Biological Control of Diamondback Moth

The Roles of Predators, Parasitoids and Insecticides

Freddy Miranda Ortiz
Faculty of Natural Resources and Agricultural Sciences
Department of Ecology
Uppsala

Doctoral Thesis
Swedish University of Agricultural Sciences
Uppsala 2011

Acta Universitatis agriculturae Sueciae
2011:35

Cover: Diamondback moth stages (eggs, larvae, adults) on a cabbage plant with predators (spider and reduviid bug) and a parasitoid ready to attack. Illustration by Hernán Guzman, UppsalAnimation, based on photographs by Karin Eklund.

ISSN 1652-6880
ISBN 978-91-576-7580-4

Print: SLU Service/Repro, Uppsala 2011

Biological Control of Diamondback Moth. The Roles of Predators, Parasitoids and Insecticides

Abstract

The diamondback moth *Plutella xylostella* L. (Lepidoptera: Plutellidae) is a serious pest of economically important crucifer crops such as cabbage. The moth has developed resistance to all tested insecticides and further studies on the potential role of factors affecting *P. xylostella* survival, including natural enemies, are urgently needed. One aim of this thesis was to identify the species that are natural enemies of *P. xylostella* and to evaluate their role in the natural biological control of this pest insect. Another aim was to gain knowledge that could be used to develop biological pest control methods with the potential for high efficacy against *P. xylostella*, thus avoiding the side effects of traditional chemical control while maintaining production and profits. This study was carried out in Estelí, Nicaragua during five cropping seasons, from 2006 to 2008. The results indicate that there is a broad spectrum of predators present in habitats within and around cabbage fields, and that these have the capacity to feed on *P. xylostella* eggs and larvae under laboratory conditions. The predators with the highest consumption rates were insect larvae (Syrphidae) and spiders in the families Linyphiidae and Salticidae. The most abundant predators, which also had the highest consumption rate and consequently the highest potential for suppressing *P. xylostella* populations, were spiders (Lycosidae) and rove beetles (Staphylinidae), although sheet weaving spiders, jumping spiders, assassin bugs (Reduviidae) and damsel bugs (Nabidae) may also be important. It is concluded that these generalist predators should be considered for further study in the field as candidate species with a role in the management of the pest *P. xylostella*. An exclusion experiment in the field showed that flying and ground dwelling natural enemies of *P. xylostella* interact negatively with each other. In another study, leaf damage was found to be higher in insecticide treated fields than in untreated fields as a combined consequence of insecticide resistance in the pest and lower predation from natural enemies which are reduced in number by the insecticide applications. In the last study, the main focus was to identify whether a combination of bio-control agents, i.e. parasitoids and a biological insecticide (*Bt*), interact additively, negatively or positively, in affecting the mortality of *P. xylostella*. It is concluded that a combination of control measures, including the promotion of predators and parasitoids, is probably needed to achieve sustainable biological control of the diamondback moth. To succeed with such approaches we must, however, learn more about the particular roles of different predators.

Keywords: spiders, generalist insect predators, consumption rate, natural enemies, *Plutella xylostella, Diadegma insulare,* biological control.

Author's address: Freddy Miranda Ortiz, SLU, Department of Ecology,
P.O. Box 7044, 750 07 Uppsala, Sweden
E-mail: Freddy.mirandae@slu.se, Freddy.miranda@una.edu.ni

Dedication

I dedicate this thesis to my beloved father, Nicolas Miranda of blessed memory, who was not educated, but who valued education greatly and did all he could so that I would receive an education.

The moral and spiritual support and examples of hard work provided by my mother Ramona Ortiz Castillo, my wife Silvia Lanuza Marquez and my daughter Fryda have been my inspiration.

Contents

List of Publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

I Miranda, F., Bylund, H., Grönberg, L., Larsson, L., and Björkman, C. 2011. Population Density and Killing Capacity of Predators of Eggs and Larvae of the Diamondback Moth in Nicaragua. Environmental Entomology 40: 333-341.

II Bylund, H., Miranda, F., Bommarco, R., and Björkman, C . Separating effects of flying and ground dwelling natural enemies on survival of *Plutella xylostella* larvae. (Manuscript)

III Bommarco, R., Miranda, F., Bylund, H., and Björkman, C. Insecticides suppress natural enemies and increase pest damage in cabbage. (Submitted manuscript)

IV Miranda, F., Björkman, C., Bommarco, R. and Bylund, H. Combined and separate effects of parasitoid release and *Bacillus thuringiensis* spraying on diamondback moth control and crop damage (Manuscript).

Abbreviations

DBM	Diamondback moth
DAT	Days after transplanting
OPS	Organizacion Panamericana de la Salud
Bt	*Bacillus thuringiensis*
IPM	Integrated Pest Management
USD	US Dollar

(This is an example of an empty page. A white rectangle is drawn on top of the page number.)

1 Introduction

1.1 The importance of Crucifer crops

Crucifers such as common cabbage (*Brassica oleracea* var. Capitata L.) are important vegetables grown throughout the world. The cabbage is an economically important vegetable in the countries of the Caribbean (Alam, 1992), in Eastern and Southern Africa (Jankowski *et al*., 2007), Asia (Talekar, 1996; Amend and Basedow, 1997), and Central America (Andrews, *et al*., 1992). In many countries, production of cabbage is greatly limited by insect pests, especially the diamondback moth [DBM]. In Nicaragua, cabbage is grown mostly by small-scale farmers in rural areas. The farmers often use high input intensive horticultural practices because the sale of these vegetables is an important source of cash income (Varela, 1991). In Central America, cabbage is produced all year-round. In some areas of the world, it is produced only during rainy seasons and fields are left fallow during the dry season, but in other areas, cabbage is continuously cropped using irrigation during the dry seasons (Andrews *et al*., 1992).

1.2 Background

The diamondback moth (*Plutella xylostella* L.) is a major pest insect in more than 100 countries across the globe; it affects cruciferous plants, especially *Brassica oleracea* crops such as cabbage, cauliflower, broccoli, Brussels sprout and turnip (Talekar *et al*., 1992; Alam, 1992; Morallo-Rejesus and Sayaboc, 1992). It is a globally important pest, causing serious yield losses to crucifers (Arora *et al*., 2000; Talekar and Shelton, 1993). In India the estimated annual crop losses due to this pest amount to 16 million USD (Mohan and

Gujar 2003) the loss has been estimated to be 40-70 million USD for cabbage and broccoli in Texas (Shelton 2004). Insecticide application is the primary method of control of DBM (Andrews *et al*., 1992; Beck and Cameron 1992; Talekar and Shelton, 1993). This insect has, however, developed resistance to virtually all commercial insecticides and the pest problem is particularly severe in many tropical and subtropical countries because of the indiscriminate use of pesticides (Cheng, 1988; Miyata *et al*., 1986; Sun *et al*., 1986; Sun, 1992), resulting in DBM resistance to insecticides throughout Central America (Andrews *et al*., 1992; Ovalle and Cave, 1989; Perez *et al*., 2000).

Agriculture in the Central American region is characterized by the excessive use of agrochemicals with the trend being towards an increase in their use. Currently, an average of 1 to 1.5 kg of agrochemicals per person per year is used in the region. In El Salvador, over the last ten years, imports of agrochemicals have doubled. In Nicaragua in 2009, 63.8 million dollars worth of agrochemicals was imported (MAGFOR, 2009) and, according to Organizacion Panamericana de la Salud (OPS) statistics, there are 67,800 cases of poisoning of humans every year. Insecticides cost the country 7.9 million USD for medical care related to poisoning each year and 1.8 million USD in environmental damage. These are considerable costs for developing countries' economies (CIEA, 2006).

Problems with insecticides were discovered not long after their large scale use was introduced to agriculture, after the Second World War (Metcalf, 1980). This sparked research activity that demonstrated how the indiscriminate use of insecticides, a common practice to control DBM (the main pest insect on cabbage in Nicaragua), is to apply insecticides, sometimes as frequently as twice per week (F. Miranda personal communication with farmers, 2007). This could lead to human health problems (Kamel and Hoppin, 2004; McCauley *et al*., 2006), increased pressure on overall biodiversity (Tilman *et al*., 2001), development of resistance in pests (Georgiou and Mellon, 1983), and problems with secondary pests (Metcalf, 1980). Secondary pests occur when insecticides eliminate non-target parasitic and predatory natural enemies so that previously low populations of herbivorous insects are released from natural control and break out as crop pests. In some cases, the emergence of secondary pests in combination with the development of insecticide resistance in the primary pest, has led to the collapse of entire cropping systems, such as that of cotton in Central America (Metcalf, 1980).

In Central America, populations of DBM in Honduras have developed resistance to the synthetic insecticides commonly used to control this insect on crucifers (Ovalle and Cave, 1989). In addition, the species has developed resistance to the "biological" insecticide *Bacillus thuringiensis*, Berliner (Tabashnik, 1994). Despite the development of resistance to insecticides, vegetable growers worldwide continue to apply pesticides in the vain hope of controlling DBM in the field (Talekar and Shelton, 1993). Farmers in Tisey, Estelí, Nicaragua, have reported high densities of DBM in fields frequently sprayed with cypermethrin and deltamethrin (F. Miranda personal communication with farmers 2007) and high levels of resistance to these compounds are now known to occur in Nicaraguan populations of DBM (Perez *et* a*l*., 2000).

Alternative control measures that are simple and inexpensive to implement have not been properly developed with the consequence that farmers continue to use chemical pesticides, thereby exacerbating problems of resistance, environmental pollution, degradation of important ecosystem services, such as control by natural enemies, and creating significant hazards to human health (Corriols *et al*., 2009).

The general biology of DBM is well known as, to some extent, are its population dynamics. However, much variation in abundance has been identified and the mechanisms involved in the species' population dynamics are poorly understood. In Central America very little is known about the mortality factors affecting DBM and the extent to which natural enemies affect population dynamics (Andrews *et al*., 1992). Such knowledge is essential when trying to develop biological control methods for insect pests.

In summary, the problems associated with insecticide use are that: insecticide application results in high toxicity of field crops; the insecticides have a wide action spectrum, strong residuality, high economic costs, pose human health hazards and have negative effects on the populations of natural enemies, as well as DBM having developed resistance to them.

The overall goal of the studies presented here was to acquire information that might serve as the basis for identifying biological control agents for use in cabbage cultivation in Nicaragua, where the investigation took place, and in neighboring countries. The need for this kind of information is, currently, very great since none of the crucifers cultivated in Central America can be exported readily to the United States and Europe due to the high level of insecticide contamination and visible damage to the product (Nicholls and Altieri, 1997). This tends to exclude large numbers of small farmers from these important markets because small farmers depend heavily on chemical pest control.

1.3 The role of parasitoids in biological control

Parasitoids are insects that lay their eggs inside or on the outside of the eggs or bodies of other insects. The parasitoid eggs hatch and the emerging larvae begin to consume the "host" insect from the inside, eventually killing the host and emerging as free-living adults. Parasitoids have been a favorite subject for biological control programs because they tend to be highly specific to one or a few species of host, and therefore can be used to target specific pests. Numerous studies have suggested that parasitoids play an essential role in the natural control of DBM (Harcourt, 1960; Pimental, 1961; Talekar and Shelton, 1993).

Many parasitoid species have been recorded on the DBM. Forty-eight species were catalogued by Thompson (1946), while Goodwin (1979) stated that there are more than 90. Among these, six species attack diamondback moth eggs, 38 attack larvae and 13 attack pupae (Lim, 1982). However, not all parasitoids are effective natural enemies. Lim (1992) reported that the potential to function as biological control agents varied between species, often depending not only on their direct relationships with their host(s) but also on interrelationships between each other (interspecific interactions) and the environment. Evaluation of the impact of natural enemies on *P. xylostella* is fundamental to the design of pest management programs (Verket and Wright, 1997; Furlong *et al.*, 2004).

Diadegma insulare (Cresson) (Hymenoptera: Ichneumonidae) is one of most important parasitoids of the DBM in North America (Biever *et al.*, 1994; Mitchell *et al.*, 1997; Godin and Boivin, 1998) and is most abundant in the United States (Mukenfuss *et al.*, 1992; Shelton *et al.*, 2002). *D. insulare* is the principal regulator of *P. xylostella* (Mustata, 1992, Azidah *et al.*, 2000). Compared with other parasitoids, it is an efficient host searcher (Xu *et al.*, 2001; Wang and Keller, 2002).

Diadegma insulare has been reared successfully in greenhouse conditions with a parasitism rate of 95% and a population comprising 45–63% females (Xu *et al.*, 2001). The strategy of biological control is to reestablish top-down control by reintroducing the natural enemies of the pest into its new range. This has been the conceptual underpinning of classical biological control for over 100 years and it continues to be applied today (Van Driesche and Bellows, 1996). Biological control agents that greatly reduce their target species while remaining host-specific will reduce their own population through density-dependent resource depletion (Pearson and Callaway, 2005).

1.4 The role of generalist predators in biological control

Predatory insects and spiders kill and consume their prey and tend to be generalists: they have a fairly broad diet of arthropod prey that they can feed on. Mortality caused by invertebrate predators and parasitoids is an important factor in the regulation and dynamics of pest populations but the role of predators has often been underestimated (Symondson *et al.*, 2002). Spiders and many generalist insect predators consume large numbers of prey, and cause no or only minor damage to plants. Such predators can reach high enough densities to be important agents in pest control, although at very high densities their numbers may be reduced by territorial behavior and cannibalism (Lee and Kim, 2001). Spiders in particular have long been considered important predators, able to help regulate the population densities of insect pests, especially in agricultural ecosystems (Pickett *et al.*, 1946, Dondale, 1956, Duffy, 1962, Kajak, *et al.*, 1968; Dondale, 1972, Tanaka, 1989). Lee and Kim (2001), for example, reported a large number of spiders, i.e. 175 genera, in 99 families, many of them with high population densities, in Korean rice fields.

Multiple predator species can interact and their combined effect on lower trophic levels may result in complex non-additive effects on prey populations and community structure (Griswold and Lounibos, 2006). Sometimes the interactions between predator species are synergistic and the impact of several species together will then be greater than the sum of the impacts of individual species (Simberloff and Holle, 1999). Some results indicate that the importance of ground-foraging predators in agroecosystems may need to be reevaluated and that positive interactions between predators must be considered in models predicting the impact of multiple predator complexes (Losey and Denno, 1998). The effect of predators needs to be considered when analyzing their effective use in the control of DBM (Polis *et al.*, 1989; Krupa, 1996).

To date, the majority of studies of natural enemies, in general, and of those attacking DBM, in particular, mainly relate to parasitoids. Predation and other sources of mortality have historically been very much ignored and are poorly understood (Lim, 1992; Talekar and Shelton, 1993; Furlong *et al.*, 2004; Ma *et al.*, 2005ab). If predators are discussed at all, they are often merely listed in terms of the species found in traps in crucifer fields (Lim, 1992) and only a few papers report experiments where predators have actually been shown to predate DBM. For example, Alam (1992) reported that the most common groups of insect predators found in cabbage field were coccinellids, chrysopids, syrphids and staphylinids. However, some recent publications have added important knowledge about the role of predators (Straub and Snyder, 2006; Furlong *et al*., 2008; Furlong and Zalucki 2010). Thus, there seems to be potential for the development of more sustainable biological control methods by learning more about generalist predators.

