treated plots is a clear indication that this synthetic pyrethroid could be harmful to natural enemies, including the parasitoids, and could be the likely explanation of low parasitism levels recorded in the farmers' fields (Seif and Löhr, 1998). Higher damage levels recorded in Karate-treated plots and the fact that cabbage yield is closely associated with the extent of

DBM infestation (Kibata, 1996) heralds a warning that Karate could be reducing cabbage yield rather than providing the necessary protection against Diamondback moth.

A rise in the population of natural enemies within a specific treatment easily moved into adjacent treatments and therefore use of larger plots which reduce parasitoid migration from one treatment to another is recommended (Van Driesche and Bellows, 1996).

2.4.3 Effect of locality and seasonality on DBM population fluctuations and parasitoid performance

Low parasitism rates on DBM, irrespective of the crop protection practices employed in Limuru Division was insufficient to regulate *P. xylostella* populations. During the first planting season parasitism due to *D. semiclausum* was below 10%, rising to a maximum of 25% during the second cabbage growing season. This level of parasitism was insufficient to regulate DBM populations (Goulloux *et al.*, 2003) and could probably be explained using prevailing climatic, topographic, or agronomical practices in this area (Wakisaka *et al.*, 1992; Oduor *et al.*, 1996). Therefore DBM population fluctuation in this site can not be satisfactorily explained using parasitoid activities only.

Rainfall has been pointed as one of the key mortality factors of *P. xylostella* (Hardy, 1938; Harcourt, 1963; Talekar and Griggs, 1986) but low precipitation experienced at both sites during time of experiments was not sufficient to affect DBM populations. Continuous presence of fog and dew on cabbage leaves has been known to reduce DBM

oviposition (Goudegnon *et al.*, 2002) and probably this phenomenon gives an explanation for low DBM counts in Limuru during the second cabbage growing season.

The highest temperature of 19.8°C corresponded with the highest DBM populations in all the four treatments at Wundanyi while low temperatures during the second season resulted in low DBM population (Fig.2.4). A mean temperature of 19.3°C, during the first cabbage planting season, was suitable for DBM survival resulting in high DBM numbers while a lower mean temperature of 16.4°C, during the second season, led to low DBM numbers in the field. Similar results have been recorded elsewhere (Chen and Su, 1986; Harcourt, 1986; Ong and Soon, 1989; Talekar, 1992).

Decrease in temperature regime from the first through the second season at both sites corresponded with reduction in DBM numbers recorded in the field. Temperatures that were recorded in Limuru were very low, with a mean of 15.5°C. Given the fact that the most suitable temperature range for development of *D. semiclausum* is between 15-25°C (Talekar, 1992), then temperature is most likely to be the factor that can explain low parasitism rates attained in this area.

2.4.4 Field egg mortality

The population of *P. xylostella* eggs within the field is adversely affected by a factor that could not be measured in the field. More than 40% of the eggs taken to the field could not be accounted for after a period of two days exposure in a cabbage field (Fig. 2.8). Egg

mortality has always not been accurately quantified and its key mortality factor has been from undetermined causes (Shigekazu *et al.*, 1990). This high-unexplained egg mortality could probably be as a result of predation or by detachment of eggs from plant surface as a result of wind, rainfall or due to other corporal factors.

Direct impact of the rains has been known to be an important egg mortality factor as a result of wash-off (Talekar and Griggs, 1986). Since there were no rains during the entire experimental period then this mortality is likely to be higher during the rainy seasons probably giving an explanation why *P. xylostella* is not a major crucifer pest during the rainy seasons (Sivapragasam *et al.*, 1988) in addition to the drowning effect of rain on small larvae (Harcourt, 1986; Wakisaka *et al.*, 1992).

Egg parasitism by Trichogrammatoideae was not observed which is an indication of its absence in the cabbage fields in the two pilot sites. Oduor *et al.*, (1996), also reported lack of egg parasitism in the field and therefore unexplained mortality factors, predation and egg viability are the key regulatory factors that determine the number of *P. xylostella* eggs that successfully develop to adult stage other than egg parasitism.

CHAPTER 3

3.0 IMPACT ASSESSMENT OF *Diadegma semiclausum* ON THE SURVIVAL OF DIAMONDBACK MOTH AND ITS LOCAL NATURAL ENEMIES

3.1 INTRODUCTION

Assessing the impact of a natural enemy on its host population is important in order to evaluate the success of a biological control project. This can be achieved using a number of methods. The limitation and applicability of these methods to different natural systems have been evaluated by many workers (Huffaker and Kenett, 1969; Hodek *et al.*, 1972; Kiritaner and Dempster, 1973; Van Lenteren, 1980; Luck *et al.*, 1988; Bellows *et al.*, 1992). These have been grouped into three different approaches;

a) Analysis of survey data using multivariate regression.

b) Comparison of field pest populations with and without natural enemies.

c) Modeling pest mortality using field life table data.

One tool within the third approach, experimental exclusion method, is the fastest and most direct way to demonstrate the impact of a natural enemy on the pest population (Boavida *et al.*, 1995).

The goal of constructing life tables for evaluating the impact of natural enemies is to obtain quantitative estimates of the mortality caused by each natural enemy or a particular agent. A life table can be defined as an organized presentation of the number of individuals of a generation or stage surviving to a fixed point in the lifecycle together with their specific causes of mortality (Deevey, 1947).

Life tables may be constructed in two ways. First data may be collected representing a cohort, typically a generation of individuals whose numbers and mortalities are determined over the course of time for each of a series of stages. The second way is the examination of the age structure of a population at one point in time and to infer from it mortalities occurring at each stage (Van Driesche and Bellows, 1996). This analytical approach provides a well-organized framework for assembling information on the set of mortalities that shape the life system of the target pest and for defining the relative importance of mortality from a specific agent of interest and its contribution toward stabilizing the population.

Examination of a preset number of hosts giving no information on the changing host density are difficult to interpret and can easily be misleading. For density relatedness to be correctly quantified and interpreted, mortality rates must be associated with the density of the host (Van Driesche, 1983; Van Driesche *et al.*, 1991). It is also important that the host is exposed to the natural enemy for the entire susceptible period of its life cycle if risk per generation is to be assessed. Parasitism is known to change the behavior of the host leading either to earlier or delayed maturity hence to avoid bias necessary sampling adjustment must be done. The greatest accuracy can be obtained by sampling a host stage between that which is attacked by the parasitoid and that from which the parasitoid emerges. Forth instar larvae or prepupae; therefore become the best stage to sample to determine percentage parasitism (Van Driesche, 1983; Bellows *et al.*, 1989; Van Driesche *et al.*, 1991).

Carter *et al.*, (1978) Criticized the use of instar distribution in the field to calculate population growth rates due to the instability of age distribution in the field and in such cases, neither one nor a series of samples of host and parasitoid densities can directly estimate generational mortality due to parasitism (Van Driesche, 1983). However, a modified approach, which involves use of caged cohorts, allows the quantification of different mortality factors (Bellows *et al.*, 1992; Hutchison and Hogg, 1985). The use of cages to evaluate the role of resident or introduced natural enemies by excluding them from the plots, plants or plant parts infested with the pest has been employed extensively (Boavida *et al.*, 1995; Hopper *et al.*, 1995, and Nechols *et al.*, 1996).

The objectives of this study were to quantify the impact of an introduced DBM exotic parasitoid, *D. semiclausum* on *P. xylostella* population and its local natural enemies with special emphasis on parasitoids, and relate this mortality with parasitoid-host density. A seven months study was carried out to investigate the variation in DBM and its parasitoid densities in the field over time. In addition field-simulated screen house studies were undertaken to look into the effect of parasitoid density on the loss of *P. xylostella* larvae and PCR-RFLP done on the *Diadegma spp.* field recovered specimens to test for the possibility of interbreeding *in situ*.

3.2 MATERIALS AND METHODS

3.2.1 Field-based life table studies

In each of the pilot sites a 0.4-hectare field was planted with cabbage based on a Latin square design, with four treatments namely Thuricide®, Karate, Neemroc® and control, replicated four times (details on pesticides applied can be found in Table 2.1). Ten plants were randomly selected from each plot and all the DBM larvae, pupae and adults on each plant counted and recorded. Samples of fourth instar larvae, pupae and parasitoid cocoons were collected after every two week from the 10 randomly selected plants in each plot. This means that 160 plants were sampled after every fortnight in every site.

The larvae and pupae were then placed in labeled plastic containers indicating collection date, site, plot number, crop and variety. Larvae were provided with food and the collected materials placed in a cool box until further sorting in the laboratory. In the laboratory the field materials were placed individually in plastic vials and the larvae provided with sections of cabbage leaves until pupation. They were checked daily for emergence of adult moths or parasitoids. All parasitoids were identified and the number of emerged adult moths and parasitoids were then used to assess the parasitation rates of the introduced and local parasitoids. Parasitism rates were evaluated before and after the release.

