Examensarbete
Institutionen för ekologi

Predators of *Plutella xylostella* in Nicaragua

Feeding capacity and potential role in biological control

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MAGISTERUPPSATS I BIOLOGI, D-NIVÅ, 20 P

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Examensarbete 2007:4
Uppsala 2007

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Abstract

The diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), is one of the most destructive insect pests of Brassica crops in both temperate and tropical regions over the world. The insect is considered as a key pest of crucifers in Central America. Due to the intense use of pesticides and because the pest occurs in all development stages at any time of the year in the tropics, *Plutella xylostella* has easily developed resistance to at least 46 different pesticides, including DDT.

Few studies have been made on the predators of *P. xylostella* and even less is known about their feeding rate. The maximum prey consumption and the density of the predators are basic elements in the evaluation of a predator as a possible biological control agent. Therefore a quantitative study was done to (1) identify possible predators of the diamondback moth and (2) estimate their feeding rate on *P. xylostella* eggs and larvae of different stages under laboratory conditions. The overall goal was to provide information that can serve as a basis for finding important biological control agents in cabbage cultivation in Nicaragua, where the investigation took place.

The result showed a broad spectrum of predators eating *P. xylostella*. The most important predators, with respect to larval consumption in the laboratory and abundance in the field and on plants, were wolf spiders (Lycosidae). Rove beetles (Staphylinidae), jumping spiders (Salticidae) and damsel bugs (Heteroptera: Nabidae) had also high consumption rates and were frequently observed on cabbage plants or in cabbage fields. The feeding rate varied least among predator groups when they were fed 2nd instar larvae (L2) compared to when fed eggs or L3. The feeding rate of predators on L2 was also significantly higher than that on L3, for all predator groups except for *Paederus* sp. (Staphylinidae). No differences in feeding capacity were found between individuals of predators, belonging to the same groups, collected in field margins and in cabbage fields.

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Introduction

The diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), is one of the most destructive insect pests of Brassica crops in tropical and temperate climates all over the world and in many different farming systems from backyard gardens to large-scale fully mechanized farms (Taleker & Shelton, 1993). In Central America, where the study took place, the insect is considered a key pest of crucifers (Andrews et al., 1991). The cabbage is cultivated all year round except in areas with no access to irrigation, where it is only produced during the wet season. As a consequence, all life stages of *P. xylostella* can be present at any time of the year.

Plutella xylostella easily develops resistance to insecticides. It was the first insect to develop resistance to DDT in 1953 in Indonesia (Taleker & Shelton, 1993) and has now developed resistance to a wide range of insecticides; it has been reported to be resistant to 46 insecticides in at least 14 countries (Miyata et al., 1986; Shelton & Wyman, 1991). In Central America, resistance has been reported in Honduras and Costa Rica, the countries neighboring Nicaragua (Andrews et al., 1991). In addition, *P. xylostella* was reported to be the first insect resistant to the microbial agent *Bacillus thuringiensis* (Taleker & Shelton, 1993). Due to *P. xylostella* having developed resistance to both chemical and biological pesticides and the absence of studied effective natural enemies, it is considered the most difficult pest in crucifers (Shelton & Wyman, 1991; Taleker & Shelton, 1993). It has also become increasingly difficult and expensive to develop new pesticides. To overcome the resistance it is common to apply combinations of insecticides, but no good combinations have been found to control resistant *P. xylostella* (Miyata et al., 1986). A higher density of *P. xylostella* is also found in the fields because of lower number of predators due to frequent use of pesticides (Guan-Soon, 1991; Ivey & Johnson, 1998; Furlong et al., 2004b). Heavy insecticide spraying may also reduce the number of spiders (Nyffeler & Sunderland, 2003). Even if there are many problems with insecticides, the lack of alternatives and the relatively low price of insecticides, has led to their use being the main control tactic for *P. xylostella*. In many areas of the world where control problems are most acute, farmers have been forced to rely more and more on biological control strategies, such as intercropping, trap cropping, rotation, clean cultivation, sprinkler irrigation, inoculative releases of parasitoids, mating disruption, cultural controls and conservation of natural enemies, where all methods still are under investigation and development (Taleker & Shelton, 1993).

Mortality caused by invertebrate predators and parasitoids is an important factor in the regulation and dynamics of pest populations (Symondson et al., 2002). To date, the majority of studies of natural enemies, in general, and of *P. xylostella*, in particular, are mainly about parasitoids. Predation and other sources of mortality have been ignored and are poorly understood (Guan-Soon, 1991; Taleker & Shelton, 1993; Furlong et al., 2004a; Ma et al., 2005ab). If predators are discussed, they often merely constitute a listing of species found in traps in crucifer fields (Guan-Soon, 1991) and only a few articles report experiments where predators actually are shown to predate *P. xylostella*. The neglect of predators is surprising, considering that manipulative field studies have demonstrated that generalist predators in 75% of the cases could reduce the amount of pest significantly (Symondson et al., 2002). Predators could also be good as biological control agents because of their capacity to rely on alternate prey during periods of low density of the target prey (Roger et al., 2000). Spiders are a potentially important group of predators of *P.*

xylostella; it is one of the most abundant predator groups recorded in grain crops and their presence cause high mortality on pest populations (Ma et al., 2005a). Few studies have been made on predators of *P. xylostella* and even less is known about the feeding rate of the predator and the effect on *P. xylostella*. The maximum prey consumption and the density of the predators are basic elements in the evaluation of a predator as a possible biological control agent (Jervis & Kidd, 1996).

Due to the poor information about the predators of *P. xylostella*, especially in Central America, a study was done to (1) identify possible predators (2) estimate their feeding rate on *P. xylostella* eggs and larvae. The overall goal of this study was to provide information that can serve as a basis for finding important biological control agents to be used in cabbage cultivation in Nicaragua, where the investigation took place, and neighboring countries.

Methods and material

Description of the pest Plutella xylostella

The diamondback moth feeds only on Brassica crops and certain weeds belonging to the Brassicaceae family. The pest is believed to have originated in the Mediterranean area, which also is the origin of many important Brassica crops. As a result of the ability to migrate long distances, *Plutella xylostella* now occurs in all countries where crucifers are cultivated. Parasitoids, which are an important source of biological control of the pest, have not been reported to migrate. In the tropics, where crucifers are cultivated all year around, there are up to 20 generations of *P. xylostella* yearly, which allows insecticide resistance to develop easily. *P. xylostella* adults become active at dusk and continue to fly into the night which also is the oviposition period. Mating takes place at dusk on the same day that they emerge. The females lay 11-188 eggs during the whole oviposition period, which starts soon after mating and continues for four days. The majority of eggs are placed on the leaf, preferentially in concavities of leafs rather than on smooth surfaces, the incubation last 5 to 6 days depending on the temperature. All larval instars feed on the foliage, preferable the undersides of leaves. From 2nd instar larvae (L2) the larvae consume all the tissue only leaving the wax layer as the characteristic windows in the leaf. The temperature regulates the development time of the larval stage of *P. xylostella*; increased temperature results in faster larval development. During the summer in Ohio the period is between 4 and 5.6 days for each larval instar. The host crop has also an influence on the development rates. After the fourth instar the larva constructs an open network cocoon on the leaf surface over a period of two days. The pupal period lasts between 4 and 15 days, depending on the temperature. The *P. xylostella* emerge in the early afternoon, between 13.00 and 16.00, and live for a few days. The adults feed on water drops or dew. All of the information above is taken from Taleker & Shelton (1993).