1.5 *Bacillus thuringiensis* in the management of DBM

Bacillus thuringiensis (Bt) is a bacterium that occurs naturally in soils worldwide. Its insect control potential was recognized in the early 1900s. The first commercial product using it was made in 1938 (Facknath, 1999). *Bt* has been extensively studied throughout the world and is now widely accepted as a pest control agent. *Bt* is the best known and most widely used pathogen acting against insects and it produces a toxin that attacks the larvae of many Lepidoptera (Brunner and Stevens, 1986). *Bt* has been shown, in some circumstances, to exert good control of DBM in association with indigenous parasitoids and introduced biocontrol agents. *Bt* has been found to be considerably more effective at controlling *P. xylostella* than the chemical insecticides cypermetrin, permetrin, methomyl and thiocard (Leibee and Savage, 1992).

In Nicaragua, two main formulations of microbiological *Bt* have been used for the control of *P. xylostella*. Dipel is a commercial formulation of the HD-1 *Bacillus thuringienses* Berliner var. Kurstaki, containing 3.2 % active ingredient. Javeling W.G is a commercial formulation of the NRD-12 strain of *Bacillus thuriengiensis* var. Kurstaki, containing 6.5% active ingredient. In Nicaragua, *Bt* applications have been shown to reduce populations of DBM without affecting natural enemies (Miranda, 1992). The absence of negative effects of *Bt* spraying on the survival of parasitoids has also been demonstrated in other studies (Andrews *et al*., 1992; Brunner and Stevens, 1986)

2 Aims of the thesis

The first aim of the work underlying this thesis was to identify the species of natural enemies that attack *P. xylostella* and to evaluate their role in the natural biological control of this pest insect. The second aim was to gain knowledge that could be used to develop biological pest control methods that are highly effective against *P. xylostella* and thus avoid the side effects of traditional chemical control, while maintaining productivity and profits.

Hopefully the results presented in this thesis can provide the basis for straightforward rational alternative methods for farmers who currently use insecticides to control *P.xylostella*. Such alternatives should be particularly useful for farmers in developing countries, allowing them to reduce their use of pest control chemicals.

The specific objectives were:

To identify and quantify the abundance of the species of natural enemies that attack *P. xylostella* and which occur in and around cabbage fields in Nicaragua.

To assess the efficiency with which predators feed on the eggs and larvae of *P. xylostella* in the laboratory and to evaluate their role in the natural biological control of this insect.

To determine whether the identified predators preferentially prey upon a particular size of *P. xylostella* larvae (small vs. large).

To determine the relative importance of and interaction between flying and ground dwelling arthropod predators and parasitoids for mortality of *P. xylostella* and thereby to identify combined effects of the two guilds.

To determine the effect of insecticides on the density of *P. xylostella* and its natural enemies in cabbage fields in Nicaragua.

To quantify the effect on cabbage yields and quality of natural enemies as biocontrol agents compared with traditional insecticides.

To determine the separate and combined effect of parasitoid release and *Bt* spraying on *P. xylostella*.

3 Study sites

The field experiments were performed in the Tisey-Estanzuela Forest Reserve in Nicaragua (N 12°59'208" W86°21'973"), in the Estelí area at an altitude of 1348 -1483 m.a.s.l. (Fig. 1). Farms in this area are usually family owned and the main crops grown are potatoes, cabbage, maize and beans.

Figure 1. Map showing the location of the research area. The white Square in the green silhouette of Nicaragua (inset) indicates the map area.

The field studies mainly took place during two periods: from June to September, corresponding to the cropping period known as 'Primera'; and from September to December, corresponding to the cropping period known as 'Postrera'. Both occur during the rainy season. Some studies were conducted in the cropping period 'Apante' during the dry season. Table 1 summarizes the cropping systems used by small-scale farmers in the different growing seasons. All experiments were conducted in three years; 2006, 2007 and 2008.

Table 1. The cropping system used by farmers on small holdings in different growing periods in Nicaragua.

Primera rainy season	**Postrera** rainy season	**Apante** dry season
May – August	August – December	December – April
Maize – Bean	Bean – Potato	Chamomile – Cabbage
Cabbage – Potato – Cucurbit	Cabbage – Potato	Potato – Cabbage – Tomato

4 DBM densities and identification of natural enemies and their efficiency

4.1 The diamondback moth in the field

The diamondback moth is one of the main pests of crucifer crops throughout the world; it can cause up to 90% crop loss (Talekar and Shelton, 1993). An import step when evaluating the efficiency of different control methods is to estimate DBM densities in the field.

4.1.1 Measuring densities of DBM and natural enemies in all field experiments

Densities per plant of natural enemies, and eggs, larvae, pupae and adults of DBM, were assessed by means of weekly visual counts. Five samples, each including ten cabbage plants, were inspected in each field. Different sampling points were selected each week. We recorded the most abundant natural enemies of the DBM on the plants. Sampling was conducted over a period of eight weeks in 2006 but, based on the results from that year, sampling was reduced to six weeks during 2007 and 2008.

The densities of all potential predators, i.e. insects and spiders (Staphylinidae, Syrphidae, Nabidae, Reduviidae, *Polybia* sp.and Lycosidae, Salticidae, Linyphiidae and Opiliones), were estimated by two methods. First, using pit fall traps in the cabbage fields, and, second, using a D-Vac (Dietrick Vacuum insect net). The latter is known to be a highly efficient technique for sampling adult Nabidae (damsel bugs), Araneae (spiders) and Staphylinidae (rove beetles) (Elliott *et al.*, 2006). Each week during the sampling period six random samples comprising 20-second applications of the vacuum sampler head to a cabbage plant were taken in each field to provide a total sampled area of 2.25 m^2.

4.1.1.1 Density of potential predators (Paper I)

The most abundant arachnid groups in this study in descending order were the Lycosidae followed by the Salticidae, Tetragnathidae, Thomisidae and Opiliones (harvestmen); the least abundant groups were the Gnaphosidae and Linyphiidae (sheet web spiders). Among the insects, the Staphylinidae, particularly adults, were the most abundant followed by Nabidae adults and nymphs; the least abundant were syrphid (hoverfly) larvae and adult wasps, *Polybia* spp. The importance of the large predatory *Polybia* spp. wasps as biological control agents seem to be acknowledged by local farmers since they never destroy their nests (F. Miranda personal communication). However, the feeding strategy of the wasps made it difficult to include them in the surveys. *Diadegma insulare* (Cresson) was the most common parasitoid of DBM found.

4.1.2 Larval parasitism rates in all field experiments

When naturally occurring *P. xylostella* larvae had reached the 3rd or 4th instar, 30 or more larvae were collected, along with any pupae located on the cabbage plants, using the recruitment method described by Van Driesche and Bellow (1988). Larvae and pupae were placed individually into small plastic cups with slices of fresh cabbage leaves as food. The growth stage of each larva collected was recorded. Reared larvae and pupae were assessed every 2nd to 3rd day and a fresh slice of leaf added if necessary. Finally larvae (*P. xylostella*) were assessed and categorized as parasitized or not parasitized by *Diadegma insulare* or *Cotesia plutellae*. Estimates of the rate of parasitism were between 32 and 49% on farms that used insecticides and between 52 and 63% on farms that did not use insecticides.

4.1.3 Estimating crop damage in all field experiments

During Primera 2006 we selected six farms, each with a cabbage field that was not treated with insecticides, and five farms, each with a cabbage field that was treated with insecticides. We repeated the survey in Postrera 2006 in five fields that were not treated with insecticides, and four treated fields, all on separate farms. We repeated the survey in Primera 2007 in five fields that were not treated with insecticide and five treated fields, all on separate farms.

Ten randomly chosen cabbage heads were collected from each experimental field. To determine the level of damage, 10 wrapper (outer) leaves were inspected from each head. The rating system was developed from that used by Chalfant and Brett (1965). The estimated mean leaf damage per head was based on percent leaf damage per leaf in six classes; 0%, 1%, 5%, 10%, 25 % and ≤ 50%. The data pertaining to mean percent damage per head were arcsine transformed for the analysis of variance. Threshold leaf damage in the market is from 0 to 10 %, and > 40 % leaf damage is unmarketable. Leaf damage was high in the fields treated with insecticides (32%) and comparatively low in fields not treated with insecticides (12%).

4.2 Killing capacity of predators

4.2.1 Methods

To determine killing capacity, individual specimens of the most common predator groups were tested one day after they were collected in the field in order to standardize their hunger level. For small predators we used 4.5 cm diameter transparent plastic cups with a volume of 30 ml and with a plastic lid; for larger predators such as Lycosidae (wolf spiders), Salticidae (jumping spiders) and Reduviidae (assassin bugs), we used 8 cm diameter cups with a volume of 250 ml. Eggs, 2^{nd} (II) or 3^{rd} (III) instar larvae were provided as food in separate experiments. Feeding on eggs and larvae was tested with different sets of individual predators. In a different study, the same individual predators were offered larvae of each instar, but in random order.

Predators were provided with 20 % - 50 % more prey than they might be expected to consume in a period of 24 hours. The number of prey killed and eaten by each predator was counted after 24 hours and then every subsequent day for up to 13 days or until the predator died or escaped (mean= 4.38 days, SE= 0.104; median 5 days). Frass and dead larvae were removed with a soft brush when new larvae were introduced. Eggs were provided still attached to the piece of leaf on which they had been laid. If a predator consumed all prey larvae supplied, the number of consumed larvae was excluded from calculations of maximum predation. Replicates in which predators died during the test were not excluded from the analysis, but only the number of prey consumed during the first day and first 5 days were used in the calculations.

4.2.2 Results of assessing predator killing capacity (Paper I)

The data collected during the study of predator killing capacity and feeding rates showed that several of the spiders and insects collected in and around cabbage fields have the capacity to feed on *P. xylostella* larvae in the laboratory. However, only insects and none of the spiders fed upon *P. xylostella* eggs under laboratory conditions. The role of the investigated predators in biological control depends on both their abundance and feeding rate. Among the spiders, lycosids were the most abundant, whilst staphylinids were the most abundant insects. Staphylinid larvae exhibited the highest feeding rate on eggs. For small (instar II) larvae, the highest feeding rate among spiders was found on linyphiids, lycosids and salticids (Figure 2). Of the insects, Staphylinids, syrphids and nabids had the highest feeding rate on small larvae (Figure 3). Among the spiders, lycosids and salticids exhibited the highest feeding rate on larger (instar III) larvae. Among the insects, reduviids, nabids and staphylinids were the most effective consumers of large larvae (Figure 3). Based on the combined information of abundance and feeding rate, wolf spiders (Lycosidae) and rove beetles (Staphylinidae) seemed to have the greatest potential to regulate *P. xylostella*. However, all of these results are only circumstantial evidence that predators play a role in the biological control of *P. xylostella*.

Ground dwelling predators, such as lycosid spiders, have previously been shown to play an important role in affecting the mortality rates of immature stages of DBM (Muckenfuss *et al.*, 1992). Laboratory studies have shown that the lycosid *Pardosa milvina* (Hentz) may consume about one larva per day of *P. xylostella* (Muckenfuss *et al.*, 1992). Our finding that the second most abundant insect predator found in the present study, the Nabidae (*Nabis* spp.), had a relatively high killing rate and might therefore be an effective natural enemy corroborates the work of Ma *et al.*, (2005b) who found that 67% of *Nabis* spp. found in broccoli fields had eaten *P. xylostella*.

Other predators of importance were the linyphiid spiders and syrphid larvae. One type of linyphiid, although rarely seen on the plants, had a high consumption rate. Syrphids have previously been identified as potential predators of *P. xylostella* (Szwejda, 2004; Wu and Miyata, 2005). Lim (1992) observed syrphids readily preying on *P. xylostella* in cabbage fields, and

Charleston *et al*. (2006) found syrphids on cabbage plants in South Africa. Other insects with a high consumption rate but not frequently found in the field were the reduviid bugs.

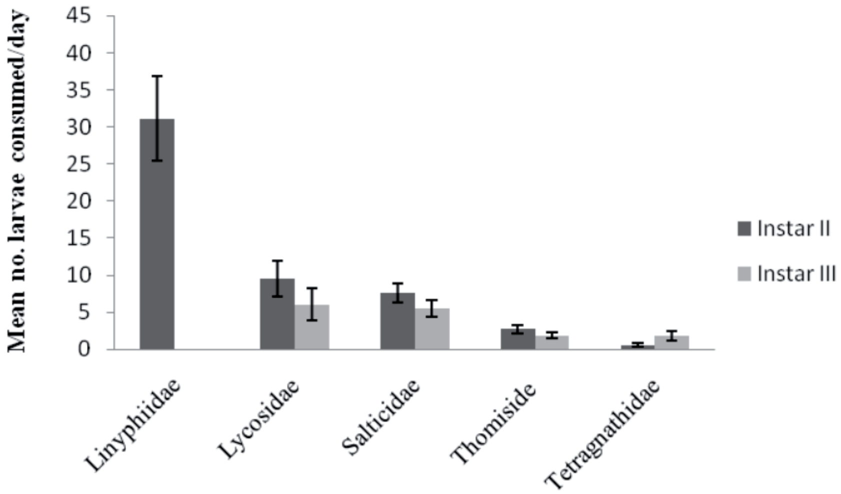

Figure 2. Killing capacity (mean no. larvae consumed /day ± SE; N>5) of different individual spider families when feeding on 2nd (dark grey bar) and 3rd (light grey bar) instar *Plutella xylostella* larvae under laboratory conditions.

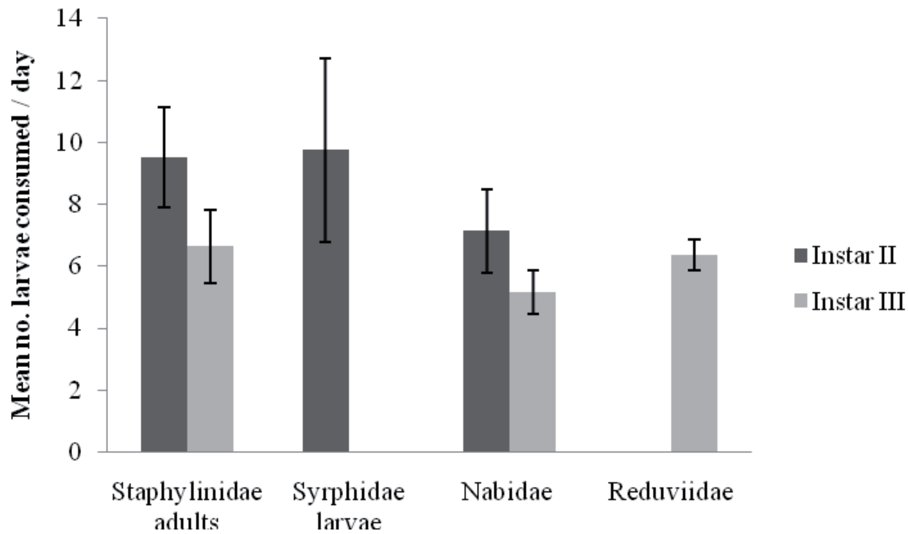

Figure 3. Killing capacity (mean no. larvae consumed /day ± SE; N>5) of different individual insects families when feeding on 2nd (dark grey bar) and 3rd (light grey bar) instar *Plutella xylostella* larvae under laboratory conditions.