Larvae that died before emergence of adults were examined for the cause of death whether it was due to disease (bacterial, fungal or viral) or parasitoids.

3.2.2 Cage exclusion experiment

Nursery trays were filled with compost manure and in each hole one cabbage seed (variety Gloria F1) was sown, and watered for a period of two to three weeks. Fully established seedlings were then transplanted to individual pots with compost manure as the substrate, watered and tendered in the green house for a period of one month or until they developed six fully extended leaves.

Sixteen of these full grown potted plants were selected and their leaves numbered from the base upwards using a marker pen. These leaves were then infested with 10 DBM neonate larvae each and were allowed one hour to settle and get established before these plants were taken to the cabbage field. Eight of these plants were coated with a ring of insect glue an inch from the soil surface level to prevent climbing up of crawling predators. The plants were then transported to the field where they were set up in cages measuring 40cm x 40cm x 40cm. Four treatments were established:

i) **Fully caged and glued-** Cages covered on all the five sides except the bottom by a muslin cloth with plants coated with insect glue one inch above the ground to exclude entry of flying and crawling DBM natural enemies.

ii) **Fully caged only** – Cages covered on all the five sides except the bottom by a muslin cloth with unglued cabbage plants.

iii) **Glued only-** Cages covered at the topside only using a muslin cloth with cabbage plants coated with insect glue an inch above the ground to exclude crawling DBM predators.

iv) **Control-** Cages covered at the topside only using a muslin cloth with cabbage plants not coated with insect glue to permit entry of all natural enemies.

For sampling the numbered leaves were randomly assigned to sampling days using a random table with a total of six collections. Samples were collected, after every other day in the month of February and for September we had a 2, 3, 2, 3, 2, 2 interval, with shorter collection intervals coinciding with periods of hot weather. At the last collection, the sixth leaf and the remainder of the plant were collected separately. DBM natural enemies as well as other insects and spiders noted on the plant during sampling were also recorded.

Egg mortality was determined in a separate experiment. Potted cabbage plants (variety, Gloria) were put in a cage with DBM adults. These were allowed to oviposit overnight. Eggs on the plant leaves were marked using a marker pen before being taken to the field for 2 days. The pots were removed and the plants were planted with the substrate between the rows of a cabbage plot of roughly the same age, where no pesticide was applied. Plants were brought back to the laboratory and eggs monitored to determine egg parasitism in the field. In the laboratory, all marked eggs were checked and missing eggs were recorded.

3.2.3 Field-simulated screen house studies

Three cages measuring 70cm x 110cm x 70cm were made using plastic pipes. Twelve-potted cabbage plants with six fully extended and numbered leaves were infested with ten neonate larvae on each of the numbered leaf and thereafter given two hours to establish before being exposed to various experimental treatments. Half of the infested plants were coated with a ring of insect glue 1 inch from the soil surface to act as a barrier for larvae which might have dropped as a result of the stinging effect of *D. semiclausum* and want to make their way back to the plant.

Four of these plants were placed in each of the three cages improvised to house the plants at spacing of 45cm x 60cm and with a soil layer that covers the pots exposing the plants above its surface. At the edge of the experimental set-up a thin layer of insect glue was applied all round as a trapping mechanism for the straying DBM larvae.

In the first two cages one and two newly emerged *D. semiclausum* pairs were introduced and provided with pure honey as food. In the third cage, which in this case acts as a control experiment, four DBM infested plants were placed without any parasitoid. The cages were then covered using fine muslin clothing to prevent escape or entry of any DBM natural enemy and the experiment was monitored for the entire DBM susceptible larval stage (L1-L4).

Plants were cut, taken to the laboratory and larvae or pupae collected from these plants using soft forceps. These samples were incubated separately in clean containers at room

temperature until emergence to determine parasitism. Larvae that dropped off to the ground during the experimental period were searched for, recorded and later dissected to check for parasitism or reared until emergence. Total number of larvae recovered, parasitoid and number of DBM that emerged were recorded in each case to unveil the effect of parasitoid density on DBM mortality.

3.2.4 Molecular identification of the *Diadegma spp.* using PCR-linked restriction fragment length polymorphism analysis (PCR-RFLP)

DNA extraction (modified after Collins *et al.*, 1987)

Twenty five adult *Diadegma spp.* from each field were examined separately using PCR-RFLP to distinguish *D. mollipla* from *D. semiclausum*. Specimens were crushed in a micro-centrifuge tube with a plastic pestle and homogenized in a grinding buffer. Proteins were removed, and then DNA centrifuged and precipitated in 95% ethanol at -20°C overnight. DNA was washed, and dried ready to be used as a PCR template.

PCR-amplification

The ribosomal ITS2 region was amplified using the primers flanking the 5.8 S and the 28 S regions (Navajas *et al.*, 1994). Amplification took place in a PCR-100 Thermocycler by an initial predenaturation step of 95°C for 4 minutes followed by 32 cycles of denaturation at 94°C for 1 minute, annealing at 52°C for 1 minute and extension at 72°C for 90 seconds.

RFLP

The amplicons of both species were digested using AluI (recognition sequence: AG□CT). Endonuclease digestion was carried out using amplified products, recommended restriction buffer (Enzyme buffer with BSA-TANGO), restriction enzyme (Alu) and distilled water. The restriction fragments were separated using 3% polyacrylamide gel electrophoresis and stained in ethidium bromide and visualized under UV light.

Restriction profiles of the ITS region indicated whether there was remarkable genetic heterogeneity within and between populations of both *D. semiclausum* and *D. mollipla* biotypes. The total number of samples that were screened in each *Diadegma* spp. were converted to proportions and the probability of accurate identification using morphological characters established.

3.3 RESULTS

3.3.1 Cage exclusion experiment

a) Wundanyi

During the first season in Wundanyi percentage DBM survival of 49% and 45% was observed in the caged and uncaged treatments respectively. There were significant differences in survival of the recovered DBM larvae/pupae (P=0.0001, df=14) between the caged and the uncaged treatments. 88% of the recovered DBM larvae/pupae in the caged treatments and 61% in the uncaged treatments emerged as DBM with mean parasitism of 17.3% and 5.3% from *D. semiclausum* and *O. sokolowskii*. Disease accounted for 5.5% while unidentified sources accounted for 6.7% of the total mortality (Table 3.1).

The second season at Wundanyi saw a drastic reduction in the number of DBM larvae/pupae recovered in the open cage treatments. A mean of 89% and 26% of the infested DBM larvae/pupae were recovered in the caged and the uncaged treatments respectively. A mean parasitism rate of 59.7% was recorded during this season with disease and unknown causes accounting for 7.1% and 4.4% respectively (Table 3.1).

A mean survival of 90.9% was observed during the first larval instar decreasing to 47.2% during the third instar and later to 33.3% in late fourth larval instar. Parasitism of 12.5% was first noted during the third larval instar stage rising to 35.4% in the fourth larval instar. Disease occurrence in this season gradually increased from 3.9% during the first larval instar to 25% during the late fourth instar (Fig. 3.1).

The second season at Wundanyi recorded the lowest DBM survival in all stages with the highest survival rate recorded being 27.5% during the first larval instar. Parasitism rates of up to 100% were recorded in this season during the fourth larval instar. Diseased larvae were observed only during the first three developmental stages, with the highest score being 21.9% during the third developmental stage (Fig. 3.2).

b) Limuru

Significantly higher numbers of larvae/pupae (P=0.0001) were recovered from caged treatments in comparison to uncaged treatments. During the first season in Limuru 54% of the infested larvae were recovered in the caged treatments whereas 51% were recovered from the uncaged treatments (Table 3.1). Among those recovered 85% in the caged treatments and 77% in the uncaged treatments emerged as DBM with an average parasitism rate of 8.6%, with disease accounting for 4.5% of the total mortality while unknown sources accounted for 10.1% (Table 3.2).

There was a reduction in the number of DBM larvae/ pupae recovered during the second season in Limuru. 62% of the infested DBM larvae were recovered in the caged experiments while only 40% were recovered in the uncaged case. 95% of the recovered DBM larvae/pupae successfully developed to adult DBM in the caged treatment compared to 77% survival in the uncaged treatments. A mean parasitism rate of 16.6% was recorded during this season whereas disease incidence and unidentified sources of mortality accounted for 1.9% and 3% of total mortality respectively (Table 3.2).

Percentage survival of the recovered DBM larvae or pupae gradually decreased from the first larval instar, with an average survival of 90%, until pupal stage which recorded 65.9% survival. This was accompanied by a stepwise increase of parasitism from 2.1%, during the second larval instar, to 16.8% at the pupal stage. Mortality from unknown sources (unidentified) remained constant throughout the entire developmental stages of diamondback moth (Fig 3.3).

There was an eminent reduction in DBM survival as it developed from one stage to the next during the second season at Limuru. Survival decreased from 100% during the first and second instar to 50% during the late fourth instar stage with parasitism of 12.5% and 50% observed during the early and late fourth larval instar respectively (Fig. 3.4).