Description of study site

The study took place between 13/10/2006 and 22/11/2006, with a preliminary study in August to October, at six small farms in the area of Tisey close to Estelí in the north-west part of Nicaragua (Lat. 12^{o} 59´ N; Long. 86^{o}22´ W), at an elevation of approximately 1400 meters above sea level. The sizes of the field plots in the study were between 0.07 and 0.11 hectare with an average of 0.09 ha (field observations). In the area for the study cabbage, potatoes and camomile were the most important cash crops, broccoli was also cultivated in the area (personal communication with local farmers).

The farms chosen for the study are also included in a part of another study by a PhD student Freddy Miranda, Universidad Nacional Agraria, UNA in Nicaragua who so far has done studies mainly about parasitoids and the population dynamics of *P. xylostella*. The field work was done together with Lina Grönberg, agronomy student at Swedish agriculture university (SLU), Sweden.

Half of the fields in the study were conventional, i.e. chemical fertilizers and insecticides were used. The other fields were not treated with insecticides but chemical fertilizers, and they were called semi-organic. The farms were not totally semi-organic or conventional. The semi-organic farms, like the conventional farms, used sometimes pesticides and insecticides depending on crop and need in the fields. This means that the preceding crop in the rotation may have been treated with chemicals in the semi-organic and not in the conventional. The exception was one farm which always was semi-organic.

The cultivated land at the farms varied from 1 to 10 'manzana' (local term) which corresponds to 0.846 to 8.46 hectares. Fruit trees, coffee bushes, forest or pasture land (except cultivated) is not included in the calculations. In addition to this, the farms often had farm land in fallow, between 0 and 6.8 ha with an average of 2.8 hectare in fallow. In Nicaragua as in Honduras, cabbage are cultivated in small plots in home gardens and in fields of up to 2 ha. The average field is approximately 0.3 ha in Honduras (Andrews et al., 1991). The amount of cabbage cultivated at the farms in the study was between 0.2 and 2.5 ha, with an average of 1.3 hectare. In comparison to other crops cultivated at the farms, 8% to 100% (average 45%) of the farm land at the farms were planted with cabbage. Other crops that were cultivated at the time were maize at 5 farms, potatoes at 4 farms, camomile at 3 farms, beans at 2 farms and pasture, broccoli and lettuce each on 1 farm. Half of the farms used oxen for the soil preparation and half used a rented tractor with disc plough/harrow. All the weeding was done by machete or hand hoe on all farms.

All the seedlings were brought up in a small nursery for one month before planting in the field. The seedlings for the conventional farms were planted close to the fields whereas the seedlings for the semi-organic were brought up in a green house. The planting date on the farms varied from 19 September to 27 September. The average plant density was 4.4 plants/m^2. For specification of weeding, application of fertilizers and pesticides in field cf. Grönberg (2007).

Sampling of possible predators

The possible predators were collected using a D-vac (vacuum insect net), both from the field and the field margins of the cabbage fields. Six samples were taken in each field and four from the field margins, one from each margin and each week during 6 weeks at six different fields. Each spot in the field for the sampling was randomly selected. The area of the sampling plot was 2.25 m^2 (1.5 m x 1.5 m). The D-vac was used for approximately 27 seconds each time and ran at maximum speed. Some predators were also caught by hand when observing of the cabbage plants was done. Observations were done on 60 plants divided at 6 spots randomly selected in each field every week.

Larval rearing

Adults and larvae of *P. xylostella* were sampled in the area of Tisey-Estanzuela and cultured in a laboratory. The adults were held in cages of net and fed a mixture of honey and water. One cabbage plant was placed in the cage every second day. After two days in the cage the cabbage plant with eggs were removed to another cage. The larvae were reared on new cabbage plants when needed. The duration of the different development stages of the larvae reared in the laboratory were estimated in the end of November; egg between day 0 and 5, egg turning into larvae at day 6, 1st instar larvae (L1) at day 7 to 9, 2nd instar larvae (L2) at day 10 to12, 3rd instar larvae (L3) at day 13 to 17 and 4th instar larvae (L4) between day 18 and 24.

Predation study

To identify the predators, all potential predators were tested in a preliminary bioassay to see if they ate larva of *P. xylostella*. Insects with stylets and big chewing mandibles and spiders were especially chosen. Small spiders were only tested a few times as they turned out to be too small to handle the prey. Afterwards a closer study was done with the predators that had eaten, in which the maximum predation rate of *P. xylostella* egg and larvae under laboratory conditions was estimated. The temperature in the laboratory fluctuated with the outdoor temperature.

To determine eating capacity, the predators were tested individually in transparent plastic 30 ml cups with a plastic lid. The diameter of the cups was 4.5 cm. Due to the large size of some predators of the Salticidae, Lycosidae and Reduviidae family, they were placed in a bigger transparent plastic cup of 250 ml, 8 cm diameter. A piece of moist paper was placed in the cups to provide the insects with water. The predation study started 1 day after the sampling of the predators to standardize hunger level. Larvae from one larval instar or eggs were provided to the predator.

The number of prey larvae provided was between 20-50% more than the expected number to be consumed by the individual in a period of 24 h. The number of prey killed and eaten by the predator was noted after 1 day and continuously every day for 1-13 days (mean 4.38 days, SE 0.104 and median 5 days). The cups were cleaned from frass and dead larvae with a soft brush at the same time as new larvae were introduced. In almost all replicates the predator had consumed the whole larvae. When providing eggs as prey, a leaf with eggs was placed in the cup. The amount of consumed eggs was estimated by counting the eggs left after 1 day.

Occasionally, an insufficient number of prey larvae were supplied to the predator. In those cases the number of consumed larvae was excluded in the calculations of the maximal predation, except if the amount was higher than the lowest observed number of larvae eaten during 24 hours for that predator. Replicates in which predators died during the test were not excluded from the test, only the number of eaten prey the last 24 hours.

Is there a difference in eating capacity between different predator groups?

To estimate any difference in the eating capacity among the different groups of predators a General Linear Model ANOVA was performed. Comparisons between all different groups were done with the Tukey Tests. The number of groups compared were 12 in all investigated prey stages; egg, L2 and L3. All groups with more than 5 individuals were included in the test. For further details about the groups see Appendix 1.

The compared groups fed eggs were; Lycosidae (n=6 individuals), Salticidae A (n=5), Araneae (n=9), Thomisidae (n=12), Nabidae *Nabis* sp. (n=8), Syrphidae larva (n=5), Staphylinidae E (n=13), *Forficulidae dorus* (n=6), Staphylinidae B (n=7), Staphylinidae larva (n=8), Pentatomidae/Miridae larva, (n=6) and Pyrrhocoridae larva (n=10).

