

**Potential of the predatory pentatomid *Eocanthecona furcellata* (Wolff) as a biocontrol agent on American bollworm in cotton in Myanmar**

Doctoral Dissertation  
to obtain the Ph. D. degree  
in the Faculty of Agricultural Sciences,  
Georg-August University Göttingen, Germany

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Göttingen, April 2008

D7

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Date of dissertation: 3<sup>rd</sup> April 2008

*This thesis is dedicated to my mother Daw Tin May,  
sisters Tin Tin Nyunt, Khin May Nyunt & her husband Alan Po*

## Acknowledgements

My profound thanks and indebtedness are due to Prof. Dr. Stefan Vidal, my “Doktorvater”, who has given this word its real meaning, through his guidance, encouragement, inspiration and positive criticism over the entire work.

I would like to thank Prof. Dr. Hans Michael Poehling for kindly using his valuable time as co-referent for this dissertation and, Prof. Dr. Teja Tschardt and PD Dr. Martin Worbes for his interest to be an external examiner.

Grateful thanks are also expressed to Dr. Bernd Ulber, Dr. Dereje Dugassa-Gobena and Dr. Joachim Möser for their helpful and constructive suggestions, critical comments and assistance during this study. I also like to thank Mrs. Dorothea Mennerich, Mrs. Angelika Metje who provided the basic material for my research: American bollworm, Diamondback moth, cotton, chickpea, wild cabbage, corn and tomato plants. My sincere thanks go to Dr. Dereje Dugassa-Gobena for reviewing the manuscript and his advices.

I wish also to accord a special debt of gratitude to all my colleagues in the Entomology Section, Department of Crop Science, for the unforgettable friendly research environment they have created and the cooperative spirits they have shown at each step of the research and writing of the thesis.

My thanks are extended to Daw Nu Nu Yi and Daw Nyo Nyo from Myanma Agricultural Service, Daw May Than Yee and colleagues from Shwe Daung Cotton Research Farm, Daw Nywe Nywe Yin from Department of Agricultural Research for collecting and providing of *Eocanthecona furcellata* eggs used to initiate the stock culture for the present research.

This research could have never been done without the generous financial support from Gottfried Daimler und Karl Benz foundation.

Finally, special thanks and gratitude are due to my mother Daw Tin May, my elder sister Khin May Nyunt and her husband Alan Po, my younger sister Tin Tin Nyunt, in addition to their endless support and encouragement during my study.

## CONTENTS

<b>GENERAL INTRODUCTION</b>	<b>7</b>
<b>CHAPTER 1</b>	
<b>PREY PREFERENCE AND PREDATION EFFICACY OF <i>Eocanthecona furcellata</i> WOLFF (HEMIPTERA: PENTATOMIDAE) ON <i>Helicoverpa armigera</i> HÜBNER LARVAE FROM DIFFERENT HOST PLANTS</b>	<b>10</b>
ABSTRACT	10
INTRODUCTION	11
MATERIAL AND METHODS	13
RESULT	16
DISSCUSSION	21
REFERENCE	23
<b>CHAPTER 2</b>	
<b>PREY CONSUMPTION AND FITNESS OF <i>Eocanthecona furcellata</i> WOLFF (HEMIPTERA: PENTATOMIDAE) ON DIAMONDBACK MOTH LARVAE AT DIFFERENT TEMPERATURE REGIMES AND PREY DENSITY</b>	<b>31</b>
ABSTRACT	31
INTRODUCTION	32
MATERIAL AND METHODS	34
RESULT	36
DISSCUSSION	48
REFERENCE	50
<b>CHAPTER 3</b>	
<b>PREY SEARCHING AND FEEDING BEHAVIOR OF <i>Eocanthecona furcellata</i> WOLFF (HEMIPTERA: PENTATOMIDAE) ON DIFFERENT PREY ITEMS, HOST PLANT SPECIES AND PLANT STATUS</b>	<b>55</b>
ABSTRACT	55
INTRODUCTION	56

MATERIAL AND METHODS	57
RESULT	60
DISSCUSION	69
REFERENCE	70
<b>GENERAL DISCUSSION</b>	<b>76</b>
<b>CONCLUSION</b>	<b>83</b>
<b>REFERENCES</b>	<b>84</b>
<b>SUMMARY</b>	<b>97</b>

The following posters in the appendix are part of this thesis:

1. Potential for Biocontrol of Diamondback moth in Myanmar by using a predatory bug
2. Predatory potential of the Pentatomid stink bug *Eocanthecona furcellata* at different temperature regimes
3. Predatory efficacy of *Eocanthecona furcellata* on *Helicoverpa armigera* larvae reared on different host plants

## GENERAL INTRODUCTION

Myanmar is one of the developing countries, where economy is mainly based on the agriculture. Cotton is one of the main crops for foreign exchange earning and self-sufficiency in Myanmar. In 1952, cotton project was planned and the new technology was introduced by the Government to improve the quality and quantity of cotton production in Myanmar. Early since then native cultivars, short staple cotton (*Gossypium arboreum*) Mahlaing 3 and Mahlaing 5 were grown for commercial cultivation (Myintzu, 1974). In 1953-1954, the Agricultural and Rural Department Corporation introduced medium staple cotton (*Gossypium hirsutum*) cultivars (MCSE, 1999). In 1980-81 seasons, Myanmar was ranked at No. 36 of the world cotton producers (Frisbie, 1983). Accordingly, cotton area, productivity and production significantly progressed during past forty years but Myanmar's current yield levels are admittedly low, which constitute only one third of world average (Pye Tin, 2003).

Local varieties of cotton (*G. arboreum*) are grown for their low susceptibility to pests and pesticides had to be applied rarely. However, medium staple cotton (*G. hirsutum*) is prone to infestation of sucking pests in the early stages and of bollworm from the initiation stage of squares up to maturing of cotton bolls (MCSE, 1999). According to the report of Myanma Cotton and Sericulture Enterprise (MCSE) in 1999, American bollworm is recorded as a major cotton pest of economic importance.

American bollworm (*Helicoverpa armigera* Hübner) which is found in all agricultural regions of Myanmar, is the most severe pest of cotton, chickpea, maize, sorghum, pigeon pea, potato, tomato, tobacco respectively (Morris and Waterhouse, 2001). Although chemical insecticides were widely applied to control this pest, reduction in the yield of seed cotton was as high as 90 percent in some cases (Myat Htwe and Mya Maung, 1992). Misuse or overuse of insecticides may results in the reduction or even eradication of predators and parasitoids in the cotton fields. Although the damage could kept low by the use of insecticides, it may Emergent impacts of multiple predators on prey unknnot economically feasible for cotton growers. On

other hand, environmental pollution may become a great problem in the long run. To overcome these problems, it is desirable to use biological control whenever feasible. Based on previous study (Khin, 2001) several effective predators can be found in the agroecosystem of Myanmar. Among them, the predatory bug *Eocanthecona furcellata* Wolff (Hemiptera: Pentatomidae) could be regarded as a potential larval predator for the whole cotton growing season.

The predatory bug *Eocanthecona furcellata* Wolff (Hemiptera: Pentatomidae) is found especially in cotton, chickpea and vegetable fields and has been found preying on larvae of leaf worm, spotted bollworm and American bollworm in Myanmar (Gillham, 1980; Nu Nu Yi and Win Kyi, 2000, and Khin, 2001). *E. furcellata* has been also reported from Southeast Asia, Japan, India, and Taiwan, and has been preying on Lepidopteran, Coleopteran and Heteropteran insects (Ahmad, 1996; Chu, 1975; Chang, 2002; Jakhmola, 1983; Prasad et. al., 1983). This predator is regarded to be a generalist, however, data on his efficacy or on prey preferences are scattered. Thus an evaluation of this predator under laboratory conditions is regarded necessary to understand its potential use as a biological control agent under field conditions.

The present work aimed, therefore, to observe the ecology and biology of *Eocanthecona furcellata* by:

1. Prey preferences and predation efficacy of *Eocanthecona furcellata* on *Helicoverpa armigera* Hübner larvae from different host plants
  - 1) The effect of host plants on the oviposition preference of *H. armigera*
  - 2) The effect of host plants on the performance of *H. armigera* larvae
  - 3) Predation efficiency of *E. furcellata* on *Helicoverpa armigera* (Hübner) reared on different host plants (Cabbage, Cotton , Chickpea, Tomato plant) and on artificial diet