3.3.2 Effects of *D. semiclausum* on the loss of DBM larvae

Results obtained from field-simulated screen house studies show that the number of larvae lost increased with increase in parasitoid density (Table 3.3). There were significant differences in the number of DBM recovered and parasitism rates at various *Diadegma* densities (P=0.0001, df=45). Survival decreased in a density-dependent linear fashion from 27.5% in one parasitoid pairs per cage to 13.1% in two parasitoid pairs per cage (Table 3.3) while parasitoid emergence increased in a similar manner from 25.6% to 34.6%.

However, there were no significant differences between the numbers of larvae lost in either one or two parasitoid pairs per cage treatments. An average of 47.4% and 47.0% of

the introduced DBM were lost in the two and one parasitoid pairs per cage treatments respectively, compared to 33.3% in control experiment. Among the larvae recovered from the ground 75% and 68% were parasitized in the two and one parasitoid pairs per cage treatments respectively (Table 3.3).

3.3.3 Effects of *D. semiclausum* on the local parasitoid fauna

A total of six hymenopterous parasitoids of *P. xylostella* were recovered from field collections conducted from the month of January to July, 2003, namely: *Diadegma semiclausum, D. mollipla, O. sokolowskii, Apanteles spp., Branchymera spp.*, and *Itoplectis spp.*

During the first season at Limuru five parasitoid species were collected, with *O. sokolowskii* constituting 67.5% of total parasitism. Other parasitoids that were recovered included: *D. semiclausum* 10.8%, *Apanteles spp.* 8.8%, *Branchymeria spp.* 8.0% and *D. mollipla* 4.8%. There was a reduction in parasitoid repertoire recovered during the second season with *D. semiclausum* being a major parasitoid, consisting of 97.1% of the total parasitism, while *D. mollipla* consisted of 1.7%, and *Branchymeria spp.* 1.3% (Table 3.4).

In Wundanyi all the six parasitoid species were recovered during the first season with *D. semiclausum* consisting of 86.2%, *O. sokolowskii* 8.6%, *Itoplectis spp.* 1.7%, *Apanteles spp.* and *D. mollipla* 1.2% each and *Branchymeria spp.*1.0% of the total parasitism. During the second season only two parasitoids, *D. semiclausum* and *O. sokolowskii* were

recovered from the field with *D. semiclausum* consisting of 98.9% of total parasitism (Table 3.4).

There was a general reduction in the number of local parasitoids recovered with increase in the number of *D. semiclausum*. For instance there was a gradual decline in the number of *O. sokolowskii* recovered from the field from the 4th week after transplanting till the end of the season (Table 3.4). During the second season parasitism due to *Oomyzus sokolowskii* was either very low or absent.

3.3.4 Molecular identification of the *Diadegma spp.* using PCR-linked restriction fragment length polymorphism analysis (PCR-RFLP)

Results obtained from the samples that were analyzed show that it is possible to distinguish between closely related sibling species, *D. semiclausum* and *D. mollipla* using PCR-RFLP of the COI gene. Similar fragment patterns were observed for all sibling species confirming the morphometric identification (Table 3.5).

Table 3.1 Mean±SE mortality of Diamondback moth larvae and pupae in cage exclusion experiments at Wundanyi Division, Coast Province of Kenya.

	Number of larvae:			Percentage mortality due to:			
Exclusion treatment	**Infested**	**Recovered**	**% Survival**	***Diadegma semiclausum***	***Oomyzus sokolowskii***	**Diseased**	**Not identified**
7 Months after release							
Caged and glued	60	30.8±3.8 a	85.2 ±1.3 a	3.8±1.5 b	0.8±0.8 a	4.2±2.5ba	6.0±1.2 a
Caged only	60	28.0±4.5 a	90.3±3.7 a	3.2± 1.4 b	0.7±0.7 a	0.8±0.8 b	5.0±2.3 a
Glued only	60	24.5 ±2.6 a	61.5± 2.3 b	18.0±2.0 a	3.9±2.6 a	10.2± 3.9a	5.8±2.3 a
Control	60	29.0 ±2.7 a	60.2±8.1 b	16.5±5.1 a	6.7 ±2.0 a	6.7±0.9ba	10.0±4.8 a
12 months after release							
Caged and glued	60	35.0 ±1.1 a	90.1± 2.5 a	0 b	0 a	4.9 ±1.2 a	2.1 ±2.1a
Caged only	60	27.5± 5.0 ba	88.0±5.2 a	2.3±2.3 b	0 a	4.9 ±3.2 a	4.9±2.1 a
Glued only	60	17.0±3.0 b	25.4±4.4 b	65.0± 2.2 a	0 a	6.2 ±2.5 a	3.4±2.0 a
Control	60	17.3±0.9 b	26.2± 3.8 b	54.3± 7.1 a	0 a	12.5±6.0 a	7.1 ±2.6 a

Means within the same column followed by a common letter are not significantly different (P>0.05), mean separation by Student Newman-Keuls test.

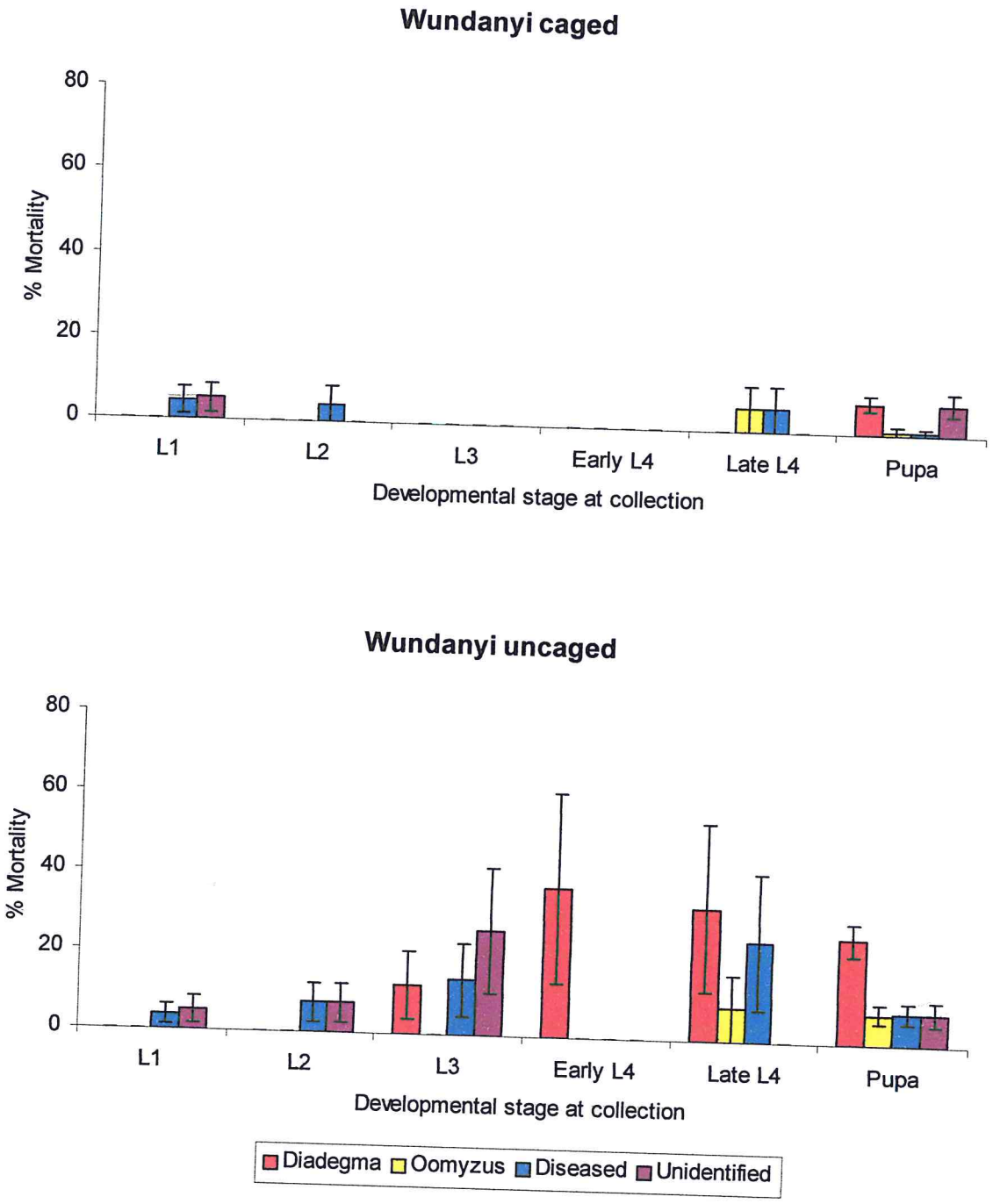


Fig. 3.1 Effect of exclusion of natural enemies on the mortality of Diamondback moth at different life stages in Wundanyi Division, Coast Province of Kenya 8 months after the release of *Diadegma semiclausum*.