4.3 Separating the effects of flying and ground dwelling natural enemies

4.3.1 Methods

Four treatments (see photographs below) were used in this field experiment: 1. Control (C), i.e. cabbage plant without any protection; 2. Excluding ground dwelling (-GD) predators; 3. Excluding flying (F) natural enemies and 4. Excluding all (-GDF) natural enemies, using a combination of the two exclosure methods. To exclude ground dwelling (-GD) predators we used a tray filled with water in which the potted plant was placed. To exclude flying predators, i.e. mainly parasitoids and *Polybia* spp. (vespid wasps) we used a small net cage (mesh size (50 cell/cm), supported by a steel wire 0.6 m x 0.6 m. The net covered the plant but allowed rain to enter. Ground-dwelling predators could enter by walking underneath the netting via a 2 cm gap from the ground that was left for this purpose.

One cabbage plant representing each of the four treatments was placed in four blocks at each farm on each occasion. Each block of cabbage plants was placed close to one edge of the field and placed between the three first parallel rows of planted cabbages in the field crop.

1. Control (C)

2. Excluding ground dwelling (-GD) natural enemies

3. Excluding flying (-FL)
4. Natural enemies

4. Excluding all (-GDF) natural
natural enemies

Each plant (of each treatment) was infested with 10 3rd instar larvae of *P. xylostella* before installing the exclosures. The larvae placed on each plant were obtained from a laboratory mass rearing. The diamondback moth culture was maintained in the laboratory using cabbage leaves (*Brassica oleracea*) as the food source.

The experimental plants were inspected after 24, 48 and 72 hours to count the remaining of *P. xylostella* larvae. All larvae remaining after 72 hours were collected and reared to pupation to determine the rate of parasitism. The same procedure was repeated twice with new plants and larvae, starting two and four weeks after the original test.

4.3.2 Survival of *P. xylostella* in cage experiments (Paper II)

The disappearance of *P. xylostella* 3rd instar larvae after 48 hours differed significantly among treatments and was highest (58 %) in the (-GD) treatment, in which ground dwelling natural enemies were excluded; the second highest value (55 %) was recorded in the control (C), which allowed all natural enemies to access the larvae. The FL treatment excluded flying natural enemies and resulted in a reduction of larvae by 42 %. In the (-GDF) treatment, which excluded all natural enemies, the rate of disappearance was 8 % (Fig 4). The same relative difference among treatments was found after 24 hours and 72 hours ($p<0.0001$) (Fig 4).

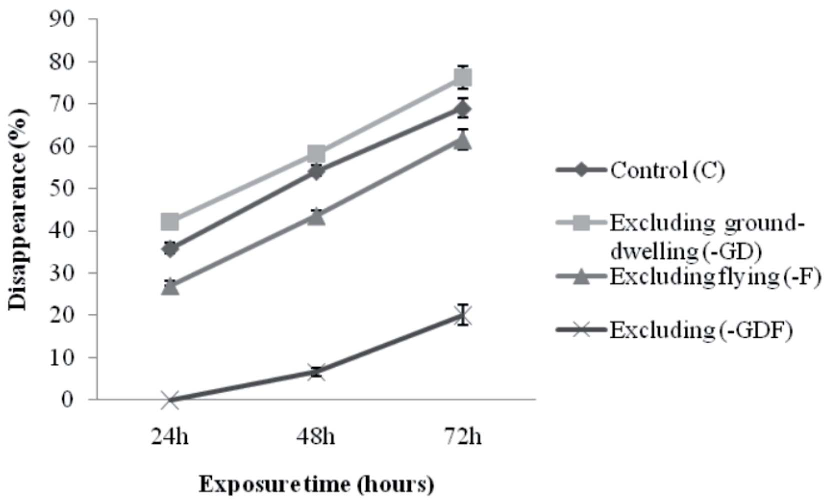

Figure 4. The disappearance of *Plutella xylostella* 3^{rd} instar larvae (mean ± SE) at three recording times (hours after transplantation into the field) recorded in an exclusion experiment in cabbage fields. The disappearance of *P. xylostella* larvae on cabbage increased significantly (p=0.0342) over time and was significantly different between treatments (p<0.0001) on all three occasions (Fig. 5).

The difference among treatments was similar during all three time replicates, but was most pronounced for the second replicate, 50 days after plants were transplanted to the field. The smallest difference was found after 30 days while the difference among treatments was intermediate after 75 days. This variation in relative difference among treatments over time was confirmed by a significant (treatment × -GD) interaction, indicating that disappearance caused by flying predators varied more over time than mortality caused by ground dwelling predators (Fig. 5).

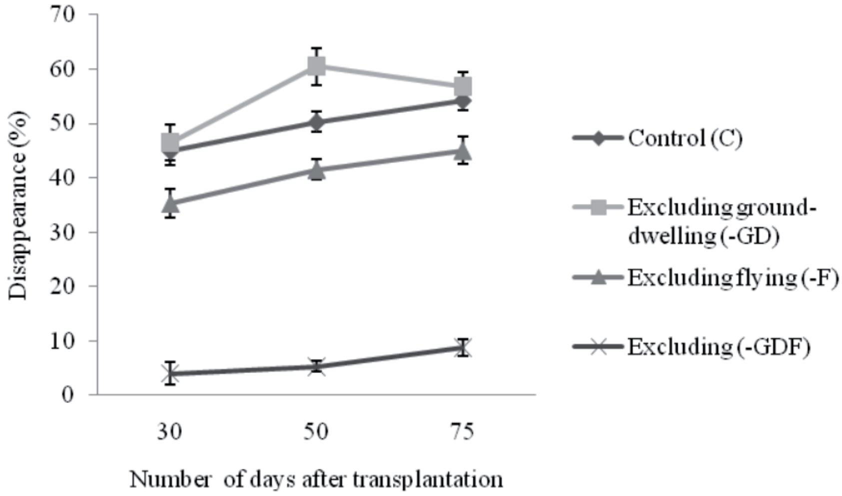

Figure 5. The disappearance of *Plutella xylostella* 3rd instar larvae (mean ± SE) at three times (days after transplantation) recorded in an exclosure experiment in cabbage fields. Means after 48 hours are presented.

5 Management of diamondback moth in cabbage fields

To assess the efficacy of insecticides in the control of DBM on cabbage and also to obtain an indication of the impacts on and benefits of natural enemies, we examined the effects of insecticide treatments on DBM, its predators and parasitoids over time. When it was conceived, the main idea of integrated pest management was that natural control alternatives should be implemented as the main control strategy with chemicals only as a backup option designed to interfere as little as possible with non-chemical options (Kogan, 1998).

The manipulation of beneficial organisms remains a very important tool in integrated pest management programs for insect pests worldwide (Orr, 2009). Here we asked: How will spraying with insecticides affect DBM, its natural enemies and crop damage? Will *Bt* spraying reduce the density of *P. xylostella* and hence damage to cabbage? Will release of parasitoids (*Diadegma insulare*) significantly increase the larval parasitism rates of *P. xylostella* compared to the background (natural) rate of parasitism in cabbage crops?

5.1 Insecticide effects on pests and natural enemies

5.1.1 Experimental design

During Primera (May-September) 2006 we selected six farms, each with a cabbage field that was not treated with insecticides, and five farms, each with a cabbage field that was treated with insecticides (Fig. 6). We repeated the experiment in Postrera (September to December) 2006 in five fields that were not treated with insecticides, and four treated fields, all on separate farms

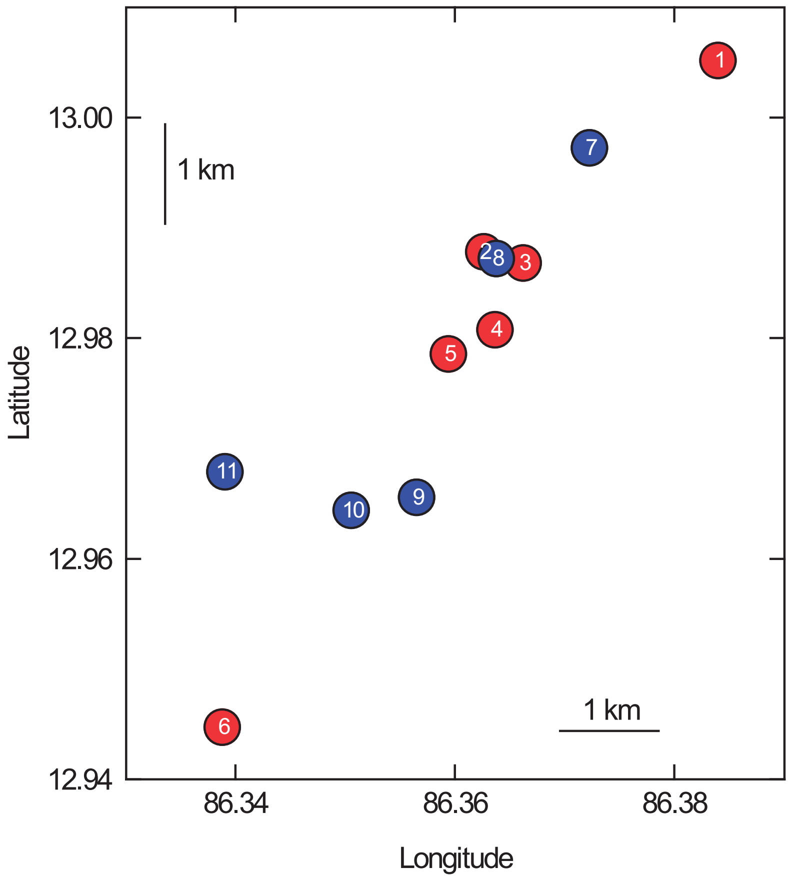

Figure 6. Locations of the fields on semi-organic (red circles, 1-6) and conventional (blue circles, 7- 11) farms used in the studies.

Farmers using insecticides in the study sprayed the experimental fields several times, as is common practice, often based on recommendations from insecticide producers and insecticide vendors. The farmers decided themselves how many times they sprayed and what substance to use to control the DBM. The farmers who did not use insecticides had ceased their use in 2005, except for one farm (Tisey) that had not used insecticide treatment since 2000. All the farms applied the herbicides Gramoxone® or Paraquat (3 l/ha) once in each growing season before transplantation.

The size of each experimental field was approximately 25 m × 60 m (0.15 ha). Head cabbage of a single variety *Brassica oleracea* var. *capitata* (Izalco) was sown in trays with small holes in a netted rearing area for farmers not using insecticides and in a seedbed for farmers using insecticides. This difference in rearing regime did not affect the seedlings or the plants in the field. All the experiments were established using seedlings, transplanted by hand into rows spaced 0.6 m apart and with 0.5 m between plants. All fields received inorganic fertilizer equivalent to 182 kg/ha (formula N P K, 12-30-10) eight days after transplantation, and an additional 95 kg/ha of nitrogen fertilizer (N, 45) was applied 30 days after transplantation.

5.2 Observed density of DBM and natural enemies in fields with and without insecticides (Paper III)

The density of DBM larvae and pupae on cabbage plants was higher on farms that used insecticides than on farms that did not (Fig. 7 and Fig. 8). The density of both larvae and pupae increased over time as shown by a significant effect of week. No significant interactions between farm type and week were found. DBM larvae per plant, which is the measure that probably best reflects the actual pest density in a field, surpassed an economic threshold of 0.3-0.5 individuals per plant (Kirby and Slosser, 1984, Diaz *et al*., 1999) in the 2006 Primera season in both the insecticide treated (Mean=1.6 DBM larvae/plant, SE=0.32, N=54) and untreated (Mean=0.82, SE=0.14, N=54) fields. In the 2006 Postrera season, the average attack level was lower and closer to the damage thresholds both in treated (Mean=0.72, SE=0.13, N=45), and untreated fields (Mean=0.48, SE=0.04, N=45). In the 2007 Primera season, the average attack level was lower and closer to the damage thresholds in both the treated (Mean=0.72, SE=0.13, N=30), and untreated fields (Mean=0.65, SE=0.13, N=30). In only one of 48 cases did we find significantly lower DBM densities in treated fields (Fig. 7, Fig. 8) than in untreated ones.

The density of DBM larvae generally increased from below the economic threshold early in cabbage crop growth to above the economic threshold as the crop developed in both Primera and Postrera seasons. The data suggest that all sites would have required additional measures to control DBM populations to ensure marketable produce.

The observed higher DBM densities in the fields treated with insecticides are probably partly due to the fact that the moth has developed resistance to insecticides (Ovalle and Cave, 1989; Branco and Gatehouse, 1997; Andrews *et al.*, 1992; Carballo *et al.*, 1989; Cordero and Cave, 1989; Cheng *et al.*, 1992; Kibata, 1996; Verkerk and Wright, 1997; Shelton *et al.*, 2000; Vastrad *et al.*, 2004; Mazlan and Mumford 2005; Zhao *et al.*, 2006; Dadang *et al.*, 2009); indeed, high levels of resistance to these compounds are now known to occur in Nicaraguan populations of DBM (Perez *et al.*, 2000).

There are additional problems with insecticide use. Lim *et al.* (1992), for example, showed that insecticides (carbaryl+malathion) are toxic to parasitoids but not to DBM. Another problem is exemplified by several studies conducted throughout Central America, confirming the widespread risks to farm workers of pesticide exposure (McConnell *et al.*, 1992; Galt, 2008). Numerous cases of human poisoning have been reported in Central America, especially from parathion. It is likely that many more cases remain unreported among farm workers in cotton and banana plantations (Metcalf, 1980). Recent studies show that acute pesticide poisoning is a public health concern in Nicaragua (Leonard, 1986; Campanhola *et al.*, 1995; Corriols *et al.*, 2009). The most common negative side-effect of pesticide application is probably reduced abundance of beneficial organisms (Orr, 2009). Kfir (2001) reported that a higher infestation level of DBM in the sprayed cabbage plots was because of partial elimination of parasitoids by the pesticide. A study by Nemoto (1993) showed increased fecundity of DBM adults that emerged from larvae and pupae exposed to sub-lethal concentrations of methomyl.