The compared groups offered L2 were; Lycosidae (n=12 individuals), Salticidae A (n=30), Araneae (n=23), Thomisidae (n=14), Tetragnathidae A (n=5), Gnaph/Club/Liocran/Anyph (Gnaphosidae, Clubionidae, Liocranidae and Anyphaenidae) (n= 6), Salticidae D (n=6), Staphylinidae *Paederus* sp. (n=10), Nabidae *Nabis* sp. (n=22), Syrphidae. larva (n=7), Staphylinidae E (n=7) and Staphylinidae larva (n=6).

The compared groups fed L3 were; Tetragnathidae B (n=6), Lycosidae (n=13), Salticidae A (n= 33), Araneae (n=14), Thomisidae (n=14), Gnaph/Club/Liocran/Anyph (n=5), Salticidae B (n=11), Salticidae C (n=5), Staphylinidae *Paederus*, (n=10), Nabidae *Nabis* sp. (n=26), Syrphidae. larva (n=5) and Reduviidae (n=7).

In the diagram predators fed on eggs n = < 5 was Tetragnathidae B (n=3), Linyphiidae A (n=2), Salticidae D (n=1), Salticidae C (n=1), Lycosidae small (n=3), Staphylinidae *Paederus* (n=4), Carabidae A (n=4), Staphylinidae C (n=2), Staphylinidae F (n=3), Staphylinidae D (n=1), Coccinellidae. larva (n=1) and Reduviidae (n=4) are also shown. Predators fed on L2 with n = < 5 in the diagram are Tetragnathidae B (n=3), Linyphiidae A (n=2), Opiliones (n=2), Araneidae (n=3), Salticidae C (n=2), Lycosidae small (n=4), Staphylinidae C (n=3), *Forficulidae dorus* (n=2), Staphylinidae D (n=1), Coccinellidae. larva (n=2), Gelastocoridae (*Gelastocoridae gelastocoris*) (n=2) individuals, Reduviidae (n=1) and Pyrrhocoridae. larva (n=3).

Which predators eat potentially most in field?

To estimate the possible impact of different predator groups in the field and on plants a hypothetical value of predation was calculated by multiplying the average eating capacity and the abundance of the predator groups. The predator density at the field and the plants was determined from the material from Lina Grönberg and are presented in appendix 2. The possible importance/predation were ranked from 1-11, where 1 is the least predation and correspond to 1-10 larvae eaten/day by individuals from one group at 60 cabbage plants or in 13.5 m^2. 2 corresponds to 11-20, 3: 21-30, 4: 31-40, 5: 41-50, 6: 51-60, 7: 61-70, 8: 71-80, 9: 81-90, 10: 91-100 and 11: 101-110 eaten larva or egg/day.

Do the predators eat more of a smaller sized larva?

In this experiment the difference in the maximal predation of larval instar L2 and L3 by the same predator individual was studied. The study only included L2 and L3 since they were in the middle of the development which was when they start to consume large amounts of cabbage. An additional reason for not using L1 was their small size and hence problems to reach correct estimates of predation rates. Also due to the broad spectrum of predators it was important to utilize intermediate size of the larvae why L4 were not used.

Ninety-one (91) predator individuals were divided into 6 groups of predators; Araneae (n=20), Lycosidae (n=12), Salticidae (n=30), Thomisidae (n=11), Staphylinidae *Paederus* sp.(n=7) and Nabidae *Nabis* sp. (n=11). One outlier was removed from the Thomisidae group and one from the Nabidae *Nabis* sp. group. In order to determine which statistical test to use the data was tested for equal variances. Variances were equal in all comparisons; and, paired t-tests in MiniTab were used to test for differences in consumption rate of L2 and L3 larvae in each predator group.

Is there a difference between eating capacity of predators found in the field margins and the field?

To determine if there was a difference between the eating capacity of the predators found in the field margins and the predators found in the field, a comparison was done using General Linear Model ANOVA test in combination with Tukey Tests including 5 different predator groups for L2 and 4 different groups for L3. For further details about the groups see Appendix 1. This test was also made to estimate if it would be necessary to adjust the results of eating capacity for the different groups depending on the distribution between predators found at the field margin or in the field.

In the group of Gnaph/Club/Liocran/Anyph and Lycosidae fed L2, 6 individuals came from the field and 5 from the field margin. For Gnaph/Club/Liocran/Anyph and Lycosidae fed L3 the corresponding numbers were 7 vs. 5. For Araneae fed L2, 15 individuals came from the field and 21 from the field margin. For Araneae fed L3, the corresponding numbers were 8 vs. 25. For Salticidae fed L2, 6 individuals came from the field and 26 from the field margin. For Salticidae fed L3, the corresponding numbers were 12 vs. 8. For Nabidae *Nabis* sp. fed L2, 8 individuals came from the field and 11 from the field margin. For Nabidae *Nabis* sp. fed L3, the corresponding numbers were 8 vs. 16. The difference in the Staphylinidae family was only studied in L2 were 6 individuals came from the field and 12 came from the field margin.

Limitations of the study

There are some limitations of the methodology of this study. First, it is a quantitative laboratory study which does not show the real predation in nature. Second, limitations of time, space, knowledge and larvae were obstacles to performing a perfect study. In the study of eating

capacity we did not separate female from male predators, and we did not have enough predator individuals to do comparisons between predators found in field margin, field and plant. In other studies of the egg predation the predation rate was determined by detecting the number of egg shells left, while we only calculated the number of intact visible eggs. The densities of larvae were not equal in all cups. Finally the predation study was not carried out in a more natural habitat e.g. on cabbage plants. However, the goal with the study was mainly to do a basic quantitative laboratory study to identify predators of *P. xylostella*, estimate their eating capacity and through that identify important predators. No other similar study have been performed in Nicaragua.

Results

All the predators that were found and predators studied with respect to predation rate are listed in appendix 2. In appendix 1 it is also possible to see the grouping of the predators

Is there a difference in eating capacity between different predator groups?

In this study the predators were identified and their eating capacities were investigated under laboratory conditions. The feeding rate varied least among predator groups when they were fed 2nd instar larvae (L2) compared to when fed eggs or L3. The feeding rate of predators on L2 was also significantly higher than that on L3, for all predator groups except for *Paederus* sp. (Staphylinidae). Staphylinidae larvae was the group with the significantly highest predation of eggs.