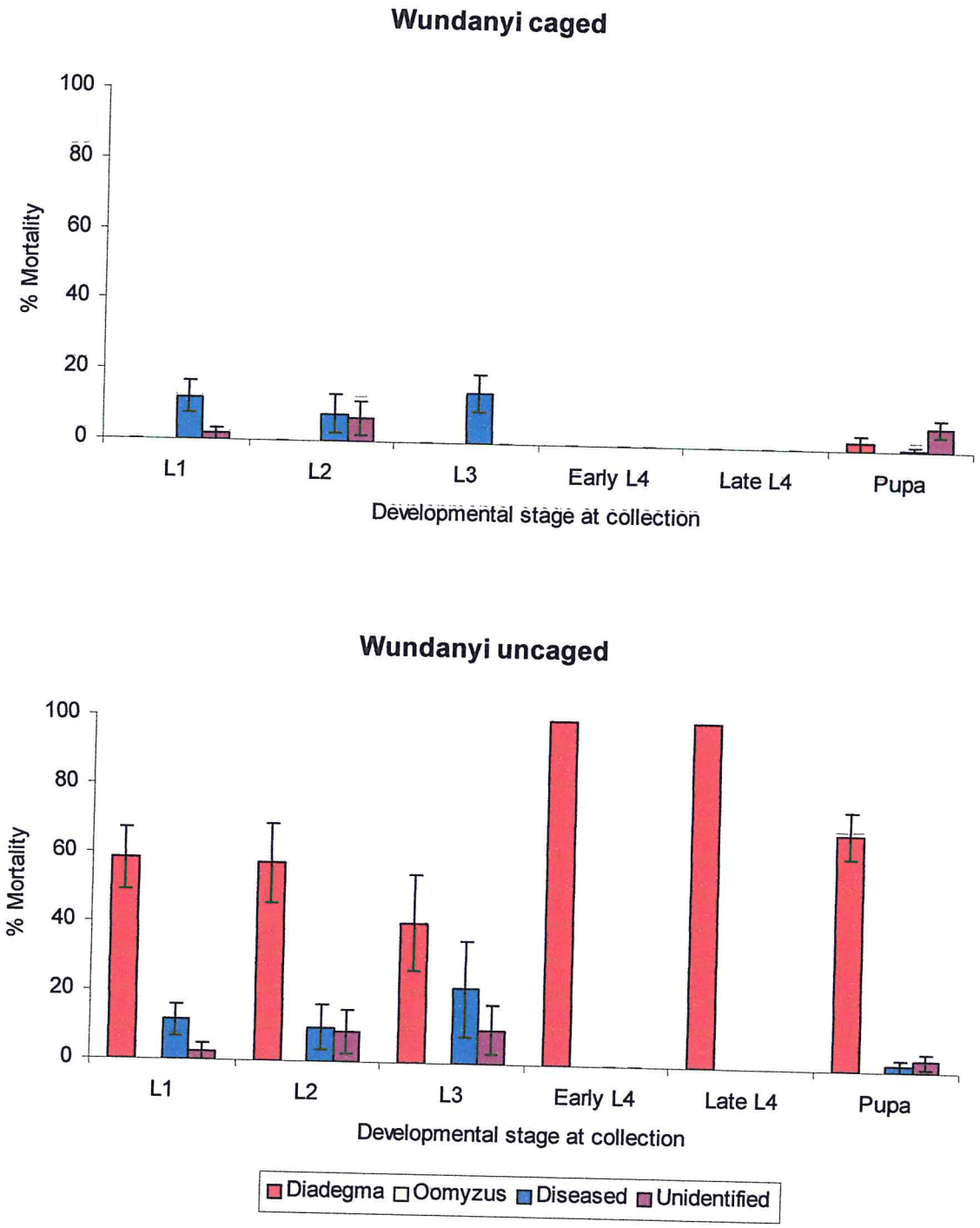


Fig. 3.2 Effect of exclusion of natural enemies on the mortality of Diamondback moth at different life stages in Wundanyi Division, Coast Province of Kenya 12 months after the release of *Diadegma semiclausum*.

Table 3.2 Mean±SE mortality of Diamondback moth larvae and pupae in cage exclusion experiments at Limuru Division, Central Province of Kenya.

	Number of larvae:			Percentage mortality due to:			
Exclusion treatment	**Infested**	**Recovered**	**% Survival**	***Diadegma semiclausum***	***Oomyzus sokolowskii***	**Diseased**	**Not identified**
8 Months after release							
Caged and glued	60	33.0±2.9 a	89.2± 2.4 a	0 b	0 a	2.0±1.2 a	8.7 ±2.2 a
Caged only	60	32.0± 3.2 a	80.2 ±4.8 a	0 b	0 a	5.9±2.8a	13.9 ±4.8a
Glued only	60	31.3± 2.9 a	78.1±2.2 a	8.0 ±1.6 a	0 a	3.8±1.8 a	10.1±2.0 a
Control	60	29.5± 0.9 a	76.9±6.9 a	9.2 ±2.7a	0 a	6.1± 2.3a	7.8 ±3.1 a
12 months after release							
Caged and glued	60	32.0±3.5 ba	95.3 ±0.8 a	1.1 ±1.1 b	0 a	1.3±0.8 a	2.3±1.6 a
Caged only	60	42.3± 4.5 a	95.1±1.7 a	0 b	0 a	1.8±0.6 a	3.2±1.5 a
Glued only	60	24.0± 4.8 b	77.8±4.8 b	18.1 ±4.4 a	0 a	2.6±1.7 a	1.5 ±1.5 a
Control	60	24.3± 1.7 b	77.0±2.0 b	16.0 ±3.4 a	0 a	2.1±1.2 a	4.9± 2.4 a

Means within the same column followed by a common letter are not significantly different (P>0.05), mean separation by Student Newman-Keuls test.

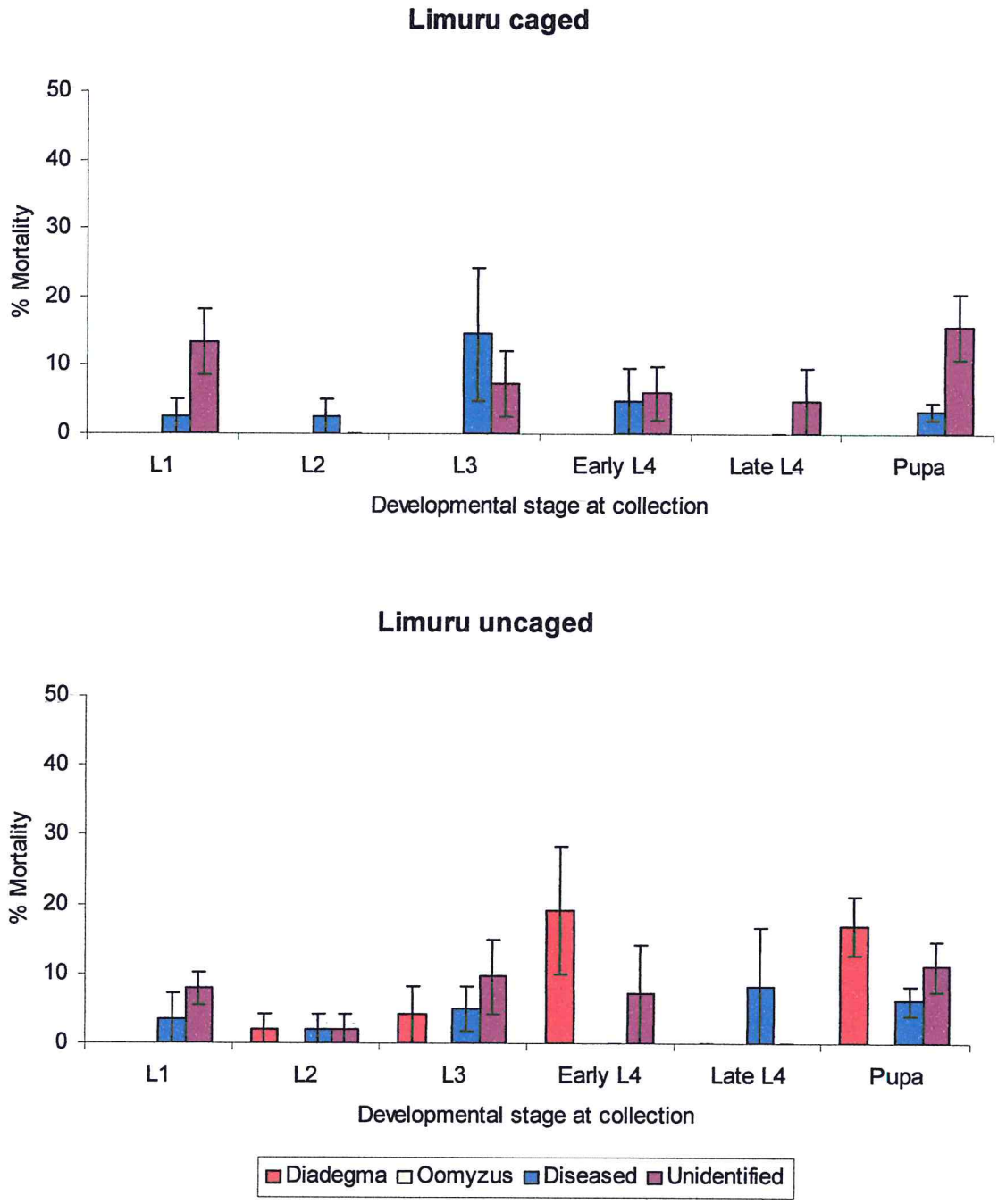


Fig. 3.3 Effect of exclusion of natural enemies on the mortality of Diamondback moth at different life stages in Limuru Division, Central Province of Kenya 8 months after the release of *Diadegma semiclausum*.