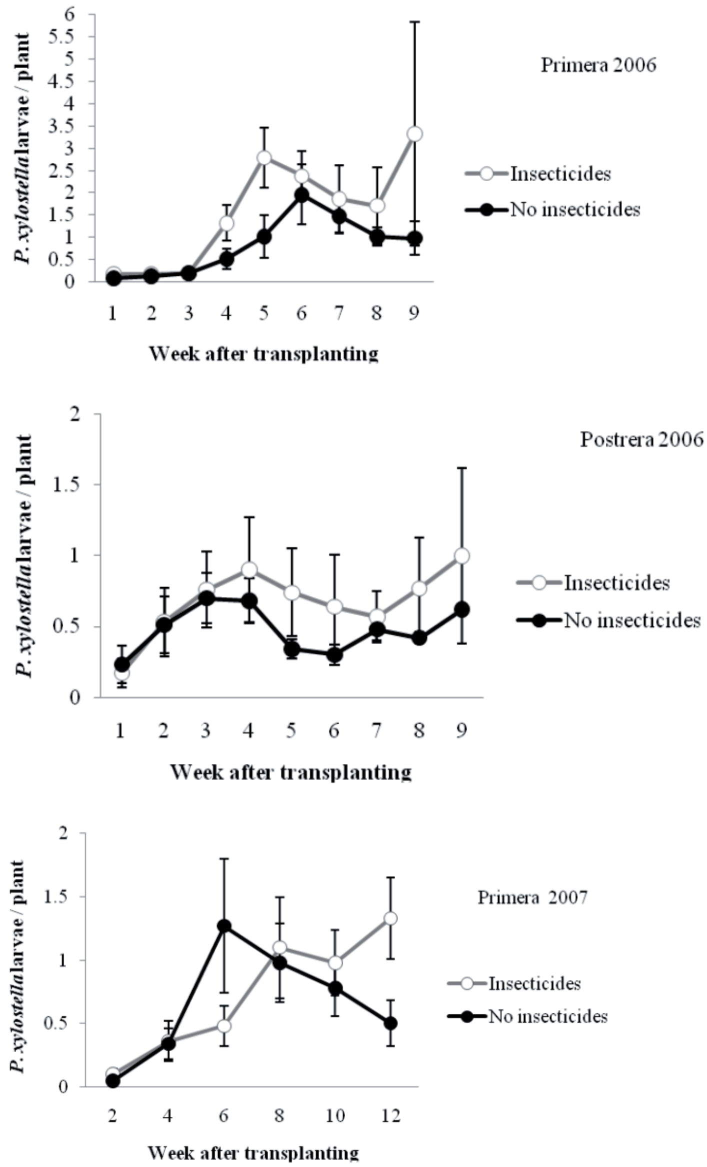

Figure 7. Mean number of *Plutella xylostella* larvae per cabbage plant in fields treated with insecticides and in untreated fields in Nicaragua. Data from Primera (June, July and August) in two years (2006 and 2007) and Postrera (October, November and December) in one year (2006) are presented. Error bars represent standard errors. N= 5-6 fields.

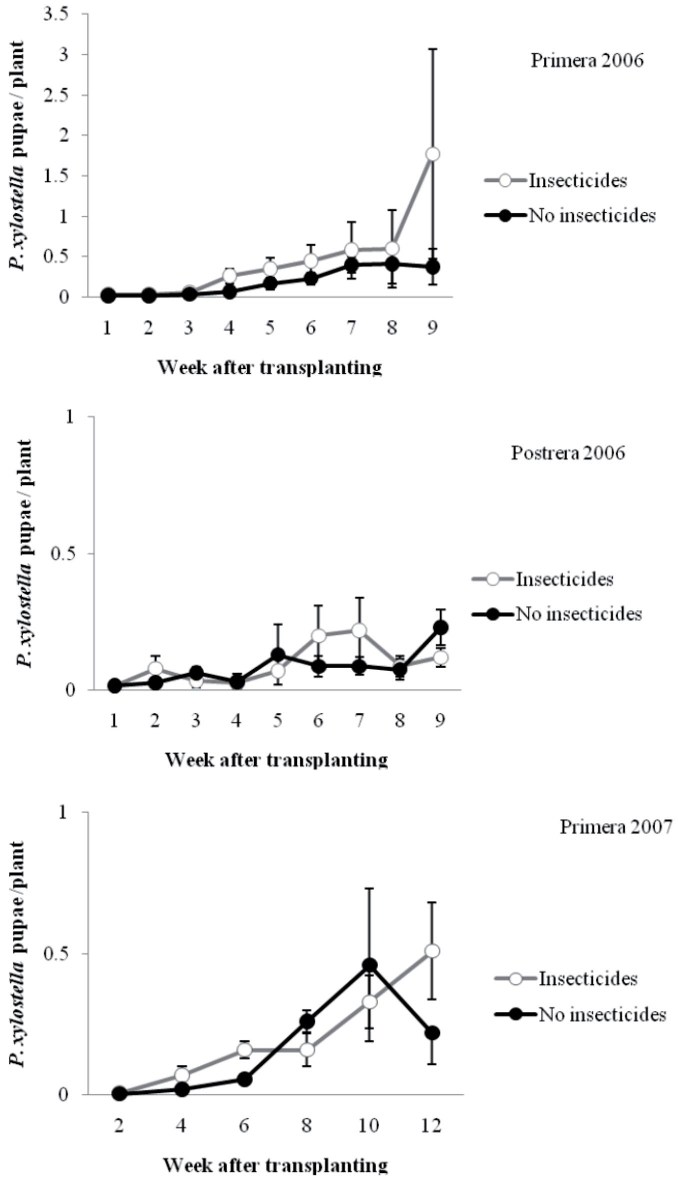

Figure 8. Mean number of *Plutella xylostella* pupae per cabbage plant in fields treated with insecticides and in untreated fields in Nicaragua. Data from Primera (June, July and August) in two years (2006 and 2007) and Postrera (October, November and December) in one year (2006) are presented. Error bars represent standard errors. N= 5-6 fields.

5.2.1 Density of *Diadegma insulare* (Paper III)

The density of pupae of the specialist natural enemy *D. insulare* was significantly higher on farms that did not use insecticides than on farms which did (Fig. 9; Table 2). The same trend was found for *D. insulare* adults. The density of both pupae and adults of this parasitoid increased over the growing season. However, the increase was faster on farms not using insecticides than on farms where they were used, as indicated by significant farm type × week interactions.

The rate of parasitism by *D. insulare* among collected *P. xylostella* was higher on farms that did not use insecticides than on farms that did (Fig. 10; Table 2). These results indicate that the farmers using insecticides to control DBM are affecting the performance of the parasitoid *D. insulare* in the cabbage fields of Nicaragua. Similar results were found for anther parasitoid attacking DBM, *Diadegma semiclausum*, in the unsprayed control plot when compared to the conventional insecticide spray plots (Wang *et al.*, 2004).
The rate of parasitism by *D. insulare* was found to vary between 62 and 82% of DBM larvae examined. Parasitized larvae feed less and cause less damage to cabbages (Okine *et al.*, 1996; Monnerat *et al.*, 2002). It has been shown that application of the insecticides enfenvalerate, methomyl and acephate results in 100% mortality to *D. insulare* (Cordero *et al.*, 2007; Hill and Foster 2000; Xu *et a*l., 2001). Ayalew and Ogol (2006) found that a higher DBM density was associated with heavy pesticide usage and a low level of parasitism.

A host specialist parasitoid is thought to have greater efficiency in locating their host or to possess a greater ability to overcome host defenses than a generalist species (Wang and Keller, 2002). In Canada *D. insulare* has been found to be the principal parasitoid of DBM, accounting for 30 to 45% of the parasitism (Braun *et al.*, 2004). In the absence of synthetic insecticides, these parasitoids suppress the pest populations effectively in different parts of the world including Indonesia and Malaysia (Talekar and Shelton, 1993) and Japan (Noda *et al.*, 2000). Many countries have developed and implemented a bio-control based IPM approach that has proved successful (Poelking, 1992; Löhr *et al.*, 2007). However, larval endoparasitoids belonging to the genera *Diadegma* and *Cotesia* are the main and most effective species in the management of DBM (Fitton and Walter, 1992) and *Diadromus collaris*, a pupal parasitoid, has also been shown to contribute significantly to DBM control (Kirk *et al.*, 2004).

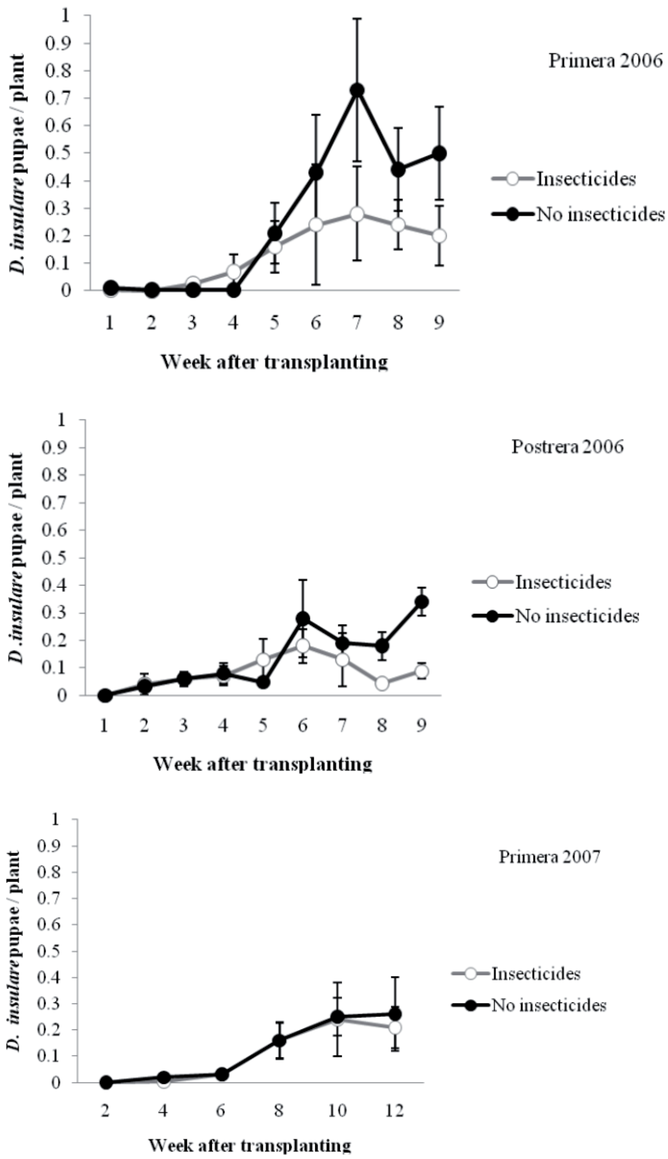

Figure 9. Mean number of *Diadegma insulare*, a parasitoid attacking *Plutella xylostella* larvae, per cabbage plant in fields treated with insecticides and in untreated fields in Nicaragua. Data from Primera (June, July and August) in two years (2006 and 2007) and Postrera (October, November and December) in one year (2006) are presented. Error bars represent standard errors. N= 5-6 fields.

Table 2. The effect of insecticide treatment on the mean density of the parasitoid *Diadegma insulare* and on other (spiders and predatory wasps) natural enemies of *Putella xylostella* in cabbage fields in two cropping seasons; Primera (June, July and August) in 2006 and 2007 and Postrera (October, November and December) in 2006.

Season Dependent Variable	**Treatment**	**Mean**	**SE**	**N**
Primera 2006				
Diadegma insulare pupae	Insecticide	0.14	0.038	54
	No insecticide	0.26	0.056	54
Rate of parasitism	Insecticide	32	2.8	20
	No insecticide	57	3.2	24
Spiders	Insecticide	0.04	0.0097	54
	No insecticide	0.072	0.010	54
Polybia sp.	Insecticide	0.004	0.0017	54
	No insecticide	0.017	0.0058	54
Postrera 2006				
Diadegma insulare pupae	Insecticide	0.083	0.017	45
	No insecticide	0.14	0.025	45
Rate of parasitism	Insecticide	49	2.9	24
	No insecticide	63	3.0	20
Spiders	Insecticide	0.044	0.0074	45
	No insecticide	0.085	0.018	45
Polybia sp.	Insecticide	0.012	0.0028	45
	No insecticide	0.017	0.0033	45
Primera 2007				
Diadegma insulare pupae	Insecticide	0.10	0.033	30
	No insecticide	0.12	0.033	30
Rate of parasitism	Insecticide	46	3.88	15
	No insecticide	52	5.24	15
Spiders	Insecticide	0.068	0.013	30
	No insecticide	0.16	0.030	30
Polybia sp.	Insecticide	0.0033	0.0014	30
	No insecticide	0.017	0.0015	30

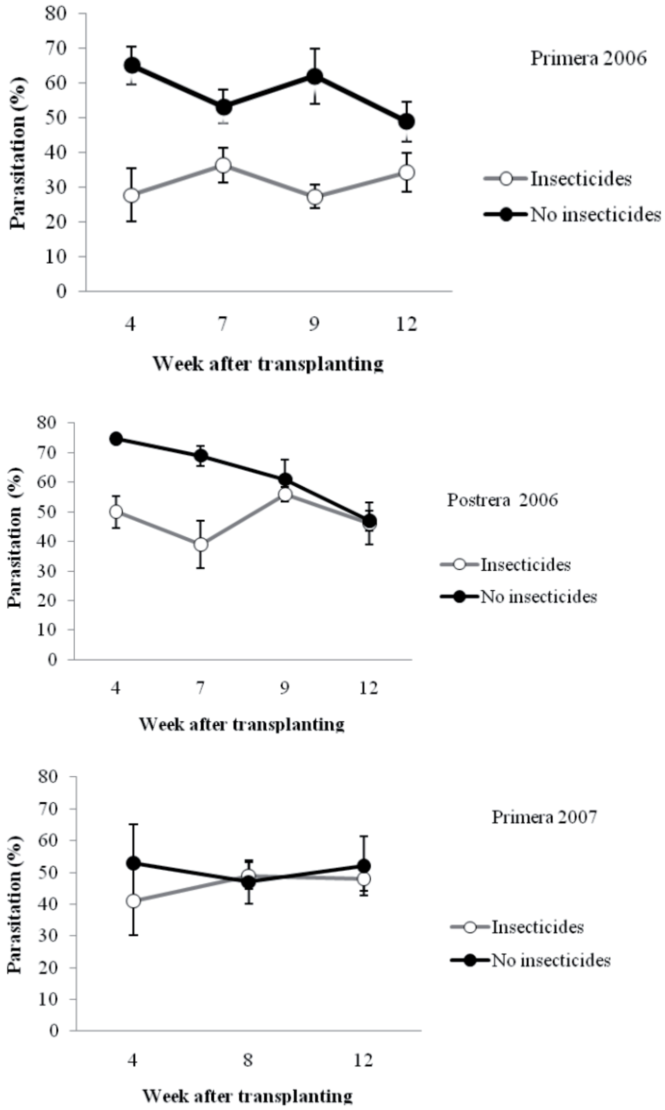

Figure 10. Level of parasitism by *Diadegma insulare* on *Plutella xylostella* in cabbage fields treated with insecticides and in untreated fields. Data from Primera (June, July and August) in two years (2006 and 2007) and Postrera (October, November and December) in one year (2006) are presented. Error bars represent standards errors. N= 5-6 fields.

5.2.2 Density of spiders and *Polybia* sp. (Paper III)

The density of generalist natural enemies – spiders (Fig. 11; Table 2) and predatory wasps, *Polybia* sp. (Fig. 12; Table 2) – was higher on farms that did not use insecticides than on farms that did. For spiders there was an increase in density over the season and the increase was greater on farms not using insecticides than on farms using insecticides, as indicated by significant farm type × week interactions. No such effects were found for *Polybia* sp. All natural enemy groups were less abundant in the insecticide treated compared to the untreated fields, although not always significantly.