- 4) Prey selection of **EO** on wrapped American Bollworm *Helicoverpa armigera* (ABW) reared on different host plants (Cabbage, Cotton and Chickpea)
2. Prey consumption and fitness of *Eocanthecona furcellata* Wolff (Hemiptera: Pentatomidae) on Diamondback moth larvae at different temperature regimes and prey density
    - 1) Evaluating the effect of three constant temperatures (25°C, 30°C and 35°C) and eight prey densities (1,2,3,4,5,6,8,and 10 Diamondback moth larvae) on the development, prey consumption and predation of *E. furcellata*
    - 2) Effect of high temperatures (15°C, 20°C 37°C and 40°C) on the development, mortality and prey consumption of the predatory bug *Eocanthecona furcellata*
3. Prey searching and feeding behavior of *Eocanthecona furcellata* Wolff (Hemiptera: Pentatomidae) on different prey items, host plant species and plant status
    - 1) Effect of different preys (American bollworm and Diamondback moth)
    - 2) Effect of different host plants (cotton and wild cabbage)
    - 3) Effect of different conditions (normal plant, wounded plant, and insect infected plant)
    - 4) Observing of host choice behavior of *Eocanthecona furcellata* (**EO**) towards American bollworm (**ABW**) and Diamondback moth (**DBM**) larvae in Y-olfactometer

## CHAPTER 1

### **Prey preference and predation efficacy of *Eocanthecona furcellata* Wolff (Hemiptera: Pentatomidae) on *Helicoverpa armigera* Hübner larvae from different host plants**

#### **ABSTRACT**

American bollworm (*Helicoverpa armigera* Hübner) is one of the most severe pests of economic important crops and even using chemical control, up to 90 percent yield loss was found in seed cotton in Myanmar.

The predatory pentatomid bug *Eocanthecona furcellata* (Wolff) is regarded a potential biological control agent against lepidopteran pests in Southeast Asia. However, no studies are available on the predation efficacy of *Eocanthecona furcellata* on *Helicoverpa armigera* larvae feeding on different host plants. This information is regarded important with regard to releasing *E. furcellata* as a biocontrol agent for control of *H. armigera* in Myanmar.

Therefore, we investigated (1) the effect of host plants on the oviposition preference of *H. armigera*; (2) the effect of host plants on the performance of *H. armigera* larvae; (3) prey selection and predation of *E. furcellata* on the polyphagous pest American bollworm reared on cotton, chickpea, tomato, wild cabbage plant, and artificial diet; and (4) Prey preference of *Eocanthecona furcellata* on wrapped American Bollworm *Helicoverpa armigera* reared on different host plants (cabbage, cotton and chickpea).

*H. armigera* preferred to lay eggs on chickpea plants as compared to tomato, cotton and wild cabbage plants. Predation efficacy of *E. furcellata* was tested with American Bollworm from four different host plants (cabbage, cotton, chickpea and tomato). Significantly more *E. furcellata* (30-60 %) directly approached towards cotton plants and preyed on *H. armigera* larvae. When *H. armigera* larvae and their

faeces were wrapped with Para film 'M', the prey selecting efficacy was reduced up to 20-40 %.

Base on these data we suggest that it is possible to release the predatory bug *Eocanthecona furcellata* in cotton fields as a biocontrol agent for controlling *Helicoverpa armigera* in Myanmar; the impact of the surrounding vegetation on releasing the predator in the fields is discussed.

Keywords: American bollworm, Biological control, *Eocanthecona furcellata*, Diamondback Moth, Host plants, Myanmar, Predation efficacy

## INTRODUCTION

In Myanmar, American bollworm is the most severe pest of economically important crops including cotton, chickpea, pigeon pea, pea and beans; the pest is found in all agricultural regions of Myanmar (Morris and Waterhouse, 2001). The American bollworm (*Helicoverpa armigera* Hübner) is known as a widespread polyphagous pest, with high mobility and fecundity (Hardwick, 1965; Fitt, 1989 and King, 1994). The larvae feed on a wide range of food, fiber, oil and fodder crops, as well as on many horticultural and ornamental plants (Pearson, 1958, Zalucki et.al., 1986 and Fitt, 1989). Reed and Pawar (1982) reported *Helicoverpa armigera* damaging 60 cultivated plant species and 67 other plant species.