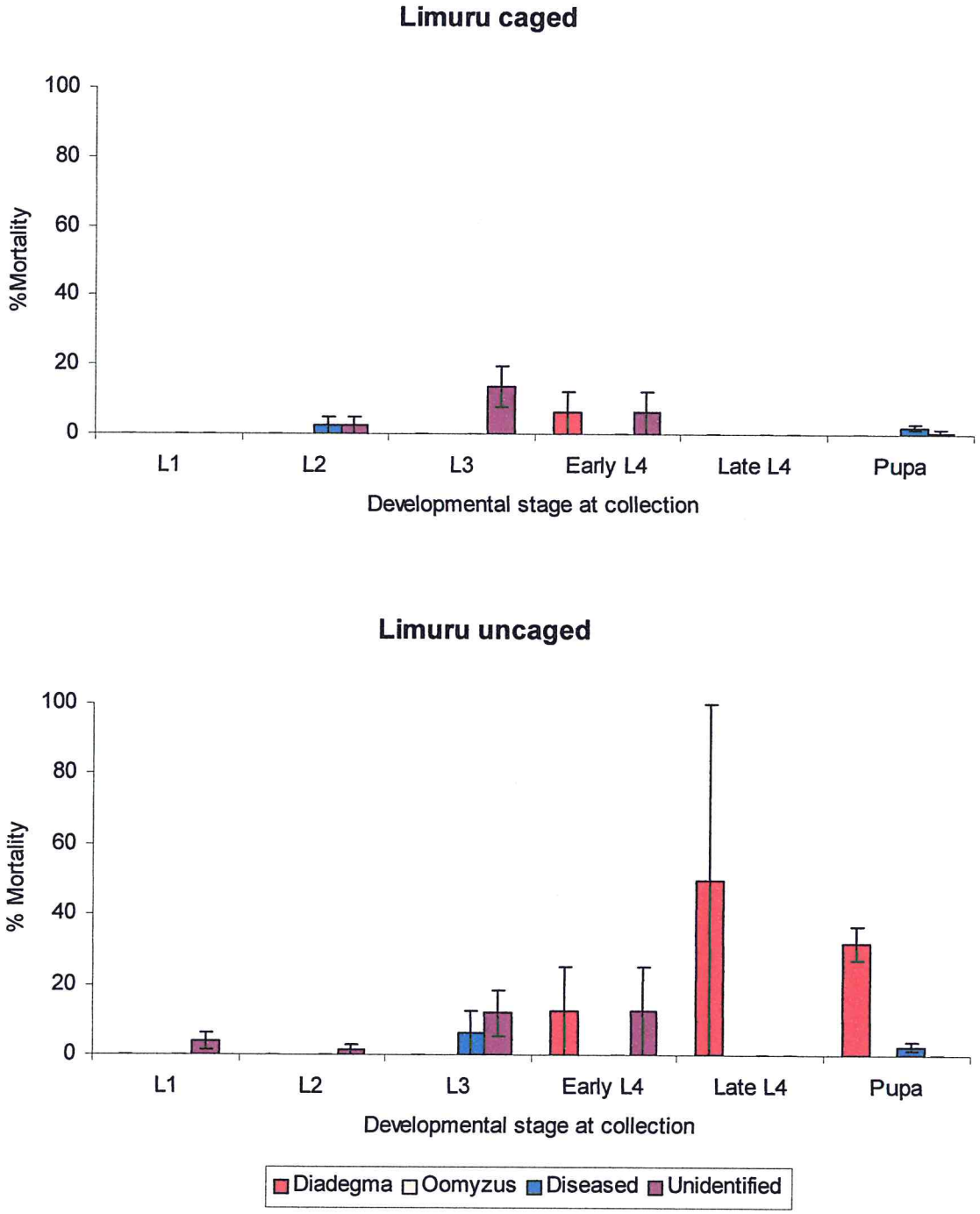


Fig. 3.4 Effect of exclusion of natural enemies on the mortality of Diamondback moth at different life stages in Limuru Division, Central Province of Kenya 12 months after the release of *Diadegma semiclausum*.

Table 3.3: Effects of parasitoid density on loss of Diamondback moth larvae from artificially infested cabbage plants.

Treatments	Percentage emergence		Percentage Larvae lost	% Parasitism of the dropped larvae
	Diamondback moth	*D. semiclausum*		
Two pairs of parasitoids				75.0
Glue at plant base	11.0±2.5 c	36.9±5.4 a	52.1±6.9 a	
No glue	15.2±3.1 c	42.1±4.2 a	42.7±5.3 a	
One pair of parasitoid				68.0
Glue at plant base	25.6±6.3 b	24.6±3.6 b	49.8±4.3 a	
No glue	29.4±8.5 b	26.5±4.8 b	44.2±5.3 a	
No parasitoid				0
Glue at plant base	61.3±7.3 a	0 c	38.8±7.3 a	
No glue	72.3±3.6 a	0 c	27.7±3.6 b	
Pr>F	0.0001	0.0001	0.04	

Means within the same column followed by a common letter are not significantly different (P>0.05), mean separation by Student Newman-Keuls test for comparison of means.

Table 3.4 Parasitism of field-collected diamondback larvae/pupae in two cabbage growing seasons (January- April and April-July, 2003) after release of *Diadegma semiclausum* at Wundanyi Division, Coast Province of Kenya.

	Seven months after release			Twelve months after release		
	n collected	% occurrence	% of total parasitism	n collected	% occurrence	% of total parasitism
DBM larvae/ pupae	1103	–	–	577	–	–
DBM adults emerged	533	48.3	–	276	47.8	–
died	163	14.8	–	22	3.8	–
total parasitoids	407	36.9	–	279	48.4	–
Parasitoid species						
Diadegma semiclausum	351	31.8±1.9ā	86.2	276	47.8±1.9ā	98.9
Diadegma mollipla	5	0.5±0.2 c	1.2	0	0±0 b	0
Oomyzus sokolowskii	35	3.2±0.9 b	8.6	3	0.5±0.7b	1.1
Apanteles spp	5	0.5±0.2 c̄	1.2	0	0±0 b	0
Brachymeria spp.	4	0.4±0.4 c	1.0	0	0±0 b	0
Itoplectis spp.	7	0.6±0.2 c	1.7	0	0±0 b	0

Means within the same column followed by the same letter are not significantly different (P>0.05), mean separation by Student Newman-Keuls test.

Table 3.5 Parasitism of field-collected diamondback larvae/pupae in two cabbage growing seasons (January- April and April-July, 2003) after release of *Diadegma semiclausum* at Limuru Division, Central Province of Kenya.

	Seven months after release			Twelve months after release		
	n collected	% occurrence	% of total parasitism	n collected	% occurrence	% of total parasitism
DBM larvae/ pupae	1202	–	–	1034	–	–
DBM adults emerged	850	70.7	–	768	74.2	–
died	103	8.6	–	27	2.6	–
total parasitoids	249	20.7	–	239	23.1	–
Parasitoid species						
Diadegma semiclausum	27	2.2±0.5 b	10.8	232	22.4±1.7a	97.1
Diadegma mollipla	12	1.0±0.3cd	4.8	4	0.4±0.4 b	1.7
Oomyzus sokolowskii	168	14.0±0.3a	67.5	0	0±0 b	0
Apanteles spp	22	1.8±0.4cb	8.8	0	0±0 b	0
Brachymeria spp.	20	1.7±0.6cb	8.0	3	0.3±0.4 b	1.3

Means within the same column followed by the same letter are not significantly different (P>0.05), mean separation by Student Newman-Keuls test.