Generalist predators, as part of complex communities of natural enemies, can make significant contributions to the biological control of pests (Obrycki and Kring, 1998; Sunderland *et al.* 1997; Symondson *et al.*, 2002) when they reach high enough densities. However, at very high densities their numbers may be reduced by territorial behavior and cannibalism (Pickett *et al.*, 1946, Dondale , 1956, Duffy, 1962, Kajak *et al.*, 1968; Dondale, 1972; Tanaka, 1989; Lee and Kim, 2001).

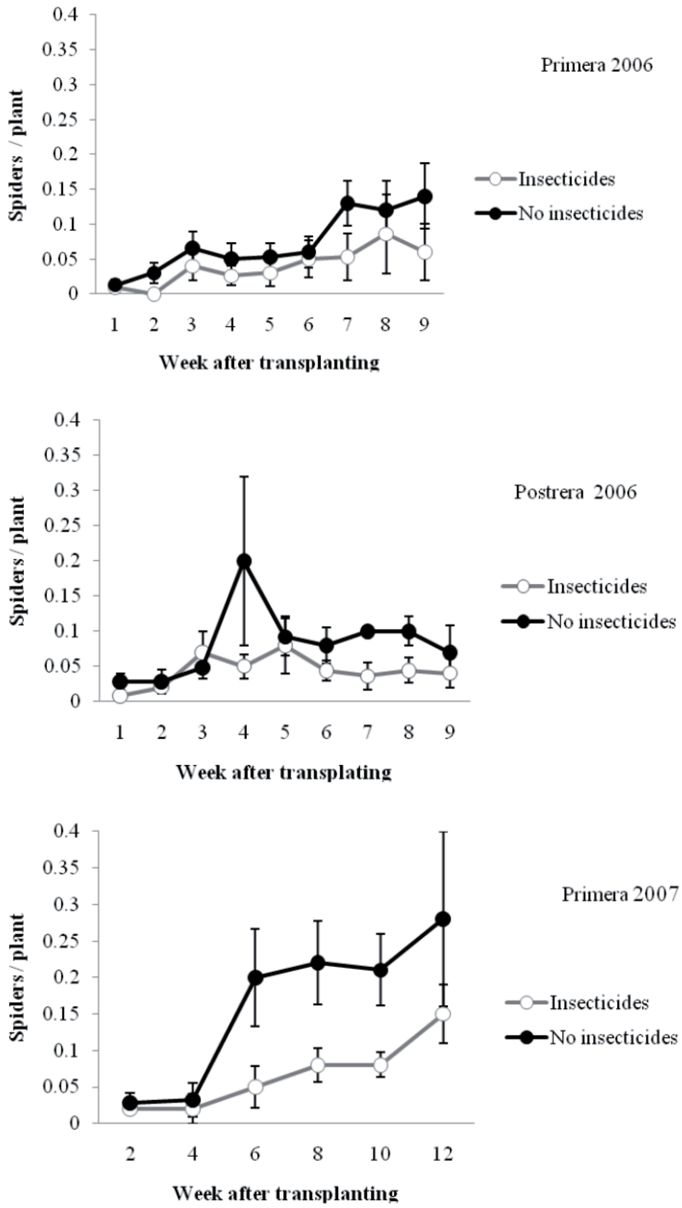

Figure 11. Mean number of predatory spiders per cabbage plant in fields treated with insecticides and in untreated fields in Nicaragua. Data from Primera (June, July and August) in two years (2006 and 2007) and Postrera (October, November and December) in one year (2006) are presented. Error bars represent standard errors. N= 5-6 fields.

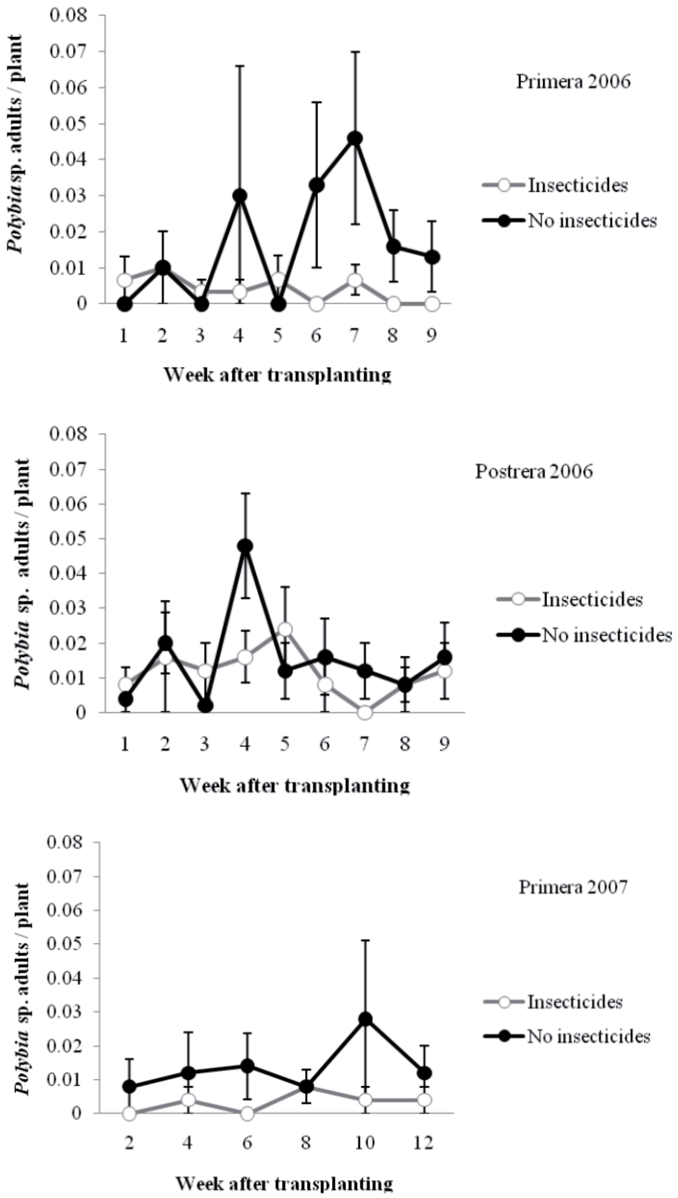

Figure 12. Mean number of predatory wasp (*Polybia* sp.) adults, per cabbage plant in fields treated with insecticides and in untreated fields in Nicaragua. Data from Primera (June, July and August) in two years (2006 and 2007) and Postrera (October, November and December) in one year (2006) are presented. Error bars represent standard errors. N = 5- 6 fields.

5.2.3 Leaf damage to cabbage crops by *P. xylostella* (Paper III)

Insect damage to cabbage, including holes chewed in leaves and heads, and deposits of excrement and body parts make cabbage unmarketable. In our studies, leaf damage was significantly higher in fields treated with insecticides than in fields not treated with insecticides over three growth seasons. There was a significant difference between treatments in all the growing seasons, Primera 2006 (F=5.4, df=1,9, P= 0.045), Postrera 2006 (F= 7.7, df=1,7, P= 0.028) and Primera 2007 (F=27, df=1,8, P= 0.0001) (Fig. 13).

The effect of insecticide use had a dual negative effect on insects in the cabbage fields studied. Density of and damage from *P. xylostella* was higher on farms using insecticides than on farms not using insecticides at the same time as the density of natural enemies and rates of parasitism were comparatively higher on the non-insecticide farms.

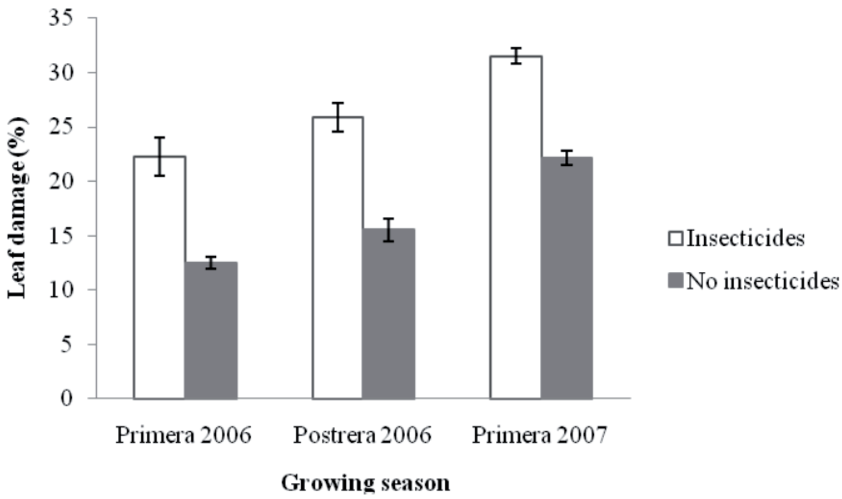

Figure 13. Average percentage leaf damage by *Plutella xylostella* on cabbage heads in fields with no insecticides (grey bars) and in fields treated with insecticides according to common practice in Nicaragua (white bars). Data from Primera (June, July and August) in two years (2006 and 2007) and Postrera (October, November and December) in one year (2006) are presented. Error bars represent standard errors. N= 5-6 fields.

Left: Photo of damage caused by *Plutella xylostella* to cabbage heads from a field treated with insecticides. Right: Photo of damage caused by *Plutella xylostella* to cabbage heads from an untreated field.

5.3 Combined and separate effects of parasitoid release and *Bt* spraying on DBM control (Paper IV)

5.3.1 Rearing of *P. xylostella*

Diamondback moths were reared *en masse* on cabbage leaves (*Brassica oleracea*) in the laboratory, in order to obtain a range of different immature life stages to feed to parasitoids (*D. insulare*) during tests of their potential for the control of *P. xylostella* in the field.

Cabbage plants used as food for the diamondback moth were obtained by sowing cabbage seeds in plastic trays and transplanting the seedlings into individual plastic pots. The plants were maintained in a nursery until they had 12-14 leaves. Most moth larvae pupated after 17 to 21 days at 22 to 25 °C with 12 h photoperiods and a relative humidity (RH) of 60 – 80 %. The conditions for the mass rearing were similar to those described by Hou (1986).

Cabbage seedlings in the greenhouse. Seedlings transplanted to pots.

5.3.2 Rearing of *D. insulare*

Rearing of the larval parasitoid *D. insulare* was carried out on a farm in Tisey using larvae of DBM reared on cabbage plants as hosts. One hundred and fifty adult parasitoids were introduced into one screened cage (50 cm × 50 cm × 50 cm) for oviposition over a period of 48 hours. The cage contained a cabbage plant with 250 host larvae in their 2nd instar. The parasitoid rearing was conducted in a room kept at 25 °C and with 56 to 85 % relative humidity (RH). Adults of *D. insulare* were fed with honey water (10%). The food source was changed once every second day. After being exposed to parasitoids for 48 h, host larvae were reared until pupation after 18 to 21 days. After three days the parasitized cocoons that remained in the early pupal stage were collected and put in 5 cl plastic cups. Later, the moth and parasitoid pupae become distinctly different. The moth pupae is tapered and changes from being pale to dark as it matures, while *D. insulare* pupae are initially pale and then turn dark brown with a characteristic white band around the middle. The conditions in the mass rearing (Fig. 14) were similar to those described by Talekar and Lin (1998).

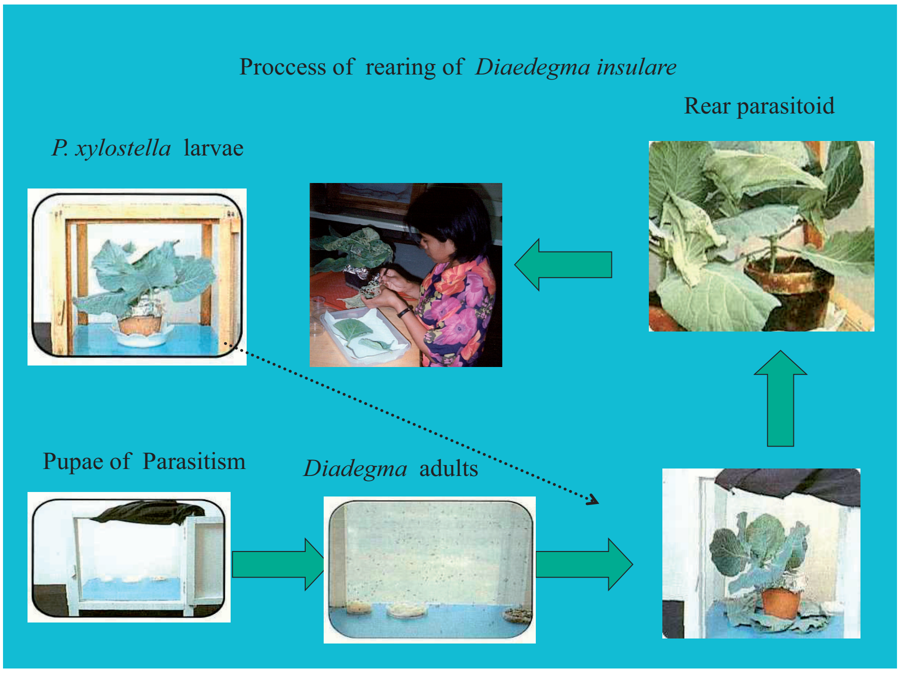

Figure 14. Process of mass rearing of the parasitoid *Diadegma insulare* in the laboratory

5.3.3 Release of parasitoids

Parasitoids were released on three occasions into each experimental field. The first release was performed by introducing 125 cocoons of *D. insulare* per field 30 days after transplanting the cabbage plants into the field. The second release was 45 days after transplanting the cabbages and involved releasing 250 parasitoids (cocoons and emerging adults) per field. For the third release, 60 days after transplanting, 125 parasitoids were released in each field. Equal numbers of parasitoid cocoons were placed in five release chambers that were evenly distributed across the field. The release of parasitoids was performed when 10 % of the adult parasitoids had emerged from the cocoons.

Parasitoids were kept in 200 ml plastic containers covered with aluminum foil to prevent light penetration. A small window was created to allow flying parasitoids to exit. Each chamber was mounted on a 0.5 m long wooden stake. All stakes were covered with adhesive tape for 2 cm above the ground in order to exclude predatory insects. A single top leaf of Maguey (*Agave americana*) was fixed on top of the containers to protect parasitoids from rain and sun.

Chamber used for releasing parasitoids mounted on a 50 cm stake covered with a piece of Maguey leaf.

The questions asked were: Will release of parasitoids reduce the density of DBM and hence damage to the cabbage plants? Is the combined effect parasitoid release and *Bt* spraying in order to control *P. xylostella* densities and reduce leaf damage in cabbage crops additive, synergistic or antagonistic?

5.4 Observed density of *P. xylostella* in fields where parasitoids were released (Paper IV)

The density of *P. xylostella* larvae on cabbage plants was significantly different between treatments in Postrera 2007 but did not show any difference in Primera 2008 (Fig. 15, Table 3). However, the density of larvae increased over the course of both seasons as indicated by the significant effect of week and the significant interaction between week and treatments. The density of DBM larvae generally increased from below the economic threshold early in the growth of the cabbage crop to above the economic threshold as crop growth progressed; this occurred during all three years in which we collected data. Our data suggest that all sites would have required additional strategies to control the DBM populations.

The density of *D. insulare* pupae in the cabbage fields differed significantly among treatments in 2007 but not in 2008 (Fig. 15, Table 3). Significant week × treatment effects in both years indicate that the relative difference between treatments varied over time. In both seasons, *D. insulare* density increased significantly over time; the density of *D. insulare* increased with increasing *P. xylostella* density (Table 3).