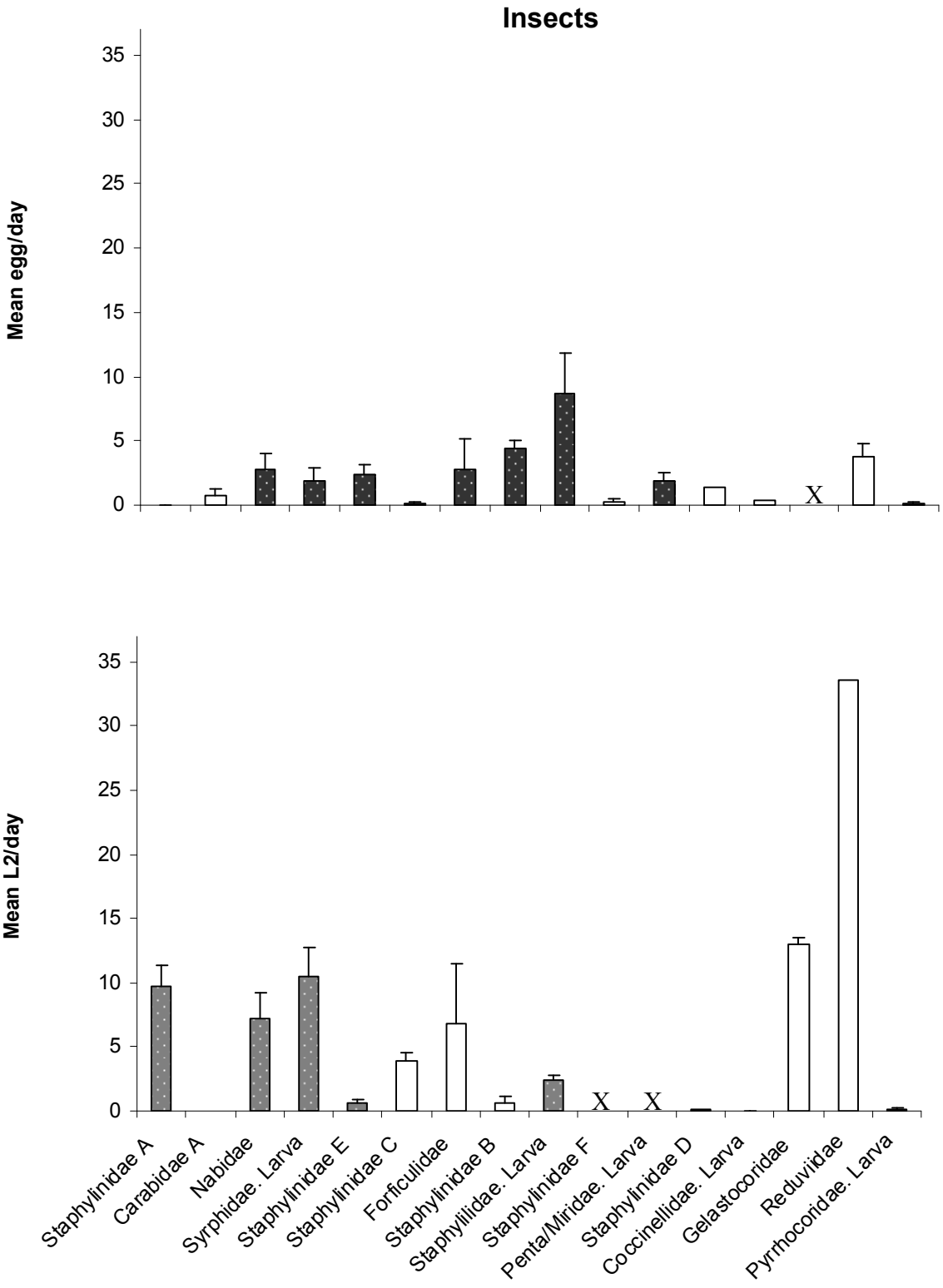

Figure 1. Eating capacity, measured as feeding rate of eggs and 2nd instar (L2) *Plutella xylostella* larvae by different groups of predatory insects collected in cabbage fields in the Tisey area, Nicaragua. The groups are ordered from left to right based on their abundance observed on cabbage plants in the field. Groups with n = >5 are grey and white if n <5. An 'X' indicates that no data was recorded for that group.

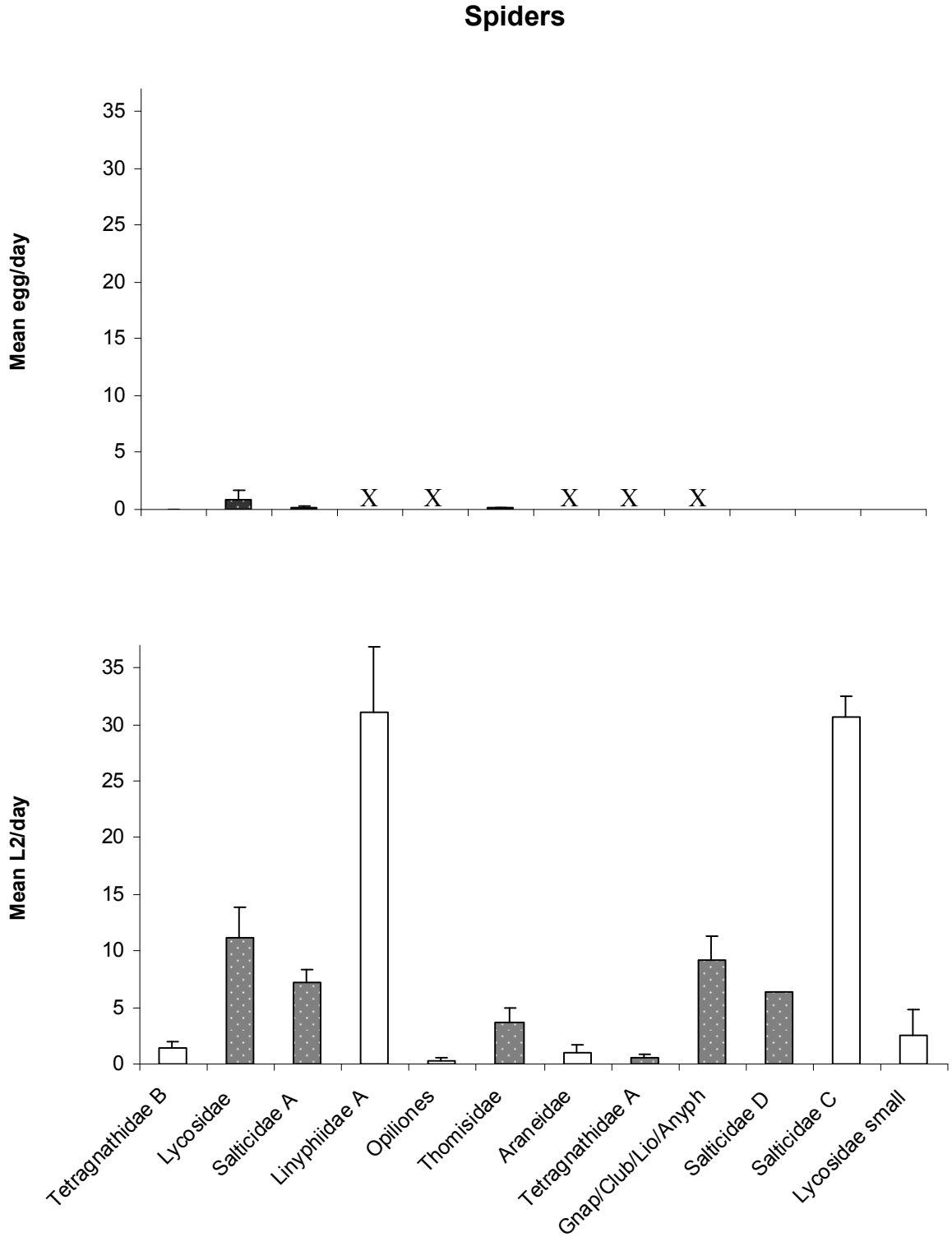

Figure 2. Eating capacity, measured as feeding rate of eggs and 2nd instar (L2) *Plutella xylostella* larvae by different groups of predatory spiders collected in cabbage fields in the Tisey area, Nicaragua. The groups are ordered from left to right based on their abundance observed on cabbage plants in the field. Groups with n = >5 are grey and white if n <5. An 'X' indicates that no data was recorded for that group.