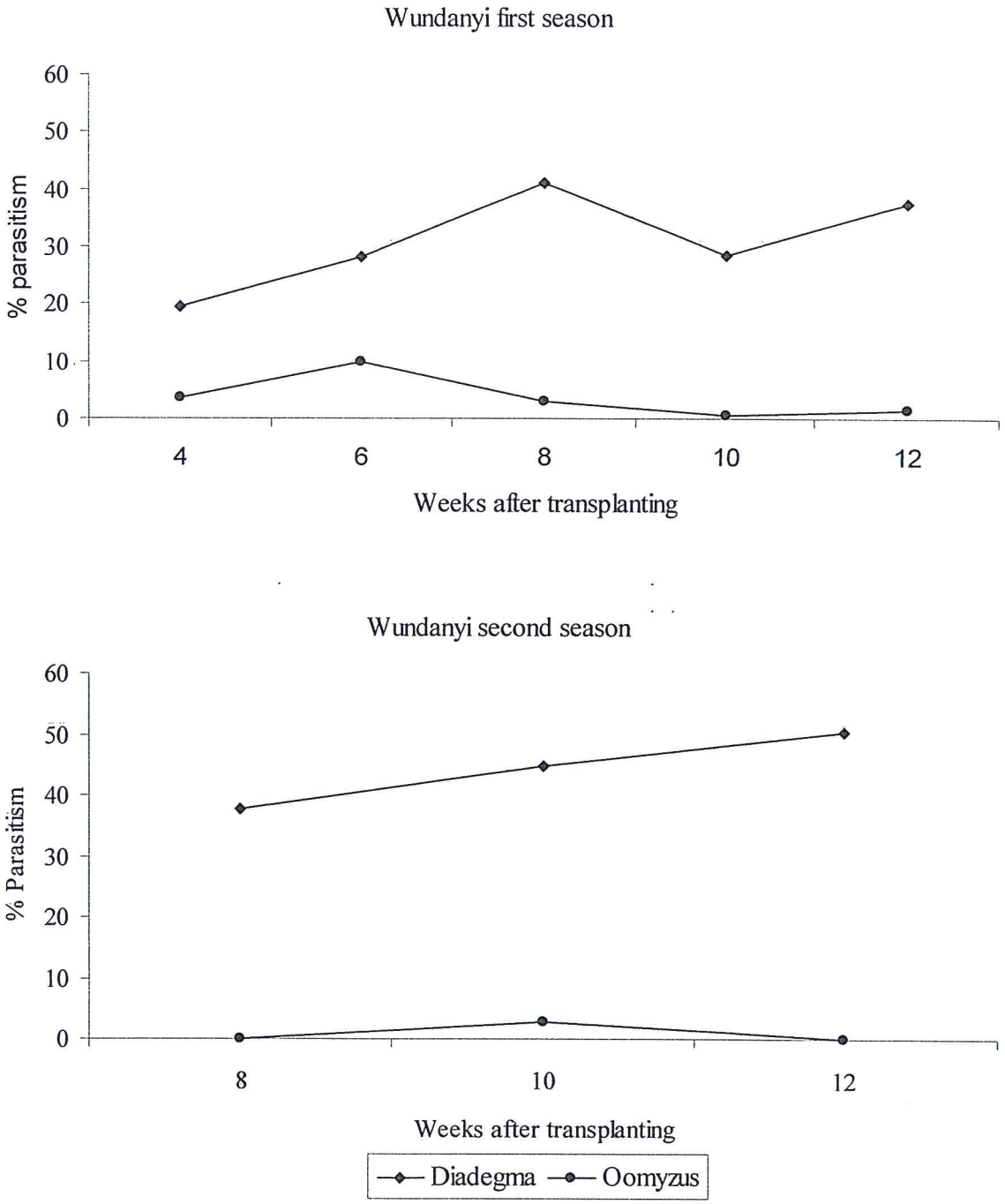


Fig. 3.5: Parasitism rates of *Plutella xylostella* by two major parasitoids (*Diadegma semiclausum and Oomyzus sokolowskii*) at Wundanyi Division, Coast province of Kenya during the first and second cabbage growing seasons (January- April and April-July), 2003.

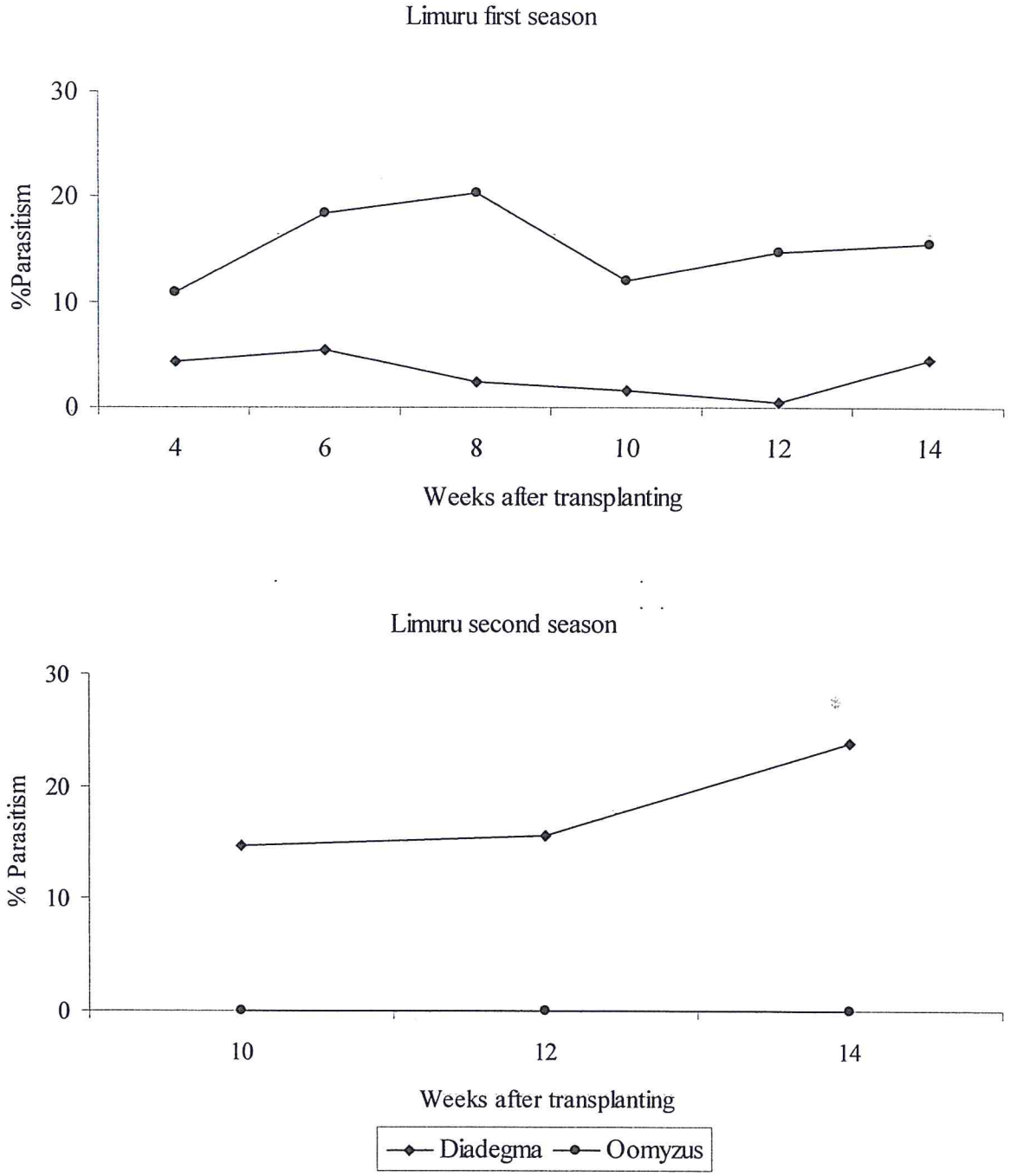


Fig. 3.6: Parasitism rates of *Plutella xylostella* by two major parasitoids (*Diadegma semiclausum and Oomyzus sokolowskii*) at Limuru Division, Central Province of Kenya during the first and second cabbage growing seasons (January- April and April-July), 2003.