The interaction between parasitoid release and *Bt* treatment was significant ($F=5.03$; $df= 1,156$, $P<0.026$) in 2007 but not in 2008. The two treatments seemed to have an adverse effect on each other (Fig. 16).

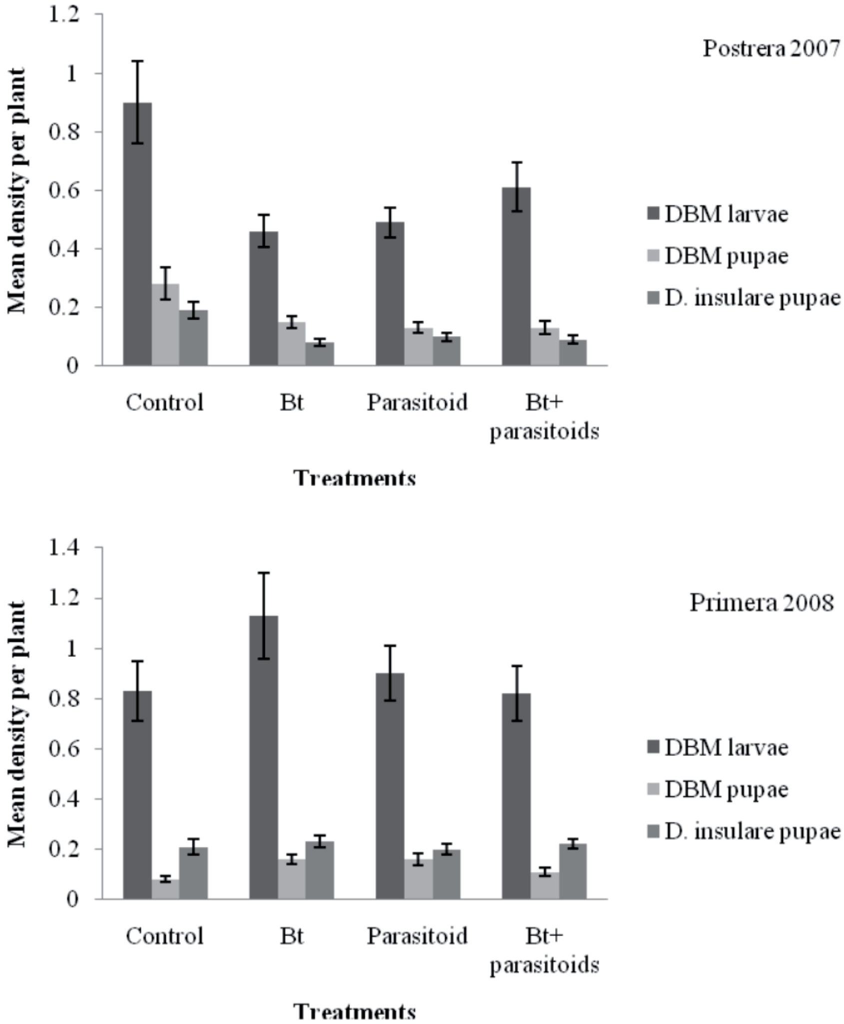

Figure 15. Mean number of *Plutella xylostella* (DBM) larvae and pupae and *Diadegma insulare*, a parasitoid attacking P. xylostella, larvae per plant in the cabbage fields exposed to four different bio-control treatments i.e. control, Bt spraying and / or parasitoid release in Nicaragua. Data from Postrera (October, November and December) 2007, and Primera (June, July and August) 2008 are presented. Error bars represent standard errors. N=4 fields per treatment and year.

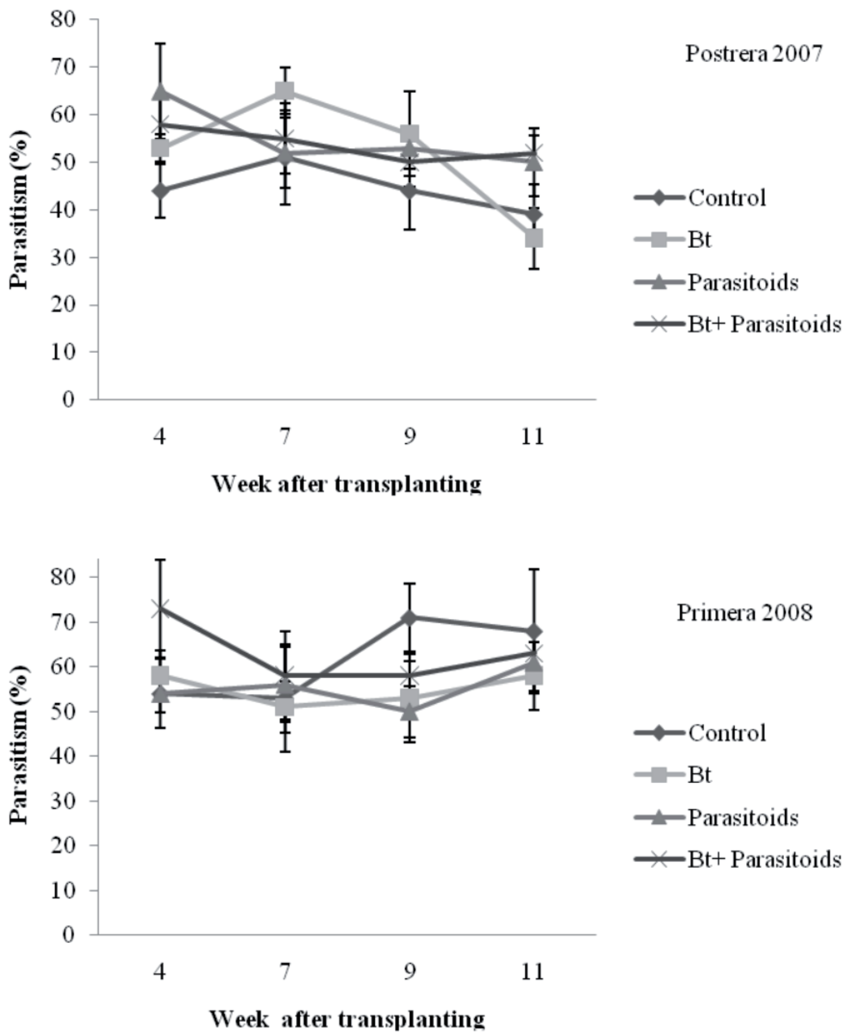

Figure 16. Level of parasitism by *Diadegma insulare* on *Plutella xylostella* in cabbage fields exposed to different bio-control treatments; i.e. control, *Bt* spraying and /or parasitoid release Results from two cropping seasons, Postrera (October, November and December) 2007, and Primera (June, July and August) 2008, are presented. Error bars represent standards errors. N= 4 fields per treatment and year.

5.4.1 The rate of parasitism by *D. insulare* in cabbage fields (Paper IV)

Diadegma insulare is considered one of the key biological control agents in an integrated pest program to combat Lepidoptera in crucifer crops in Guatemala (Biever *et al.*, 1994). The high rates of parasitism reported herein (Fig. 16) indicate that this parasitoid may also be important in Nicaragua.

There was, on average, 55 % parasitism by *D. insulare* in the cabbage fields where the species was released in Postrera 2007 and the level was between 50 and 65 %. Compared with control treatments the parasitism occurred lower on average (44%), ranging between 38 and 50 %. A similar result was obtained for the *Bt* + parasitoid treatment with an average parasitism of 54%, ranging between 50 and 58 %. The *Bt* treatment was comparable to the parasitoid release treatment, with an average of 52% and a range of 56–58 %. No significant interaction between time and treatment was found (Fig. 16, Table 3).

There was, on average, 59 % parasitism by *D. insulare* in the cabbage fields where the species was released in Primera 2008 and the level was between 56 and 64 %. Compared with control treatments the parasitism occurred lower on average (50%), ranging between 39 and 58 %. A similar result was obtained for the *Bt* +parasitoids treatment with an average of 64 %, ranging between 64 and 67 %. The *Bt* treatment was comparable to parasitoid release treatment, with an average of 58% and a range of 56–59 %. No significant interaction between time and treatment was found (Fig.16, Table 3).

Khan et al. 2005 reported that *D. insulare* was the major parasitoid of DBM, and parasitism levels ranging from 0 to 58.5% were found in a study of collard cropping in South Carolina. Parasitism of *Plutella xylostella* 3rd and 4th instars was evaluated in a cabbage field in Geneva, NY in 1999, over the entire season; average parasitism was 33.4% for the 3rd instar larvae and 53.6% for 4th instar larvae and the main parasitoid was *D. insulare* (Xu *et al.*, 2001). The occurrence of the DBM and its parasitoids has also been studied in Ethiopia: overall parasitism ranged from 3.6% to 79.5% but there were large differences between areas (Ayalew and Ogol, 2006)

Egg parasitoids have been found to be highly compatible with *Bt*, because the egg is not a target for the microbe. Successful examples include *Trichogramma platneri* against *Boarmia selenaria* (Lep., Geometridae) in avocado (Wysoki, 1989) and the release of *Trichogramma* in a *Bt* based IPM program for tomato (Trumble and Alvarado- Rodriguez, 1993). *Bt* spraying has been shown, in several studies, to have no effect on several beneficial insects (Hassan and Smith, 1995; Kring and Smith 1995; Muckenfuss and Shepard, 1994).

Due to el Niño climatic events the weather was unusually dry in 2006 with a total precipitation of 208 mm in Primera and 238 mm in Postrera. Dry weather usually enhances DBM attacks as generation time decreases with the warm weather and the insects are not disturbed by rainfall. But during October Postrera 2007, the precipitation was unusually high, in total 1035 mm. (Fig. 17).

It is possible that climatic conditions influenced the possibilities to detect relationships between density of *D. insulare* and *P. xylostella* population growth rate (Fig. 2). This variation in detectability of density dependence among seasons highlights the importance to replicate studies over time.

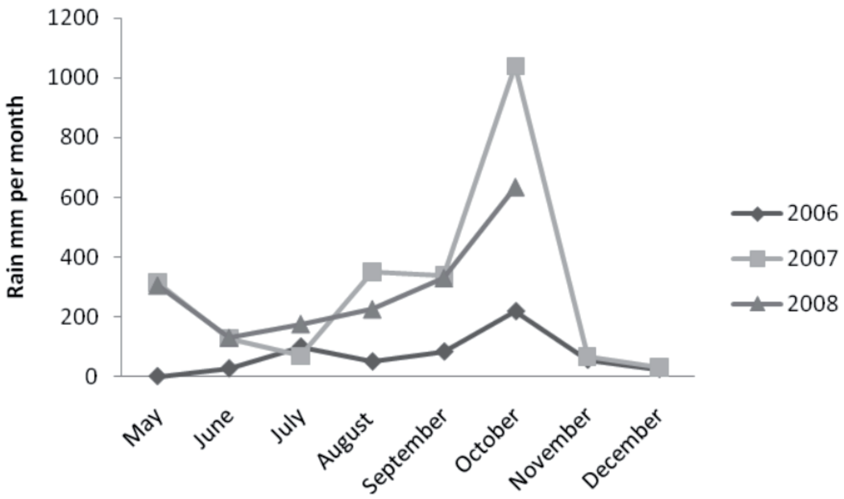

Figure 17. Mean monthly precipitation (mm rainfall) in the Tisey, Nicaragua in two growing seasons, Primera (June to end August) and Postrera (October, to December in three years (2006, 2007 and 2008).

Table 3. The effects of bio-control (i.e. control, *Bt* spraying and/or parasitoid release) treatments on DBM larval density, *D. insulare* pupal density and rate of parasitism in cabbage fields in two cropping seasons: Postrera 2007 and Primera 2008. Result of GLM ANOVA.

Dependent variable	Effect	df	F	P
Postrera 2007				
DBM larvae	Treatment	3,3	3.81	0.0135
	Week	3,5	10.70	<0.0001
	Week*Treatment	5,15	1.02	0.4327
Diadegma pupae	Treatment	3,3	8.45	0.0001
	Week	3,5	37.09	<0.0001
	Week*Treatment	5,15	1.86	0.0254
Parasitism rate	Treatment	3,3	2.26	0.0922
	Time collection	3,3	3.09	0.0346
	Time*treatment	3,9	0.97	0.4736
Primera 2008				
DBM larvae	Treatment	3,3	1.47	0.2305
	Week	5,5	18.35	<0.0001
	Week*Treatment	3,15	1.77	0.0376
Diadegma pupae	Treatment	3,3	0.32	0.8112
	Week	3,5	70.47	<0.0001
	Week*Treatment	5,15	1.71	0.0465
Parasitism rate	Treatment	3,3	1.32	0.2797
	Time collection	3,3	0.27	0.8484
	Time*Treatment	3,9	0.16	0.996

6 Limitations of the study

There are some limitations associated with the methods used in this research. First, the quantitative estimates of killing capacity are from a laboratory study, rather than real predation in natural conditions. In the study of killing capacity, time, space and availability of larvae were obstacles to designing a better experiment.

Secondly, in the release experiment, the number of parasitoids released may have been too low to affect the density of DBM in the field. Neighboring farms spraying insecticide to control DBM may have affected the treatment. In addition, *D. insulare* undertakes active migration and may have moved to neighboring fields with other treatments. If this is true there is a need to conduct release experiments on a larger scale, e.g. at the landscape level. Furthermore, DBM may have developed resistance to *Bt* in Nicaragua.

A confounding factor is that DBM has fully overlapping generations, and mortalities due to parasitoids and other factors may change over time. This can be accounted for by comparing different pest densities, cropping practices or sites to determine the factors which promote or limit biological control, as was done partly in the research described herein

7 Conclusions

This study has shown that a broad spectrum of predators collected in and around cabbage fields in Nicaragua have the capacity to feed on *P. xylostella* eggs and larvae under laboratory conditions.

The predator group with the highest potential for regulating *P. xylostella*, due to its high consumption rate and high abundance, was lycosid spiders, but linyphiid spiders, staphylinid beetles, salticid spiders and nabid bugs may also be important.

Our results show that flying and ground dwelling natural enemies of *P. xylostella* interact negatively. Further studies on the potential role of factors affecting *P. xylostella* survival, including natural enemies, are urgently needed. A combination of control measures, including the promotion of predators and parasitoids, is probably needed to achieve a real sustainable control.

Positive effects on natural enemies of not using insecticides against DBM were found both for generalist predators (spiders and predatory wasps) and for parasitoids. The results suggest that increased leaf damage in insecticide treated fields is a combined consequence of insecticide resistance in the pest and lower predation from natural enemies that are suppressed by the insecticide applications.

The need to learn more about predators is reinforced by the fact that *P. xylostella* has developed resistance to almost all known insecticides and that there is little evidence that parasitoids alone can solve the problem.

Despite this fact, integrated pest management is not implemented in the cabbage fields of Nicaragua, nor is it in many other developing countries.
A major focus in future studies should be identifying combinations of natural enemies that interact additively or positively, resulting in increased mortality of *P. xylostella*.