Survival of the larvae is dependent on appropriate host selection by females (Fitt & Boyan, 1991 and Fitt, 1991) because host plants may account for the high variation in offspring performance (Jallow & Zalucki, 2003). Larvae mostly feed on the growing point and reproductive parts of the host plants; therefore the economic loss due to American bollworm is very high on cotton, soybeans, tobacco, chickpea and pigeon pea throughout the world (Hardwick, 1965; King et.al., 1982 and Fitt, 1989). Although chemical insecticides are widely applied to control *Helicoverpa armigera*, reduction in the yield of seed cotton was as high as 90 percent in some cases in Myanmar (Myat Htwe and Mya Maung, 1992). In India, 18 to 26% yield losses due to *H. armigera* was reported by Rawat et.al. in 1970. Moreover, 41 to

56% yield losses were found in cotton (Kaushik et.al 1969) and 40 to 50% yield losses in tomato (Srinivasan 1959). The management of *H. armigera* is difficult and in many crops, including cotton and pigeonpea, relies heavily on the use of insecticides (King 1994; Shanower *et al.* 1997). Multiple insecticide applications have led to high levels of resistance to major groups of active compounds (Fitt 1989; Armes *et al.* 1996). To solve this problem, one promising option could be to manage *Helicoverpa armigera* by releasing natural enemies.

Biological control is recognized as one of the best alternatives to the use of chemical insecticides for controlling insect pests. Pest control with natural enemies has been increasing due to environmental, economical, social and ecological problems with insecticides. Heteropteran predators are important biological control agents on leaf worms (De Clercq *et. al.*, 2003; Lemos *et. al.*, 2003), beet armyworms (De Clercq and Degheele 1994), Colorado potato beetles (Biever, *et. al.*, 1992; Hough-Goldstein *et. al.*, 1996; Tipping, *et. al.*, 1999; Westich and Hough-Goldstein, 2001), and southern green stinkbugs, respectively (De Clercq *et. al.*, 2002), on soybean caterpillars (Marston, *et. al.*, 1978).

Naturally occurring predators and parasitoids are regarded important in regulating the numbers of *Heliothis* in the field (Van den Bosch and Hagen 1966, Lingren *et.al.* 1968, and Van den Bosch *et.al.* 1969). The predatory bug *Eocanthecona furcellata* Wolff (Hemiptera: Pentatomidae) is found especially in cotton, chickpea and vegetable fields and has been found preying on larvae of leaf worm, spotted bollworm and American bollworm in Myanmar (Gillham, 1980; Nu Nu Yi and Win Kyi, 2000, and Khin, 2001). *E. furcellata* has been also reported from Southeast Asia, Japan, India, and Taiwan, and has been preying on Lepidopteran, Coleopteran and Heteropteran insects (Ahmad, 1996; Chu, 1975; Chang, 2002; Jakhmola, 1983; Prasad *et. al.*, 1983). This predatory bug can be easily reared on larvae of *Pieris rapae* (Chu, 1975) and frozen preserved larvae of *Spodoptera litura* (Yasuda and Wakamura, 1992). The host plants of prey affect the predation rate and searching behavior of predatory Hemiptera (Yasuda, 2000 and Perdikis *et.al.* 2004). However, there is no publication on the predation efficacy of *Eocanthecona*

*furcellata* with regard to *H. armigera* larvae feeding on different host plants. This may be important with regard to the surrounding vegetation for release of *E. furcellata* as a biocontrol agent to control *H. armigera* in Myanmar.

In this study, we investigated (1) the effect of host plants on the oviposition preference of *H. armigera*; (2) the effect of host plants on the performance of *H. armigera* larvae; (3) prey selection and predation of *E. furcellata* on American bollworm larvae reared on cotton, chickpea, tomato, wild cabbage, and artificial diet; and (4) prey preference of *Eocanthecona furcellata* on wrapped American Bollworm larvae reared on different host plants (cabbage, cotton and chickpea).

## **MATERIALS AND METHODS**

### **Culture of insects**

Eggs and adults of the pentatomid *Eocanthecona furcellata* (Wolff) (Hemiptera: Pentatomidae) were originally collected on November 2004 from cotton fields in Myanmar. They were released into the rearing cages (75 x 55 x 75 cm) and were kept on Diamondback moth infested wild cabbage plants because of the high egg laying capacity of Diamondback moth adults, maintaining the population density for the experiments at room temperature ( $22 \pm 1$  °C) under laboratory condition at the Entomology Section, Georg-August University, Goettingen, Germany. 10 male and 10 female adults of *E. furcellata* were collected randomly from the rearing cage and maintained individually in Petri-dish and starved 24 hours before the experiment.