Table 3.6 Number of *Diadegma semiclausum* and *Diadegma mollipla* screened using the Molecular technique (PCR-RFLP) to access the accuracy of morphological identification of the *Diadegma spp.*

	Morphological identification		**Molecular identification (PCR-RFLP)**		
Site and Sampling period	***D. semiclausum***	***D. mollipla***	***D. semiclausum***	***D. mollipla***	**% morphometric identification error**
Wundanyi					
First season	8	5	9	4	7.69
Second season	13	0	13	0	0
Limuru					
First season	7	6	7	6	0
Second season	9	4	9	4	0
Totals	37	15	38	14	7.69

Key: First season= January-April, 2003, Second season=April-July, 2003

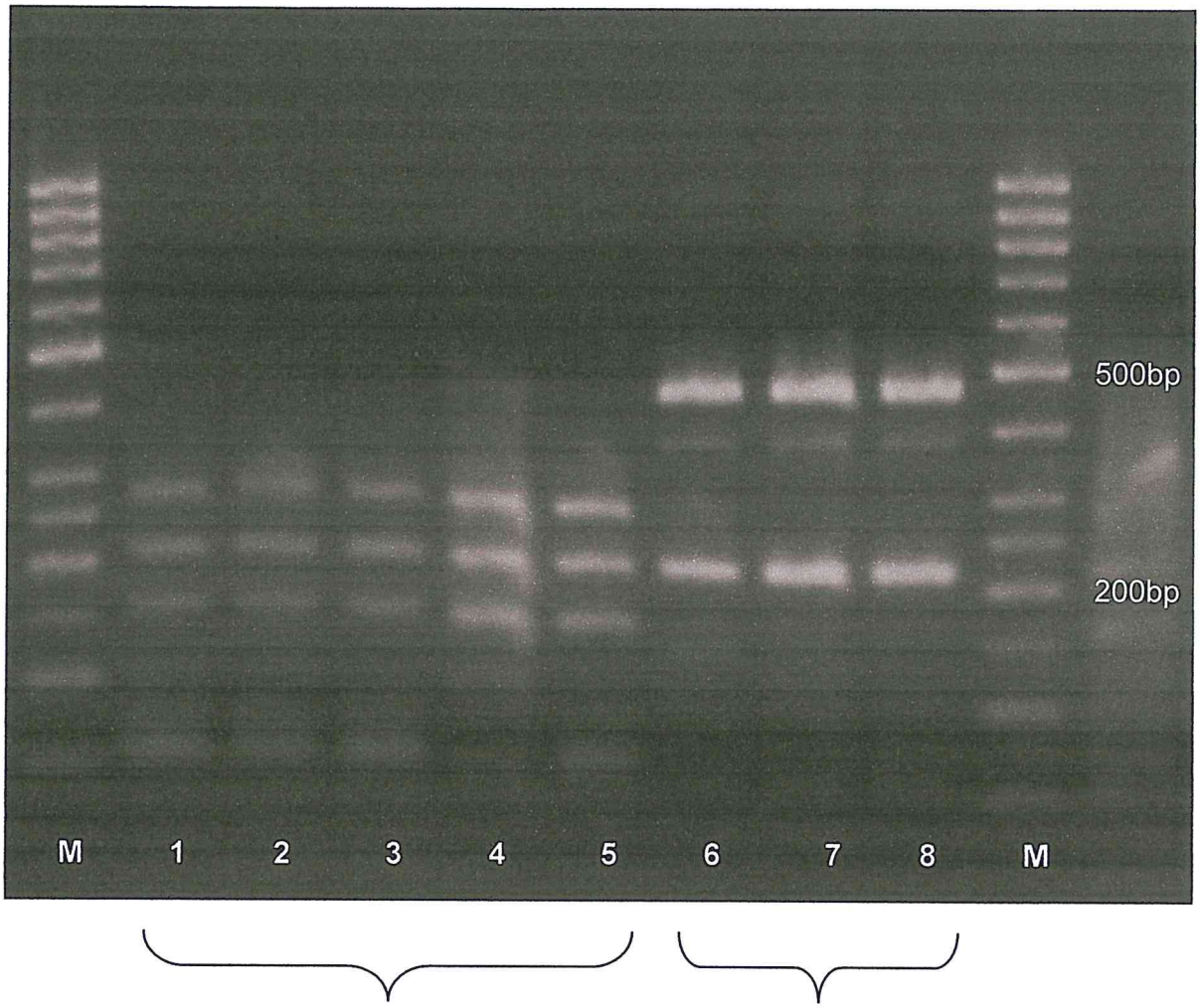


Plate 3.1 Restriction profiles of the ITS region showing the difference between *D. mollipla* and *D. semiclausum.*

Key: M= Size Marker

3.4 DISCUSSION

3.4.1 Contribution of *D. semiclausum* to overall DBM mortality

Evaluation of biological control projects and acquiring of biological information to portray the success or failure of a project is of primary importance to any scientist undertaking any biocontrol program. It is therefore imperative to note that increase in parasitoid activity lead to increased unexplained mortality probably due to increased parasitoid disturbance as a result of their heightened stinging effect on DBM. Apart from the obvious hosts used in progeny development (often recorded as percentage parasitism rate) others die as a result of mechanical trauma during oviposition whereas others, parasitized or not parasitized, are subjected/made more susceptible to other sources of mortality such as predation, death due to starvation following disturbance by foraging parasitoids, or disease (Godwin and Shields, 1984; Jones, 1987).

It is of paramount importance to note that the presence of natural enemies do enhance levels of mortality and often these mortality levels may even exceed losses attributed to demonsratable parasitism (DeBach, 1943; Pacala *et al.*, 1990; Hassell *et al.*, 1991; Jervis *et al.*, 1996). Therefore, this becomes critical in explaining successes of biological control agents (Neueschwander *et al.*, 1986; Briggs *et al.*, 1995). In such cases laboratory or green house experimental data, or field exclusion experiment comes in handy in explaining mortality caused by the host oviposition/feeding behavior and thus in shedding more light when evaluating the overall impact of a natural enemy in a system.

A low level of parasitism observed at Limuru was a demonstration that the introduced parasitoid, *D. semiclausum*, did not establish as quickly at Limuru as it did in Wundanyi. High DBM recoveries coupled with low parasitism rates at this site (Tables 3.1 and 3.2) sheds more light to our postulate that high unexplained mortality in Wundanyi area and subsequent low DBM population was as a result of increased parasitic activities of the introduced parasitoid. Results obtained from the screen house-based field simulated studies reveal that increase in parasitoid density lead to increased unexplained mortality (Table 3.3) and thus it becomes clear that unexplained loss of DBM larvae increase with increase in the number of parasitoids in the field.

The above fact is supported by the fact between 68% and 75% of the larvae recovered from the ground were parasitized. This finding makes it clear that this unexplained disappearance of DBM larvae is due to parasitoid disturbance by foraging parasitoid. The insect glue barrier method was used to measure the effect of ground dwelling predators that do not readily disperse through flight (Chiverton, 1987). By the fact that higher numbers of DBM larvae were lost in the plants with insect glue at its base than those without insect glue (Table 3.3) is probably an indication that some DBM larvae that drop to the ground as a result of parasitoid activity are able to regain access back to the plant.

By the fact that a number of larvae are lost as a result of parasitoid disturbance strongly point to another fact that it is very difficult to estimate or measure the mortality attributable to the parasitoid in question by merely determining field parasitism rates. A traditional approach of sampling field materials to assess parasitism rates and thereafter

assign mortality due to the parasitoid has always been known to be of limited value (Van Driesche *et al.*, 1991) and in this case it was shown to be an inaccurate measure of parasitoid related mortality because it cannot quantitatively measure the number of larvae lost due to parasitoid disturbance/ stinging effects.

It is important to quantify correctly the proportional losses due to the natural enemy and to associate this mortality with parasitoid and host density (Luck *et al.*, 1988; Van Driesche and Bellows, 1988; Simon and Minkenberg, 1994). Accurate measurement of mortality attributable to the parasitoid in question does require accurate estimate of recruitment to both the host stage susceptible to attack and to the pool of parasitized hosts (Van Driesche *et al.*, 1991). Consequently mortality attributable to parasitoid activities can only be measured with a certain level of accuracy by jointly determining the number of larvae that are lost as a result of parasitoid activities, those that die of parasitism during larval or pupal stage and those successfully utilized for parasitoid breeding in the field.

The above predicament can be surmounted by adding the number of larvae lost due to parasitoid disturbance to the established parasitism levels that were recorded from the recovered DBM. In this case the exclusion experiment approach provided a clear assessment of the degree to which *D. semiclausum* affects the average DBM density survival. Difference in recovery between the caged and the uncaged treatments give a better estimate of the number of larvae lost due to parasitoid disturbance (De Bach and Huffaker, 1971; Neuenschwander *et al.*, 1986; Dennis and Wratten, 1991). For instance during the second season at Wundanyi (Table 3.1) parasitism levels of around 65% were

achieved but this does not fully describe mortality due to *D. semiclausum* until 23%, percentage number of larvae lost due to the parasitoid foraging behavior, is appended to the established parasitism levels.

It is apparent that the differences in recoveries within seasons were due to increase in parasitism levels. For instance during the first season in Wundanyi the differences between the caged and the uncaged treatment was only 4% eight months after release increasing to 23% twelve months after release. In Limuru it was 3% eight months after release and 22% twelve months after release. Cage exclusion method is the fastest and most direct way to demonstrate the impact of a natural enemy on the pest population (Boavida *et al.*, 1995).

Disease occurrence on the recovered DBM larvae was relatively higher in open cage treatments compared to closed cages giving an indication that insects act as disease vectors or agents of disease dissemination from one plant to another as they look for food or host.