8 Future perspectives

The experience of using chemical controls is that strong suppression of pests tends to decline because the pest evolves. Chemical control also has negative impacts on natural enemies and causes health problems in humans. Therefore, future research should focus on answering questions such as:

- How can farmers achieve local conservation of natural enemies that attack *P. xylostella*?
- To what extent are changed management practices, especially those affecting densities of natural enemies, responsible for changes in pest abundance?
- What is the role of field margins in promoting natural enemies of *P. xylostella*?

The results and new insights presented in this thesis will, hopefully, serve as a basis for biological control systems that ultimately prove to be effective management methods for pests like *P. xylostella* in the cabbage fields of Nicaragua and elsewhere.

References

Alam, M. M. 1992. Diamondback moth and its natural enemies in Jamaica and some other Caribbean Islands. Pp. 233-243. *In* Talekar, N. S. (ed.). Diamondback moth and other crucifer Pests: Proceedings of the Second International Workshop, Tainan, Taiwan, Asian Vegetable Research and Development Center, Shanhua, Taiwan.

Amend, J. and Besedow, Th. 1997. Combining release / establishment of *Diadegma semiclausum* (Hellen) (Hym., Ichneumonidae) and *Bacillus thuringiensis* Berl. For control *of Plutella xylostella* (L.) (Lep., Yponomeutidae) and other lepidopteran pests in the Cordillera Region of Luzon (Philippines). J. Appl. Entomol. 121: 337-342.

Andrews, K. L., Sanchez, R. J and Cave, R. D. 1992. Management of diamondback moth in Central America. Pp. 487-497. *In* Talekar, N.S. (ed.). Diamondback moth Management: Proceedings of the Second International Workshop.

Arora, R., Battu, G. S., and Bath, D. S. 2000. Management of insect pests of coliflower with biopesticides. Indian Journal of Ecology.27: 156-162.

Ayalew, G. and Ogol, C. K 2006. Occurrence of the diamondback moth (*Plutella xylostella*) and its parasitoid in Ethiopia: Influecie of geographical region and agronomic traits. J. Appl. Entomol. 130: 343-348.

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

Beck, N. G. and Camerom, P. J. 1992. Developing a reduced- spray program for brassicas in New Zealand. Pp. 341-350. *In* Talekar, N. S. (ed.). Diamondback moth and other crucifer Pests: Proceedings of the Second International Workshop, Tainan, Taiwan, Asian Vegetable Research and Development Center, Shanhua, Taiwan.

Biever, K. D., Hostetter, D. L. and Kern, J. R. 1994 Evolution and implementation of biological control-IPM system for crucifers. 24 year case history. American. Entomol. 40: 103-108

Branco, M. C., and Gatehouse, A. G. 1997. Insecticide resistance in *Plutella xylostella* (L.) (Lepidoptera: Yponomeutidae) in the Federal District, Brazil. Ann. Soc. Entomol. Brazil. 26: 75-79.

Braun, L., Olfert, O., Soroka, J., Mason, P. and Dosdall, L. M. 2004. Diamondback moth biocontrol activities in Canada. Pp. 144-146. *In* Kirk A. A. and Bordat, D. (eds). Improving Biocontrol of *Plutella xylostella*

Proceedings of the International Symposium, 21-24 October 2002. Montpellier, France.

Brunner, E. and Stevens, P. F. E. 1986. The control of diamondback moth with Thuricide. Pp. 213- 219. *In* Talekar, N. S., and Griggs. T. D. (eds). Diamondback Moth Management: Proceedings of the First International Workshop. Asian Vegetable Research and Development Center, Shanhua, Taiwan.

Campanhola, C., Moraes, G.J. D. and DeSa, L. N. 1995. Review of IPM in South America. P121-151. In Management, Mengech, A.N. Saxena, K. N and Gopalan (eds). Integrated Pest Manegement in the tropicsccurrent Status and Future Prospects, New York. John Wiley & Sons, 171 p.

Carballo, M,. Hernandez, M. and Quezada, J. R. 1989. Efecto de los insecticidas y de las malezas sobre *Plutella xylostella* (L.) y su parasitoide *Diadegma insulare* (Cress) en el cultivo de repollo. Manejo Integrado de Plagas 11: 1-20.

Chalfant, R. B., and Brett, C. H. 1965. Cabbage loopers and imported cabbage worms; feeding damage and control on cabbage in western North Carolina. J. Econ. Entomol. 58: 28-33.

Charleston, D. S., R. Kfir, M. Dicke, L .E. M. Vet. 2006. Impact of botanical extracts derived from *Melia azedarach* and *Azadirachta indica* on populations of *Plutella xylsotella* and its natural enemies: A field test of laboratory findings. Biological Control 39:105-114.

Cheng, E.Y. 1988. Problems of control of insecticide - resistant *Plutella xylostella*. Pestic. Sci., 23: 177-188.

Cheng, E.Y. Kao, C.H. and Chiu, C.S. 1992. Resistance of diamondback moth in Taiwan: Recent developments. Pp. 465-478. *In* Talekar, N. S. (ed.). Diamondback moth Management: Proceedings of the Second International Workshop.

CIEA, 2006. Learning for sustainable action. Program Promipac, Centro America. 25 International course on vocational training and education in agriculture. 1-8 p.

Cordero, J., and Cave, R. D., 1992. Natural enemies of *Plutella xylostella* (Lep. Plutellidae) on crucifers in Honduras. Entomophaga 37: 397- 403.

Cordero, R. J., Bloomquist, J. R. and Kuhar, T. P. 2007. Susceptibility of two diamondback moth parasitoids, *Diadegma insulare* (Cresson) (Hymenoptera; Ichneumonidae) and *Oomyzus sokolowskii* (Kurdjumov) (Hymenoptera; Eulophidae), to selected commercial insecticides. Biological Control 42: 48-54

Corriols, M., Marin, J., Berroteran, J. Lozano, L. M., and Lundberg, I. 2009. Incidence of acute pesticide poisonings in Nicaragua: a public health problem. Occup. Environ. Med. 66: 205-210.

Cortez, M. E., and Macias, C. J. 2007. Parasitism natural de la palomilla del repollo dorso de diamante *Plutella xylostella* en Canola (*Brassica napus* L.) en el norte de Sinaloa Mexico. Agrociencia 41: 347-354.

Cihoh, Y., Uefune, M., and Takabayashi, J. 2008. Diamondback moth females oviposit more on plants infested by non-parasitized than by parasitized conspecifics. Ecol. Entomol. 33: 565-568.

Dadang, E. Fitriasari, D. and Prijono, D. 2009. Effectiveness of two botanical insecticide formulations to two major cabbage insect pests on field application. J. ISSAAS. 15: 42-51.

DeBach, P. 1964. Biological control of insect pests and weeds. Chapman and Hall, London, U.K. 844 pp.

Diaz, J., Guharay, F., Miranda, F., Molina, J., Zamora, M., and Zeledón, R. 1999. Manejo Integrado de Plagas en el cultivo de Repollo. Manual Tecnico No 38 Managua, Nicaragua.

Dondale, C. D. 1956. Annotated list of spiders (Araneae) in Nova Scotia apple orchards. Can. Entomol. 88: 697-700.

Dondale, C. D. 1972. Laboratory breeding between European and North American populations of the spider *Philodromus rufus* Walckenaer (Araneidae: Thomisidae). Bull. Br. Arachnol. Soc. 2: 49-52.

Duffy, E. 1962. A population study of spider on limestone grassland. J. Anim. Ecol. 31: 571- 599.

Elliott, N. C., Tao, F. L., Fuentes-Granados, R., Giles, K. L., Elliott, D. T., Greenstone, M. H., Shufran, T. A. andRoyer, T. A. 2006. D-vac sampling for predatory arthropods in winter wheat. Biological Control 38: 325-330.

Facknath, S. 1999. Control of *Plutella xylostella* and *Crocidolamia binotalis* through the combined effects of *Bacillus thuriengiensis* and botanical pesticide. AMAS. Food and Agricult. Research council, Reduit, Mauritius. 87-92.

Fitton, M. G. and Walter, A. K. 1992. Hymenopterous parasitoids associated with diamondback moth: the taxonomic dilemma. Pp. 225-232 *In* Talekar, N.S. (Ed) Diamonback moth and other crucifer pest: Proceeding of the second international workshop, Tainan, Taiwan.

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

Furlong, M. J. and Zalucki, M. P. 2010. Exploiting predators for pest management: the need for sound ecological assessment. Entomologia. Experiementalis *et* Applicata. 135: 225-236.

Furlong, M. j., Ju, K. H., Su, P. W., Chol, J. K., Il, R. Ch. and Zalucki, M. P. 2008. Integration of endemic natural enemies and *Bacillus thuringiensis* to manage insect pests of Brassica crops in North Korea. Agric. Ecosyst. Environm.125: 233-238.

Galt, E. R. 2008. Pesticides in export and domestic agriculture: Reconsidering market orientation and pesticide use in Costa Rica. Science Direct. Geoforum39:1378- 1392

Georgiou, G. P. and Mellon, R. B. 1983. Pesticide resistance in time and space. Pp.1-46 *In* Georgiou, G. P and Satio, T. (eds). Pest resistance to insecticides. Plenum publishing, New York.

Godin, C. and Boivin, G. 1998. Lepidopteros pest of Brassica and their parasitoids SouthWestern Quebec. J. Environ. Entomol. 27: 1157-1165.

Goodwin, S. 1979. Changes in numbers in parasitoid complex associated with the diamondback moth, *Plutella xylostella* (L.) (Lepidoptera) in Victoria. Aust. J. Zool. 27: 981- 989.

Griswold, M. W. and Lounibos, L. P. 2006. Predator identity and additive effects in a treehole community. Ecology 87: 987-995.

Hassan, E. and Smith, S. 1995. Toxity of endosulfan, esfenvelerate and *Bacillus thuringiensis* on adult of *Microplitis demolitor* Wilkinson and *Tricogrammatidae bactrae* Nagaraja. Z. Pflanzenk. Pflanzensch. 102: 428-442.

Harcourt, D. G. 1960. Biology of the diamondback moth, *Plutella maculipennis* (Curt) (Lepidoptera: Plutellidae), in easter Ontario. III Natural enemies. Can. Entomol. 29: 419-428.

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

Hou, R. F. 1986. Mass rearing of Diamondback moth. Biological control of diamondback moth. Pp. 89- 95. *In* Talekar, N. S. and Griggs. T. D. (eds). Diamondback Moth Management: Proceedings of the First International Workshop. Asian Vegetable Research and Development Center, Shanhua, Taiwan.

Jankowski, A., Mithöfer, D., Löhr, B. Weibel, H. 2007. Economic of biological control in cabbage production in two countries in East Africa. Conference on International Agricultural Research for Development, tropentag. 8 pp.

Kamel, F. and Hoppin, J. A. 2004. Association of pesticide exposure with neurologic dysfunction and disease. Env. Health Perspective. 112: 950-958.

Kajak, A., Andrezejewska, L. A. and Wojcik, Z. 1968. The role of spiders in the decrease of damage caused by Acridoidea on meadows-experimental investigations. Ecol. Pol. A. 16: 755-765.

Kfir, R. 2001. Prospects for biological control of *Chilo partellus* in grain crops in South Africa. Insect Science and its Application 21: 275-280.

Khan, M. F. R., Griffin, R. P., Carner, G. R. and Gorsuch, C. S. 2005. Susceptibility of diamondback moth, *Plutella xylostella* (L.) (Lepidoptera:

Plutellidae), from collard fields in South Carolina to *Bacillus thuringiensis*. J. Agric. Urban Entomol. 22: 19-26.

Kibata, G. 1996. Diamondback moth *Plutella xylostella* (Lepidoptera: Yponomeutidae), a problem pest of Brassica crops in Kenya. Pp. 1-11. *In* Farrel, G. and Kibata, G. N. (eds). Proceedings of the 1st Biennial Crop Protection Conference. Nairobi, Kenya.

Kirk A. A., Mercadier, G., Bordat, D., Delvare, G., Pichon, A., Arvanitakis, L., Goudegnon, A. E. and Rincon C. 2004. Variability in Plutella and its natural enemies: implications for biological control. Pp. 71-77 *In* Endersby, N. M. and Ridland, P. M. (eds). The management of diamondback moth and other crucifer pests. Proceedings of the Fourth International Workshop, 26-29 November 2001. Melbourne, Australia: Department of Natural Resources and Environment.

Kogan, M. 1998. Integrated pest management: Historical perspectives and contemporary developments. Ann. Rev. Entomol. 43: 243-270.

Kring, T. J. and Smith, T. B. 1995. *Trichogramma presiosum* efficacy in cotton under Bt- insecticide combinations. Proceedings of the Beltwide Cotton Conference, National Council, San Antonio, Texas, USA. 2: 856- 857.

Krupa, J. J. 1996. Direct and Indirect effects of multiple enemies on water strider mating dynamics. Ecology 105: 179-188.

Lang, A. 2003. Intraguid interference and biocontrol effects of generalist predators in a winter wheat field. Oecologia 134: 144- 153.

Lee, J-H, and Kim.,S-T. 2001. Use of spider as natural enemies to control rice in Korea. Pp. 441-744. Entomology Program, School of Agricultural biotechnology, Seoul National University, Suwon, Korea.

Leibee, G. L. and Savage, K. 1992. Insecticide resistance in diamondback moth in Florida, pp. 427-435. *In* Talekar, N. S. (ed.). Diamondback Moth and Other Crucifer Pests: Proceedings of the Second International Workshop. Tainan, Taiwan, 10-14 December 1990. Asian Vegetable Research and Development Center, Shanhua, Taiwan.

Leonard, H. J. 1986. Natural Resources and Economic Development in Central America: A Regional Environmental, Profile. New Brunswick, HJ: Transactions Books International.

Lim, G. S. 1992. Integrated pest management of diamondback moth. Pp. 565-576. *In* Talekar, N. S. (ed.). Diamondback Moth and Other Crucifer Pests: Proceedings of the Second International Workshop. Tainan, Taiwan, 10-14 December 1990. Asian Vegetable Research and Development Center, Shanhua, Taiwan.

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

Losey, J. E. and Denno, R. F. 1998. Positive predator- predator interactions: enhanced predation rates and synergistic suppression of aphid populations. Ecology 79: 2143-2153.

Ma, J., Li., D., Keller.,M., Schmidt, O., and Feng, X. 2005a. A DNA marker to identify predation of *Plutella xylostella* (Lepidoptera: Plutellidae) by *Nabis kinbergii* (Hem., Nabidae) and Lycosa sp. (Araneae, Lycosidae). J. Appl. Entomol. 129: 330-335.

Ma, J., Li, Y. Z., Keller, M., and. Ren, S. X. 2005b. Functional response and predation of *Nabis kinbergii* (Hemiptera: Nabidae) *to Plutella xylostella* (Lepidoptera: Plutellidae). Insect Science 12: 281-286.

McCauley L. A., Anger W. K., Keifer M., Langley R., Robson M. G. and Rohlman, D. 2006. Studying health outcomes in farm worker populations exposed to pesticides. Environm. Health Perspectives 114:953-960.

Macintosh, S. C.., Stone, T. B. andJokerst, R. L. 1991. Binding of *Bacillus thuringiensis* proteins to a laboratory selected strain of *Heliothis virescens*. Proc. Nat. Acad. Sci. USA. 88: 8930- 8933.