In general no big differences were found in eating capacity within the groups fed on eggs. Only Staphylinidae larvae ate significantly more in comparison with Pyrrhocoridae larvae ($p = 0.002$), Syrphidae larvae ($p = 0.0343$), Pentatomidae/Miridae larvae ($p = 0.0203$), Araneae ($p = 0.0002$), Lycosidae ($p = 0.0035$), Nabidae *Nabis* sp. ($p = 0.0398$), Salticidae A ($p = 0.0021$), Staphylinidae E ($p = 0.0056$) and Thomisidae ($p = 0.0001$).

Within groups fed L2 only, Lycosidae ate significantly more than Araneae ($p = 0.0146$) and Staphylinidae E ($p = 0.0192$).

Groups fed L3 showed a bigger difference among the groups. Salticidae C ate significantly more than; Salticidae A ($p = 0.0011$), Thomisidae ($p = 0.0001$), Lycosidae ($p = 0.0143$), Nabidae *Nabis* sp. ($p = 0.0048$), Tetragnathidae B ($p = 0.0006$), Araneae ($p = 0.0001$). Reduviidae ate significant more than; Araneae ($p = 0.0045$), Tetragnathidae B ($p = 0.0334$), Salticidae A ($p = 0.0011$) and Thomisidae ($p = 0.0004$). Salticidae B ate significant more than; Tetragnathidae B ($p = 0.0013$), Salticidae A ($p = 0.0000$), Thomisidae ($p = 0.0000$), Araneae ($p = 0.0001$), Nabidae *Nabis* sp. ($p = 0.0073$) and Lycosidae ($p = 0.0380$). Staphylinidae *Paederus* sp. ate significant more than; Thomisidae ($p = 0.0019$), Tetragnathidae B ($p = 0.0334$), Salticidae A ($p = 0.0050$) and Araneae ($p = 0.0045$).

Which predators eat potentially most in field?

The hypothetically most important group of predators based on larval consumption in the laboratory and abundance in fields and on cabbage plants is the Lycosidae. It has a high ranking as an important predator on both plants and in the field for egg, L2 and L3. Other important predator groups in the field and on the plants appear to be Staphylinidae, Nabidae *Nabis* sp. and Salticidae. Linyphiidae have a high ranking eating L2 because of a high feeding rate, on average 31.1 L2 /day ($n = 2$). The larvae of Syrphidae also ate quite much 10.4 L2 /day on average ($n = 7$) and 1.8 egg/ day on average, see figure 1.

Table 1: Calculated hypothetical predation capacity by the best seven potential predators, with respect to abundance on cabbage plants in the Tisey area, Nicaragua. The groups are ordered by highest feeding rate at the top of the list. The predation capacity is symbolized by an index, the lowest number, 1 = lowest predation, 1-10 larva or egg/day and so on with respect to the sampled area, 60 plant observations, 2=11-20, 3=21-30, 4=31-40, 5=41-50, 6=51-60, 7=61-70, 8=71-80, 9=81-90, 10=91-100 and 11=101-110 larva or egg/day.

Egg		L2		L3	
Lycosidae	1	Lycosidae	6	Staphylinidae A	3
Nabidae	1	Linyphiidae	5	Lycosidae	3
Syrphidae. larva	1	Staphylinidae A	4	Tetragnathidae B	2
Carabidae A	1	Salticidae A	3	Salticidae A	1
Forficulidae	1	Tetragnathidae B	2	Gnap/Club/Lio/Anyph	1
Staphylinidae E	1	Salticidae C	1	Nabidae	1
Salticidae A	1	Nabidae	1	Syrphidae. larva	1

Note: Linyphiidae (L2) not tested in L3
Carabidae A (egg) not tested in L2 or L3
Gnap/Club/Lio/Anyph (L3) not tested on eggs
Salticidae C (L2) no observations in field

Table 2: Calculated hypothetical predation capacity by the best seven potential predators with respect to abundance in cabbage field in the Tisey area, Nicaragua. The groups are ordered by highest feeding rate at the top of the list. The predation capacity is symbolizes by an index, the lowest number, 1 = lowest predation, 1-10 larva or egg/day and so on with respect to the sampled area, 13,5 m^2. See Table 1 for a more detailed description.

Egg		L2		L3	
Staphylinidae B	5	Lycosidae	11	Lycosidae	5
Staphylinidae. Larva	4	Nabidae	4	Nabidae	3
Nabidae	2	Salticidae B	4	Salticidae A	2
Lycosidae	1	Linyphiidae	3	Staphylinidae A	2
Staphylinidae E	1	Araneae	2	Araneae	2
Carabidae A	1	Lycosidae small	2	Staphylinidae. larva	1
Penta/Miridae. larva	1	Staphylinidae A	2	Tetragnathidae B	1

Note: Linyphiidae (L2) not tested in L3
Carabidae A(egg) not tested in L2 or L3
Staphylinidae B (egg) not tested in L3
Penta/Miridae (Pentatomidae and Miridae) larvae (egg), not tested in L2 and L3 no observations on plants
Lycosidae small (L2) no observations on cabbage plants
Staphylinidae B (egg) no observations on cabbage plants
Staphylinidae larvae (egg) no observations on cabbage plants

Do the predators eat more of a smaller sized larva?

The number of larvae eaten varied with the instar of the prey; all predator groups except Staphylinidae *Paederus* sp. ($p = 0.45$) ate significantly more of L2 than of L3 (Fig. 3).