3.4.2 Effect of *Diadegma semiclausum* on local parasitoids fauna

Plutella xylostella is associated with a large number of hymenopterous parasitoids with more than 90 nominal species having been recorded from *P. xylostella* in various parts of the world (Goodwin, 1979; Fitton and Walker, 1992; Löhr and Kfir, 2002). A number of local parasitoids were recovered in the course of the study, including parasitic Hymenopterans such as *D. mollipla* Holmgren (Hymenoptera: Ichneumonidae), a larval

parasitoid, *Oomyzus sokolowskii* Holmgren (Hymenoptera: Eulophidae), *Cotesia spp.* (Hymenoptera: Braconidae), *Itoplectis spp.* (Hymenoptera: Ichneumonidae), which are larval-pupal parasitoid, and *Brachymeria spp.* (Hymenoptera: Chalcididae) which is a pupal parasitoid (Kibata, 1996; Oduor *et al.*, (1996); Ayalew *et al.*, 2002; Löhr and Kfir, 2002).

Local parasitoid densities were high during the first season particularly in the early part of the season decreasing drastically during the second season in both sites. *Oomyzus sokolowskii,* a parasitoid that has been described by Oduor *et al.*, (1996) as the most predominant parasitoid in Central Kenya was out rightly displaced by *D. semiclausum* during the second season though it had contributed 67.5% of total parasitism during the first season. *Apanteles spp.* which had contributed 8.8% of total parasitism during the first season was also displaced by *D. semiclausum* during the second season.

Out of the total number of larvae and pupae collected in Limuru division there was also a drastic decline in the number of *D. mollipla*, and *Branchymeria spp.* recovered during the second season. *D. semiclausum* contributed 10.8% of total percentage parasitism during the first cabbage growing season which increased to 97.1% within a span of only four months. In Wundanyi four of the local parasitoids recovered during the first season; *D. mollipla, Apanteles spp, Branchymeria spp.* and *Itoplectis spp.* were displaced during the second cabbage growing season and the contribution of *O. sokolowskii* to total parasitism decreased from 8.6% during the first season to 1.1% in the second season.

It is then clear that after the establishment of *D. semiclausum* there was an apparent and sudden decline in the population of the local parasitoids. It is evident from this study that *D. semiclausum* is gradually displacing the local parasitoids in Kenya.

Similar results were observed after the introduction of braconid parasitoid of the genus *Opius* (Bess *et al.,* 1961). First O. *longicaudatus* (Fullaway) was introduced to control *Dacus dorsalis* (Hendel) but parasitism was low. When *O. vandenboschi* Fullaway became established it virtually eliminated O. *longicaudatus* (Fullaway) through high percentage parasitism and *O. oophilus* (Fullaway) after introduction rapidly increased in numbers and caused a much higher percentage parasitism and eventually eliminated the other two. The same has been observed in America between *Cochliomyia megacephala* (Fabricius) and *C. macelaria* (Fabricius) (Greenberg and Szyska, 1984), in Florida between *Cotesia plutellae* (Kurdjumov) and *D. semiclausum* (Hu *et al.*, 1998) and in China between *C. plutellae* and *O. sokolowskii* (Shu *et al.*, 2000).

Diadegma semiclausum has become the most abundant and dominant parasitoid associated with *P. xylostella* and is currently a key (most variable) mortality factor of diamondback moth in the two pilot study sites barely one year after its release. This has suppressed the host population to a point which local parasitoids can not continue to persist.

Though the introduced parasitoid has significantly contributed to the mortality of *P. xylostella* in Kenya as shown by the preceding section, this perceived displacement of the

local parasitoids by *D. semiclausum* raises serious ecological concern over their fate. However, parasitism rates achieved by *D. semiclausum* were by far greater than those achieved by all other local parasitoids combined, leaving unanswered question whether DBM is the primary host of these parasitoids. *D. mollipla* has been reported to parasitize *Phthorimaea operculella* (Zeller) (Potato tuber moth) (Azidar *et al.*, 2000), and it is likely that these parasitoids switched from other hosts and can as well go back to their original hosts. A recommendation is made that more work be done along this line to establish if these parasitoids switched from other hosts to DBM.

3.4.3 Molecular identification of the *Diadegma spp.* using PCR-linked restriction fragment length polymorphism analysis (PCR-RFLP)

Molecular taxonomy is the identification of species based on molecular rather than morphological characteristics. Molecular techniques have been known to provide a rapid means of identifying obscure members of species and to differentiate species that are morphologically indistinguishable (Black and Munstermann, 1994).

This study was undertaken to examine sequence variation of the second internal transcribed spacer/ ITS2 of the ribosomal DNA within and among the two species of *Diadegma* using the PCR-linked restriction fragment length polymorphism analysis (PCR-RFLP). PCR-RFLP is a simple and inexpensive technique compared with normal sequencing of the ITS2 region. This has been used to distinguish *D. mollipla* from *D. semiclausum* (Wagener *et al.*, 2002).

Poorly resolved taxonomy still remains a major factor in limiting exploitation of *Diadegma spp.* in biocontrol of *P. xylostella* (Azidah *et al.*, 2000). Many species in this genus have been misidentified with many of the characters used for species discrimination in the past having proved unreliable due to its high variability (Azidah, 1997). For that reason, molecular technique based on PCR-RFLP is a more reliable technique that can give clear/indicative differences between *D. semiclausum* and *D. mollipla* (Wagener *et al.*, 2002).

The two species of *Diadegma* (*mollipla and semiclausum*) can be correctly identified using morphometric characters such as the veins of the forewing and the color of the posterolateral part of the postpetiole of the first metasomal tergite (Azidar *et al.*, 2000). However this requires taxonomic expertise and the identification error construed using genetic technique is a clear indication that morphometric identification can be confusing and therefore the need for an additional and more accurate method to distinguish the two species. It is reasonable that PCR-RFLP be used as a supplement to the commonly used morphological keys to distinguish between the two *Diadegma spp.* (Wagener *et al*, 2002)

CHAPTER 4

4.0 GENERAL CONCLUSSIONS AND RECCOMENDATIONS

The introduction of *D. semiclausum* in Kenya has resulted in the increase of parasitism of *P. xylostella* recorded in the field from the conservative 15% to 60% only a year after its introduction. This is a clear indication that this form of biological control is viable and will in the long term contribute significantly to the control of diamondback moth to economically insignificant levels in Kenya.

Results from field simulation studies pointed out that parasitoid disturbance lead to a significant increase in unexplained loss of larvae thus explaining why fewer DBM were recovered in subsequent open cage exclusion experiments and cabbage growing seasons. It is obvious from this study that the rate of parasitism is a big underestimation of the killing power of *D. semiclausum* and hence its effect on the host population. Our data indicate that more parasitized larvae abandon the plant and perish than pupate and produce an adult parasitoid.

Displacement of indigenous parasitoids by the introduced *D. semiclausum* was documented at both pilot sites from the exclusion experiments and even more clearly from the samples collected from the study plots. Among the indigenous parasitoids only *O. sokolowskii* can be considered a specific diamondback parasitoid and this may be the reason why it persisted. The next most important local species, *D. mollipla,* was shown to have no preference for cabbage plants, no matter whether infested with the host or not (Löhr and Rossbach, 2004). We therefore assume that this species as well as the even

less frequent parasitoids *Itoplectis* sp. (Hym.: Ichneumonidae) and *Apanteles* sp. (Hym.: Braconidae) may disappear completely from the cabbage environment.

It is therefore necessary that these studies be carried further to investigate what becomes of the local parasitoids especially after a long period of competition between them and the introduced exotic parasitoid, *D. semiclausum*. This study is a likely indication of what will take place in the population dynamics of *P. xylostella* and other local parasitoids in the field after the introduction of *D. semiclausum* in Kenya.

The overall average egg loss was almost 50% and hence of great importance for population development. No egg parasitoids were recorded in our trials even though trichogrammatids have been reported on diamondback moth in East Africa (Abera *et al.*, 2002) and elsewhere (Kasuki Miura, 2003). Therefore, natural occurrence of egg parasitoids seems to be scarce and of little importance. Since eggs do not normally detach on their own, predation is likely to be the cause of egg disappearance.

The role of predators in diamondback moth control has been stressed by Oatman and Platner, (1969) however; there was no direct evidence from our experiments that predators play a major role. Crawling predators did not seem to have an effect on DBM larvae survival as the differences in losses between glued and non-glued plants in cage exclusion experiments were not significant.

The role of diseases as mortality factor was very low in view of their potential as documented by Oduor *et al.*, (1996). However, considering the fact that the trials were conducted in the dry season, this is not surprising, as diseases have been found to be more prevalent during the rainy seasons (Lim, 1992; Riethmacher *et al.*, 1992).

It is hoped that the results of this study will also give an indication of the potential of *D. semiclausum* on the control of DBM in Kenya. Thus it is recommended that further studies be carried out along this line to provide the understanding of the eventuality of this perceived displacement competition.

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