McConnell, R., Cedillo, L., Kiefer, M. and Palomo, M. R. 1992. Monitoring organophoste insecticide-exposed workers for cholinesterase depression: New technology for office or field use. J. Occ. Env. Med. 34: 34-36.

MAGFOR, 2009. Informe de produccion Agropecuaria acumulado a Junio 2009. Ministerio Agropecuario y Forestal Direccion de Estadistica pp. 14.

Mazlan, N. and Mumford, J. 2005. Insecticide use in cabbage pest management in Camerum Highlands, Malaysia. Crop Prot. 24: 31-39.

Metcalf, L. R. 1980. Changing role of insecticides in crop protection. Ann. Rev. Entomol. 25: 219-256.

Mitchell, E. R., Tingle, F. C., Navasero-ward, R.C. and Kehat, M. 1997. Doamondbacm moth (Lepidoptera: Plutellidae): Parasitism by *Cotesisa plutellae* (Hymenoptera: Braconidae) in cabbage. Florida Entomol. 80: 477-489.

Miranda, F. 1992. Assessment of the effect of biological and botanical insecticide against *Plutella xylostella* in cabbage *Brassica oleracea* L. in two regions of Nicaragua. Thesis MsC. ISSN 0347-3236 (51 p).

Mojan, M. and Guhar, G. T. 2003. Local variation in susceptibility of the diamondback moth, *Plutella xylostella* (L.) to insecticides and detoxification enzymes. Crop Prot. 22: 495- 504.

Monnerat, RG., Santos, RC., Batista, AC. And Berry, C. 2002. Isolamento e caracterizao de estirpes de *Bacillus thuriengiensis* endofiticos do algodao. Brasilia. Embrapa. 4p. Circular tecnica no. 10.

Morallo-Rejesus, B. and Sayaboc, A. S.1992. Management of Diamondback moth with *Cotesia plutellae*: Prospects in Philippines. Pp. 279-286. *In*

Talekar, N.S (ed.). 1991. Diamondback moth Management: Proceedings of the Second International Workshop.

Muckenfuss, A. E., Shepard, B. M. and Ferrer, E. R. 1992. Natural Mortality of Diamondback Moth in Coastal South Carolina. Pp. 27-36. *In* Talekar, N. S. (eds) Diamondback moth and other crucifer Pests: Proceedings of the Second International Workshop, Tainan, Taiwan, 10-14 December 1990. Asian Vegetable Research and Development Center, Shanhua, Taiwan.

Muckenfuss, A. E., and B. M. Shepard .1994. Seasonal abundance and response of diamondback moth, *Plutella xylostella* (L) (Lepidoptera: Plutellidae) and natural enemies to Esfenvarate and *Bacillus thuringiensis* ssp. kurstaki Berliner in Costal South Carolina. J. Agric. Entomol. 11: 361-373.

Mustata, G. 1992. Role of parasitoid complex in limiting the population of diamondback moth in Moldavia, Romania. Pp. 203-211. *In* Talekar, N. S. (ed.) Diamondback moth and other crucifer Pests: Proceedings of the Second International Workshop, Tainan, Taiwan, Asian Vegetable Research and Development Center, Shanhua, Taiwan.

Miyata, T., Saito, T, and Noppun, V. 1986. Studies on the mechanism of diamondback moth resistance to insecticides, Pp. 347- 357. *In* Talekar, N. S., and Griggs. T. D. (eds). Diamondback Moth Management: Proceedings of the First International Workshop. Asian Vegetable Research and Development Center, Shanhua, Taiwan.

Nemoto, H. 1993. Mechanism of resurgence of the diamondback moth, *Plutella xylostella* (Lepidoptera: Yponomeutidae). JARQ 27: 27-32.

Nicholls, C. I. and Altieri, M. A. 1997. Conventional agricultural development models and the persistence of the pesticide treadmill in Latin America. Int. J. Sust. Developm. World Ecol. 4: 93-11.

Noda, T., Miyai, S., Takashino, K. and Nakamura, A. 2000. Density suppression of *Plutella xylostella* (Lepidoptera: Yponomeutidae) By multiple release of *Diadegma semiclausum* (Hymenoptera: Inhneumotidae) in cabbage fields in Iwate, northern Japan. Appl. Entomol. Zool. 35: 557- 563.

Obrycki, J J. and Kring, T. J. 1998. Predaceus coccinellidae in biological control. Annu. Rev. Entomol. 43: 295- 321.

Okine, J. S., Mithchell, E. R. and Hu, G. Y. Low temperature on viavility *of Diadegma insulare* (Hymenoptera: Ichneumonidad) pupae and effect of this parasitoid on feeding rate of diamondback moth larvae (Lepidoptera: Plutellidae). Agriculture Research Service, U.S Department of Agriculture. Gainesvile, FL 32606. pp. 503-509.

Orr, D. 2009. Biological control and integrated pest management. Integr. Pest Managem.: Innov.-Developm. Proc. 207-239.

Ovalle, O. G. and Cave, R. D. 1989. Determinacion de resistancia de *Plutella xylostella* L. (Lepidoptera: Plutellidae) a insecticidas comunes en Honduras. CEIBA. Vol. 30: 119-128.

Pearson, D. E. and Callaway, R. M. 2005. Indirect no target effect of host-specific biological control agents: Implications for biological control. Biological Control 35: 288- 298.

Perez, C. J., Alvarado, P., Miranda, F., Narváez, C., Hernández, L., Vanegas, H., Hruska, A. and Shelton, A. M. 2000. Assessment of insecticide resistance in five insect pests attacking field and vegetable crops in Nicaragua. J. Econ. Entomol. 93: 1779-1787

Pickett, A. D., Patterson, N. A., Shultz, H. T. and Lord. F. T. 1946. The influence of spray programs on the fauna of apple orchards in Nova Scotia: I. An appraisal of the problem and a method of approach. Scient. Agric. 26: 590-600.

Pimental, D. 1961. Natural control of caterpillar populations on cole crops. J. Econ. Entomol. 54: 889-892.

Poelking, A., 1992. Diamondback moth in the Philippines and its control with *Diadegma semiclausum*. Pp. 347- 357. *In* Talekar, N. S. and Griggs. T. D. (eds). Diamondback Moth Management: Proceedings of the First International Workshop. Asian Vegetable Research and Development Center, Shanhua, Taiwan.

Polis, G. A., Myers, C. A. and Holt, D. 1989. The ecology and evolution of intraguild predation: Potential competitors that ate each other. Ann. Rev. Ecol. Syst. 20: 297- 330.

Shelton, A. M., Tang, J. D. Roush, R. T., Metz, T. D. and Earle, E. D. 2000. Field tests on managing resistance to *Bt*-engineered plants. Nature Biotechn. 18: 339-342.

Simberloff, D. and Von Holle, B. 1999. Positive interactions of non indigenous species: invasional meltdown? Biological Invasions. 1: 21-32.

Straub, C. S. and Snyder, W. 2006. Species identity dominantes the relationship between predator biodiversity and herbivore suppression. Ecology 87: 277-282.

Straub, C. S., Finke, D. L., and Snyder, W. E. 2007. Are the conservation of natural enemy biodiversity and biological control compatible goals? Biological Control 45: 225- 237.

Sun, C. N. 1992. Insecticide resistance in diamondback moth. Pp. 419-426. *In* Talekar, N. S. (ed.) Diamondback moth and other crucifer Pests: Proceedings of the Second International Workshop, Tainan, Taiwan, 10-14 December 1990. Asian Vegetable Research and Development Center, Shanhua, Taiwan.

Sun, C. N., Wu, T.K., Chen, J.S. and Lee, w. T. 1986. Insecticide resistance in diamondback moth. Pp. 359- 372. *In* Talekar, N. S., and Griggs. T. D. (eds). Diamondback Moth Management: Proceedings of the First International Workshop. Asian Vegetable Research and Development Center, Shanhua, Taiwan.

Sunderland, K. D., Axelsen, J. A., Dromph, K., Freier, B., Hemptinne, J-L., Holst, N. H., Mols, P. J. M., Petersen, M. K., Powell, W., Ruggle, P., Triltch, H. and Winder, L. 1997. Pest control by community of natural enemies. Acta Jutlandica 72: 271-326.

Symondson, W. O., Sunderland, K. D. and Greenstone, M. H. 2002. Can generalist predators be effective biocontrol agents? Ann. Rev. Entomol. 47: 561-594.

Szwejda, J. 2004. Review of pests and natural enemies actually occurring on cabbages in Poland Nowosci warzywnicze 39: 97- 104.

Tabashnik, BE. 1994. Evolution of resistance to *Bacillus thuringiensis* Ann. Rev. Entomol. 39: 47-79.

Talekar, N. S. 1996. Biological control of diamondback moth in Taiwan. A review. Plant Protection Bull. 38: 167- 189.

Talekar, N. S. and. Shelton, A. M 1993. Biology, ecology and management of the diamondback moth. Ann. Rev. Entomol. 38: 275-301.

Talekar, N. S. and Lin, M-Y. 1998. Training in Mass rearing of the natural enemy and its periodic releases. Asian Vegetable research and Development Center Shanhua, Tainan 741, Taiwan. Publication No. 98-472. pp 67.

Talekar, N. S., Yang, J. C., and Lee, S. T. 1992. Introduction of *Diadegma semiclausum* to control with diamondback moth in Tiawan. Pp. 263-272. *In* Talekar, N. S. (eds) Diamondback moth and other crucifer Pests: Proceedings of the Second International Workshop, Tainan, Taiwan, 10-14 December 1990. Asian Vegetable Research and Development Center, Shanhua, Taiwan.

Tanaka, K. 1989. Movement of the spiders in arable land. Plant Protection 43: 34-39.

Thompson, W. R. 1946. A catalogue of the parasites and predators of insect pests. Seccion 1; Parasite Host Catalogue: Part 7; Parasites of Lepidoptera (G-M):259-185. Imperial Agricultural Bureau, Belleville, Ontario, Canada.

Trumble, J. and Alvarado-Rodriguez, B. 1993. Development of economic evaluation of an IPM program for frest market tomato production in Mexico. Agric. Ecosyst. Environ. 43: 267- 284.

Tilman, D., Cassman, K. G., Matson, P.A., Naylor, R. and Polasky, S. 2002. Agricultural sustainability and intensive production practices. Nature 418: 671-677.

Van Driesche, R. G, and Bellows, T. S., Jr. 1996. Biological control. Chapman & Hall, New York.

Varela, G 1991. Plicultivo (Repollo- Tomate; Erpollo-Zanahoria) y la incidencia de *Plutella xylostella* y sus enemigos naturalesen el repollo. Tesis MsC Turrialba, CATIE Costa Rica

Vastrad, A. S. Lingappa, S. and Basvangoud, K. 2004. Olvicides for Managing Resistant populations of Diamonback moth, Plutella xylsotella. Resist. Pest Manag. Newsl., 14: 3-5.

Verkerk, R. H. J. and Wright, D. J. 1997. Field- basic studies with the diamondback moth tritrophic system in Camerum Highlands of Malaysia: implication for pest management. International J. Pest Managem. 43: 27-33.

Waage, J. and Cherry, A. 1992. Quantifying the impact of parasitoids on diamondback moth. Pp. 245-253. *In* Talekar, N. S. (ed.) Diamondback moth and other crucifer Pests: Proceedings of the Second International Workshop, Tainan, Taiwan, 10-14 December 1990. Asian Vegetable Research and Development Center, Shanhua, Taiwan.

Wang, X., Duff, J., Keller, M. A. Zalucki, M. P., Liu, S. S. and Bailey, P. 2004. Role of *Diadegma semiclausum* (Hymenoptera: Ichneumonidae) in controlling *Plutella xylostella* (Lepidoptera: Plutellidae). Cage exclusion experiment and direct observation. Biocontrol Sci. Techn. 14: 571- 574.

Wang, X. G. and Keller, M. A. 2002. A comparison of host searching efficiency of two larval parasitoids of *Plutella xylostella*. Ecol. Entomol. 27: 105- 114.

Wu, G. and x Miyata, T. 2005. Susceptibilities to methamidophos and enzymatic characteristics in 18 species of pest insects and their natural enemies in crucifer vegetable crops. Pestic. Biochem. Physiol. 82: 79-93.

Wysoki, M. 1989. *Bacillus thuringiensis* preparations as a means for the control of lepidopterous pests in Israel. Isr. J. Entomol. 23: 119- 129.

Xu, J., Shelton, A. M., and Cheng, X. 2001. Variation in susceptibility of *Diadegma insulare* (Hymentoptera: Ichneumonidae) to Permethrin. J. Econ. Entomol. 94:541-546

Zhao, J. Z., Collins, H. L., Li, Y. X., Mau, R. F. L., Thompson, G. D., Hertlein, M., Andaloro, J. T., Boykin, R. and Shelton, A. M. 2006. Monitoring of diamondback moth (Lepidoptera: Plutellidae) resistance to spinosad, indoxacarb, and emamectin benzoate. J. Econ. Entomol. 99: 176-181

Acknowledgements

I would like to express my sincere gratitude to all great colleagues, teachers, and friends at the Department Ecology and Department of Crop Production Ecology and to my family that in many ways contributed to this thesis. Your support during these years have been encouraging and I would not be here today without you.

Many thanks to:

I thank Professor Christer Björkman, my main supervisor, and Associate Professors Helena Bylund and Riccardo Bommarco for all their valuable support and critical advice, and for English corrections, that were essential for me to accomplish this work. They have made me much more confident as a scientist and researcher. It has been a great pleasure for me to study and conduct this work under their supervision. Finally, I thank you for your kindness, for always keeping an open door to your office, and for your efforts in helping me finish.

The National Agrarian University (UNA) for given opportunity and the education leave for my PhD study.

This study was supported within the SIDA/ SAREC project UNA-SLU program.

I thank Lars and Eva Ohlander for you special help to me.

I extend my special thanks to Ing. Adriana Esther Diaz Valery, Sr. Dimas y Franklin Cerrato for field assistance.

Muchas Gracias a productores de la zona del Tisey Estelí. Los hermanos Salvador, Marcos Dimas y Vicente Cerrato Jiron; Lesther Navarro, Ariel Salgado, Modesto Mendoza, Bayardo Lopez, Eric Cruz, Wilfredo Valdivia, Herman Cerrato, Isabel Rayo, Victor Rivera, Gustavo Cerrato, Ervin Cerrato y Juan Rayo. Tambiem al Dr. Emilio Perez por colaborar con mi tisis.

Thank you Lina and Linda for you valuable work in Estelí Nicaragua

I will always be grateful to Professor Barbara Ekbom; Dr. N. S. Talekar and MsC. Enilda Cano for their support and motivation at the beginning of my career.

Mucha gracias a las familias de Nicaragua que viven en Uppsala, Gracias por recibirme y brindarme una linda Amistad: Famlia Nestor Altamirano, Edwind Briceno, Margarita Cuadra y de Chile Luis Miranda, y esposa (La Nana).

Tambien a todos los colegas MSc. estudiantes de Nicaragua- Ulises Blandon, Roldan Corrales, Isabel Herrera, Gerardo Murillo, Bryan Mendieta, por su solidaridad, Muchas gracias.

Tambien gracias a Dios por darme fortaleza cada dia.