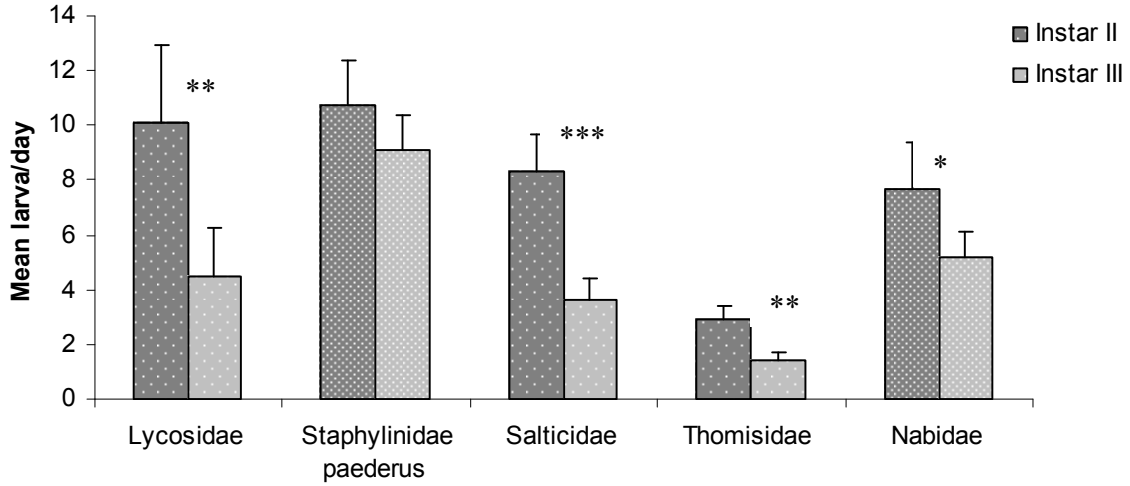

Figure 3: Difference in feeding rate (larvae/day) of 2nd instar (L2) and 3rd instar (L3) *P. xylostella* larvae in different predator groups, ordered from left to right based on their abundance observed on cabbage plants in the field. * = $p<0.05$, ** = $p<0.01$ and *** = $p<0.001$.

Araneae, a group with a mixture of different spiders also showed a significantly bigger predation of larval L2. In addition to the result of only Staphylinidae *Paederus* sp. ($p = 0.45$). A group of a mixture of different Staphylinidae (n =5) and Staphylinidae *Paederus* sp. (n=7) were also tested with no significantly difference between L2 and L3 ($p = 0.271$).

Is there a difference between eating capacity of predators found in the field margins and the field?

No significant difference in feeding rate was found between predator individuals collected in the field margins and individuals collected in the field. A comparison by General Linear Model ANOVA test gave $p = 0.671$ for L2 and the same test for L3, $p = 0.076$. There was no interaction between eating capacity of the different groups of predator and the field margin and the field (L2 $p = 0.149$ and L3 $p = 0.344$).

Discussion

This study showed 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 feeding rate varied least among predator groups when they were fed 2nd instar larvae (L2) compared to when fed eggs or L3. The feeding rate of predators on L2 was also significantly higher than that on L3, for all predator groups except for rove beetles (Staphylinidae *Paederus* sp.). No differences in feeding capacity could be established between individuals of predators collected in the field margins and in the fields. Wolf spiders (Lycosidae) seemed to be the predator group with the highest potential for regulating *P. xylostella.*

The most important predators with respect to larval consumption in the laboratory and abundance in field and on plants were spiders from the family Lycosidae (see Table 1 and 2). It had a high ranking and was, thus, an important predator both on plants and in the field for eggs, L2 and L3. One species of Lycosidae has been noted as the most abundant predator in an article about *P. xylostella* by Muckenfuss and Shepard (1994). This spider family has also been found in high frequency in pit fall traps placed in brassica fields in Nicaragua (Grönberg, 2007) and in Australia by Furlong et al. (2004b). This indicates that this ground dwelling spider family is active in the field which may be an important factor in the regulation of *P. xylostella* larvae. In one study made by Ma et al. (2005a) it was also proved that 76.6 % of the Lycosidae found in a broccoli field had been consuming *P. xylostella*. In addition, Lycosidae was the second most abundant predator found on the cabbage plants in Nicaragua (see appendix 2). The most frequently seen spider on the plants were long jawed orb-weavers (Tetragnathidae) because they often constructed nets between the cabbage plants, but they did not eat much; 1.4 L2 larvae/day in comparison to 11.2 L2/day for Lycosidae (see Appendix 2).

Other important predator groups in the field and on the plants appeared to be Rove beetles (Staphylinidae), jumping spiders (Salticidae) and damsel bugs (Nabidae *Nabis* sp.) (see Table 1 and 2) Staphylinidae was also similar to Lycosidae frequently found in pit fall traps both in Nicaragua (Grönberg, 2007) and Australia (Furlong et al., 2004b). Staphylinidae are reported to feed on eggs and larvae of the fly *Delia radicum* (Szwejda, 2004). Salticidae was the fourth most frequent predator on the cabbage plants (see appendix 2) and it is named as a predator found in cabbage field in Japan (Nemoto, 1986).

The feeding rate of Nabidae *Nabis* sp., which was the fourth most frequently found predator family in the field and the eighth most frequently found on the cabbage plants (see appendix 2), has also been investigated by Ma et al. (2005b) under laboratory conditions . The *Nabis* sp. showed a high predation rate, up to 131 eggs/day or 95 L2/day on average. The *Nabis* sp. tested in Nicaragua ate 3 eggs/day or 7 L2/day on average. In a second study by Ma et al. (2005a) it was shown that 67% of the same *Nabis* sp. found in the broccoli field had eaten *P. xylostella*.

Other predators which can be of importance are spiders from the sheet weavers/money spiders (Linyphiidae) family and larvae of hover flies (Syrphidae). One type of Linyphiidae was not often seen on the plants but had a high feeding rate (see Figure 2 or Appendix 2); it consumed on average 31.1 L2/day (n = 2). The larvae of Syrphidae also had a high rate of consumption; 10.4

L2/day on average (n = 7) or 1.8 eggs/day in average, which may seem low but one individual consumed 20 eggs in one day. Syrphidae are mentioned as potential predators in articles involving *P. xylostella* (Szwejda, 2004; Wu & Miyata, 2005). Further, Guan-Soon (1991) reported syrphids to predate readily on *P. xylostella* in cabbage fields, but is not further discussed and Charleston (2006) found syrphids on cabbage plants in South Africa. Other insects with a high feeding rate but not frequently found in the field were assassin bugs (Reduviidae), toad bug (*Gelastocoridae gelastocoris*) and two bigger types of Salticidae (B and C).

The bigger Salticidae (B and C) consumed significantly more L3 than the smaller Salticidae A (no statistical comparisons were made on feeding rate of L2 due to low n-values). The size of the predator is important for the feeding capacity. Usually predators prefer to attack prey smaller than themselves (Roger et al., 2000). Huseynov (2005) show that the most frequent prey taken by Salticidae were arthropods with a size of about 50-100 % of the spider body length.

The size of the prey also affected the predation rate. All groups of predators except Staphylinidae consumed significantly more L2 than L3 in this study (see Figure 3). That the prey size affects feeding rate was also demonstrated in a study of Nabidae, *Nabis* sp., which consumed more of the smaller larvae (Ma, et al. 2005a). In the preliminary study some predators were provided prey of different size in order to determine which size to use during the main predation study. The result of the study was that the L2 seemed to be the most popular. In the study of feeding capacity, there are bigger differences between the predator groups consuming L3 than when predators were fed L2. The reason could be that strong predators like, Salticidae B and C and Reduviidae (see figure 2 and appendix 2), were not included in the statistical test. However, it can also show that the predation is more equal between the predator groups because it is favorable for the predators to consume medium-sized larva.

In a study on predator preference by Furlong et al. (2004a) plants with different *P. xylostella* larval instars were presented to natural enemies. The L1 had the highest disappearance rate, which indicates a higher predation of L1. 2nd instar larvae (L2) and L4 had the same disappearance rate. The rate of egg disappearance was lower, ranged from 14.5 to 56.5 % for the egg in comparisons with 39.4 to 58.6 % for the larvae. In comparison, the highest predation rate was found on eggs followed by L1, L2, L3 and last L4 in laboratory studies on spotted ladybird beetle by Roger et al. (2000) and on *Nabis* sp. by Ma et al. (2005b). It is, however, difficult to know the real preference of predators and their consumption rate in the field. In the laboratory the predators are provided larvae in large amounts close to them. In the field, generalist predators may have many options of prey and might not chose a certain prey if another prey have a higher energetic value, are more abundant or are easier to catch. As a result, generalist predators may change their consumption rate over time. Prey density may also affect predation rate, e.g. a high density of prey have been observed to provoke high consumption rates both under laboratory and field conditions.

It is difficult to estimate the real effect of the predators on *P. xylostella*. In some studies it has been shown that predators can cause 68% or more of the larval mortality of the pest (Guan-Soon, 1991), whereas in other experiments no influence of predators was possible to detect (Furlong et al., 2004a). One difficulty with predation studies in the laboratory is that it is impossible to evaluate the feeding rate of for example ants (Formicidae) and wasps (Vespidae *Polybia* sp.), both potential important predators. Wasps were in some fields observed relatively frequently at

the cabbage plants during the study in Nicaragua. One wasp came also into the laboratory to catch a larva from a cabbage plant. Ants were observed at a plant in the laboratory. The ants had made a nest in the soil and came up to capture a pupa. Ants were as well observed in large numbers in the field and in the pitfall traps.

Rove beetles (Staphylinidae) and harvestmen (Opiliones) were found in large amounts in the pitfall traps (Grönberg, 2007). Opiliones was furthermore occasionally found at the cabbage plants in field, but it was impossible to evaluate their eating capacity since they died quickly in the laboratory. The same problem occurred with lady beetles (Coccinellidae). However, only one individual of the Coccinellidae was found in the field, more were found at the field margins. Coccinellidae is often mentioned as a possible predator of *P. xylostella* (Guan-Soon, 1991; Eigenbrode et al., 1995; Eigenbrode et al., 1996; Eigenbrode & Kabalo, 1999; Sreekanth et al., 2000; Liu & Sengonca, 2002; Ferry et al., 2003; Szwejda, 2004; Furlong et al., 2004b; Wu & Miyata, 2005). In a more detailed study made by Roger et al. (2000) Coccinellidae was demonstrated to be a predator of *P. xylostella*. Another predator often mentioned in articles on *P. xylostella* is Chrysopidae (Guan-Soon, 1991; Eigenbrode et al., 1995; Eigenbrode et al., 1996; Eigenbrode & Kabalo, 1999; Reddy et al., 2002; Szwejda, 2004; Furlong et al., 2004b). No Chrysopidae were found in the fields, only one adult was observed visiting the laboratory. A small trial with laboratory reared Chrysopidae demonstrated that they predate *P. xylostella* larvae. The feeding rate of young Chrysopidae larvae was low, less than one each day but when older just before turning into pupa the predation capacity was up to three big L3 larvae a day.

In this study, and others discussing *P. xylostella* (Nemoto, 1986; Guan-Soon, 1991; Furlong et al., 2004b), many possible predators have been identified but the total destruction of cabbage plants by *P. xylostella* is dependent on when and if natural enemies can attack the pest. If the predators prefer smaller larvae or eggs they are more effective in pest control. If the predators prefer to predate late in the development of the larvae it might not affect the plant damage significantly. In the tropics, where the pest constantly is present at the crop or on weeds, it is always important to control the pest. A preference for smaller larvae or eggs will also give the largest reduction of the pest if the feeding rate is higher on them. It is important to continue the investigations of different predator preference in order to know which predators are more important, in order to minimize damage to the crop.

Less effective pest control can be caused by interpredation among the predators. In one study made by Prasad & Snyder (2004) on predators feeding on eggs of the fly *Delia* spp., it was shown that large carabid species rarely consumed eggs but that smaller species of Carabidae consumed the dipteran eggs readily. The smaller Carabidae was also susceptible to being preyed upon by larger guild members. Therefore, encouragement of the large Carabidae may result in less predation of the eggs. It is common with intraguild predation and other negative predator-predator interactions but it has not been widely examined which impact it has on biological control. Additional important factors which can regulate the amount of predators, such as field margins, are also needed to be studied so favorable natural enemies can be supported. It is important also to have a broader view and improve the often poor information given to the farmers about how to control *P. xylostella* in a more sustainable way and to use more of the integrated pest management (IPM) that is available. In one study where farmers used the IPM programme, the number of applications of synthetic pesticides could be reduced from nine to two per crop cycle with the same or better control of *P. xylostella* (Andrews et al., 1991).

The role of predators in agroecosystems is important to understand but very few detailed studies have been done about predators controlling *P. xylostella*. Not even basic studies on different predators, especially spiders, have been done and predators remain poorly investigated and understood (Ma et al., 2005a). This study was done to (1) identify possible predators of *P. xylostella* and (2) investigate their eating capacity under laboratory conditions. More studies have to be done about the real impact of predators, but this study is as an important step towards learning and understanding the complex system of how to control *P. xylostella* in Nicaragua.

In conclusion, 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. Predators with the highest consumption rate were assassin bugs (Reduviidae), sheet weavers/money spiders (Linyphiidae) and a bigger type of jumping spiders (Salticidae). The predator groups with the highest potential for regulating *P. xylostella* due to high consumption rate and high abundance were especially wolf spiders (Lycosidae) but sheet weavers/money spiders (Linyphiidae), rove beetles (Staphylinidae *Paederus* sp.), jumping spiders (Salticidae A) and damsel bugs (Nabidae) could also be important predators.

Acknowledgments

I am grateful to my supervisors Christer Björkman and Helena Bylund for all the support and all the time you have spent on me. Special thanks to Christer for believing in me, giving me the opportunity to discover the insect world and to Helena for the time in Nicaragua. Thank you Freddy Miranda PhD-student at SLU and UNA in Nicaragua, for all useful information and for letting me take a part of your study. Also many thanks to Richard Hopkins for the final opinions and corrections in the text. I would also like to thank Magnus Halling and Victor Aguilar Bustamante coordinators for the PhD programme, a cooperation between Universidad Nacional Agraria, UNA and the Swedish University of Agriculture, SLU, supported by Swedish international development cooperation agency, SIDA. I am very grateful to all the farmers letting me use their fields and answering my questions. I am especially grateful to all the warm people I got to know at the Tisey farm which was my home for the stay in Nicaragua. I would also like to thank everyone helping me with the identifications of insects; Åke Lindelöw at SLU for the first inspiring lessons, Alex A. Cerrato Cuarezma at UNA for identification of insects and Jonas Sandström at Artdatabanken for help with identification of the spiders and Christer Solbreck at SLU. For support and computer assistance thank you Miquel Anglès-Marín. Also many thanks for the financial support from the Minor Field Studies at SIDA and the NL-faculty at SLU. Last but not least I will give my special thanks to Lina Grönberg my dear friend and companion for all the laughs and her patience.

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Appendices

Appendix 1

Groups of possible predators of *Plutella xylostella* found in Tisey, Nicaragua.

Group	Order	Family	Comments
Araneae	Araneae		Mixture of spiders many looks like Linyphiidae type
Araneidae	Araneae	Araneidae	Mixture of different Araneidae
Gnap/Club/ Lio/Anyph	Araneae	Gnaphosidae/Clubionidae/ Liocranidae/Anyphaenidae	Mixture of different types Gnaphosidae, Clubionidae, Liocranidae and Anyphaenidae
Lycosidae	Araneae	Lycosidae	Mixture of different Lycosidae
Lycosidae small	Araneae	Lycosidae	Smaller type of Lycosidae probably a younger individual
Linyphiidae	Araneae	Linyphiidae	One genera of the Linyphiidae, yellow
Salticidae A	Araneae	Salticidae	Medium sized Salticidae mixed genera
Salticidae B	Araneae	Salticidae	One genera of Salticidae of bigger type, black
Salticidae C	Araneae	Salticidae	One genera of Salticidae of bigger type, metallic green
Salticidae D	Araneae	Salticidae	Small sized Salticidae, probably one genera
Tetragnathidae A	Araneae	Tetragnathidae	Mixture of different Tetragnathidae
Tetragnathidae B	Araneae	Tetragnathidae	One genera of Tetraghathidae
Thomisidae	Araneae	Thomisidae	Mixture of different Thomisidae
Carabidae A	Coleoptera	Carabidae	Smaller type of Carabidae
Forficulidae	Dermaptera	Forficulidae	Dorus genera
Gelastocoridae	Hemiptera	Gelastocoridae	Gelastocoris genera
Coccinellidae. Larva	Coleoptera	Coccinellidae	Larva of Coccinellidae genera
Syrphidae. larva	Diptera	Syrphidae	Larva of Syrphidae baehar type
Staphylinidae. Larva	Coleoptera	Staphylinidae	Larva of Staphylinidae
Staphylinidae A	Coleoptera	Staphylinidae	Paederus genera
Staphylinidae B	Coleoptera	Staphylinidae	Small and black
Staphylinidae C	Coleoptera	Staphylinidae	Probably Paederus genera
Staphylinidae D	Coleoptera	Staphylinidae	Probably Paederus genera
Staphylinidae E	Coleoptera	Staphylinidae	Probably Tachinus genera
Staphylinidae F	Coleoptera	Staphylinidae	Probably Paederus genera
Lygaeidae. larva	Hemiptera	Lygaeidae	Larva of Pachybrachius genera probably
Nabidae	Hemiptera	Nabidae	Nabis genera
Reduviidae	Hemiptera	Reduviidae	Probable only Sinea genera
Penta/Miridae. Larva	Hemiptera	Pentatomidae/Miridae	Larva of Pentatomidae or Miridae genera
Pyrrhocoridae. Larva	Hemiptera	Pyrrhocoridae	Larva of Pyrrhocoridae genera
Opiliones	Opiliones		Mixture of different Opiliones

Appendix 2

Eating capacity (Mean and SE Mean) number of individuals tested in each larval stage and the sum of their average abundance on the cabbage plants and in the field.

Insect	Sum		Egg			L1			L2			L3			L4		
Group	Plant	Field	n	Mean	SE Mean	n	Mean	SE Mean	n	Mean	SE Mean	n	Mean	SE Mean	n	Mean	SE Mean
Araneae	7	38	9	0.30	0.16	6	1.28	0.78	23	3.13	0.71	14	1.91	0.55			
Araneidae	4	0							3	1.01	0.62	3	1.33	0.88			
Gnaph/Club/Liocran/Anyph	3	5							6	9.25	1.9	5	8.03	2.84			
Lycosidae	29	56	6	0.87	0.87				12	11.15	2.62	13	4.94	1.53	9	4.15	1.01
Lycosidae small	0	38	3	0	0				4	2.57	1.13	3	0.4	0.31			
Linyphiidae	8	4	2	0	0				2	31.12	5.68						
Salticidae A	18	30	5	0.13	0.13				30	7.18	1.12	33	2.85	0.50	2	0.66	0.04
Salticidae B	1	0										11	10.76	2.01	5	6.2	1.52
Salticidae C	1	0	1	0					2	30.7	2.3	5	13.1	3.93	1	6	
Salticidae D	1	3	1	0					6	6.42	1.85	4	1.04	0.43			
Tetragnathidae A	3	2							5	0.52	0.32	2	0.5	0.5			
Tetragnathidae B	65	28	3	0	0				3	1.41	0.63	6	1.47	0.65			
Thomisidae	5	23	12	0.08	0.08				14	3.73	1.16	14	1.50	0.29			
Carabidae A	4	49	4	0.76	0.54	2	0.29	0.04									
Forficulidae	1	0	6	2.83	2.29				2	6.8	4.7	1	1.2				
Gelastocoridae	0	1							2	13.04	0.46	2	9.8	3			
Coccinellidae. larva	0	1	1	0.33					2	0	0						
Syrphidae. larva	2	1	5	1.84	0.99				7	10.42	2.39	5	7.47	0.87			
Staphylinidae. larva	0	25	8	8.69	3.11	1	1		6	2.41	0.32	2	1.8	0.6			
Staphylinidae A	22	9	4	0	0				10	9.76	1.62	10	8.91	1.09			
Staphylinidae B	0	62	7	4.41	0.58	1	0		4	0.63	0.51						
Staphylinidae C	1	14	2	0.14	0.14				3	3.96	0.65	2	2.2	0.2			
Staphylinidae D	0	6	1	1.33					1	0.1							
Staphylinidae E	1	17	13	2.40	0.81				7	0.68	0.17	2	0.5	0.5			
Staphylinidae F	0	10	3	0.28	0.28												
Lygaeidae. larva	0	0	4	0.75	0.75				1	0.14							
Nabidae	4	31	8	2.77	1.21				22	7.22	1.96	26	4.89	0.60			
Reduviidae	0	1	4	3.83	1				1	33.6		7	10.54	3.05			
Penta/Miridae. larva	0	7	6	1.86	0.69												
Pyrrhocoridae. larva	0	0	10	0.13	0.10				3	0.19	0.10						
Opiliones	8	17				1	0.67		2	0.28	0.28						