American bollworm (*Helicoverpa armigera* Hübner; Lepidoptera: Noctuidae) eggs were obtained from Bayer A.G., Germany, and second instar larvae were used for the host preference experiment. 100 second instar larvae were reared on cotton plants, wild cabbage plants, chickpea plants and tomato plants for the predation efficacy test. *Helicoverpa armigera* larvae were also reared on a modified diet according to Shorey and Hala (1965).

A stock culture of Diamondback moth (*Plutella xylostella* Linnaeus) larvae has been reared on wild cabbage plant in the rearing cage at room temperature ( $22 \pm 1$  °C) under laboratory condition.

### **Culture of plants**

Cotton (*Gossypium hirsutum* cv. MCU 9), wild cabbage (*Brassica oleracea* var. *viridis*), Chickpea (*Cicer arietinum* L.) and tomato (*Solanum lycopersicum* variety Suso R2) were grown under controlled greenhouse conditions; plants were grown in 13 cm diameter pots (Sand: Clay 50: 50) for these experiments. 25 day after emergence of seedlings (25 DAE) plants were used for the oviposition experiment and four to eight weeks old plants were used for the other experiments. Seeds of *G. hirsutum* cv. MCU 9 were provided from Myanmar; seeds of the other plants originated from Germany.

#### **1. Oviposition preference of the American Bollworm**

Chickpea, cotton and tomato, and wild cabbage plants were planted at the same date and plants being 25 day old were used in this experiment. Four plant species with four replicate were set at a random position in rearing cages at room temperature ( $22 \pm 1$  °C) and one pair of *H. armigera* were released into these cages. The number of *H. armigera* eggs and their distribution on the plants were recorded daily. The experiment was replicated 4 times.

#### **2. Performance of American Bollworm larvae on different host plants**

For this experiment 100 American Bollworm larvae were reared on cabbage, chickpea, cotton and tomato in different rearing cages and on the artificial diet at room temperature ( $22 \pm 1$ °C) to study the performance of *H. armigera* till the adult stage.

#### **3. Predation efficiency of *Eocanthecona furcellata* on American Bollworm reared on different host plants (cabbage, cotton, chickpea and tomato)**

American Bollworm (*Helicoverpa armigera*) larvae were reared on cabbage, chickpea, cotton and tomato plants in different rearing cages and on the artificial diet at room temperature ( $22 \pm 1^\circ\text{C}$ ). Larvae were kept on these plants for 20 days. Thereafter ten males and ten females of adult *E. furcellata* were used in this experiment, which was replicated ten times. All *E. furcellata* were starved 24 hour before the experiment started. *H. armigera* larvae were fixed with tape and placed in a  $15 \times 22 \times 3 \text{ cm}^3$  plastic box and ten *E. furcellata* adults were transferred to the center of the arena. The movement of *E. furcellata* was observed and recorded at room temperature.

#### **4. Prey preference of *Eocanthecona furcellata* on wrapped American Bollworm larvae reared on different host plants**

This experiment was set up to understand in detail the effect of the prey previously feeding on different host plants on the predation by *E. furcellata*. American Bollworm larvae were reared on cabbage, chickpea and cotton, in different rearing cages at room temperature ( $22 \pm 1^\circ\text{C}$ ). The same size of third and fourth instar larvae of *H. armigera* from different host plants were used in this experiment; ten male and female *E. furcellata* adults, were added in ten replications. All *E. furcellata* were starved 24 hours before the experiment. *H. armigera* larvae and their faeces were wrapped with Para film 'M' and placed randomly in a  $15 \times 22 \times 3 \text{ cm}^3$  plastic box and ten *E. furcellata* adults were placed in the center of the arena. The movement of *E. furcellata* was observed and recorded at room temperature.

#### **Statistical analyses**

Analysis of variance (ANOVA) was used to determine statistical differences with regard to the development data among the host preference and predation preference tested (SPSS Inc., 2004). Means of treatments were separated by Bonferroni adjustment.

## RESULT

### 1. Oviposition preference of the American Bollworm moth *Helicoverpa armigera*

The number of *H. armigera* eggs placed on the 4 crop plants tested was significant different (df = 3; F= 31.079; P= 0.000). The highest numbers of *H. armigera* eggs was recorded on chickpea plants ( $46.8 \pm 11.63$ ), followed by tomato, cotton and cabbage plants (Fig. 1). The majority of the *H. armigera* eggs was recorded on the upper side of the leaves of all plant species; however eggs were also found to some extent on the under side of the leaves and on the stems of all plant species.

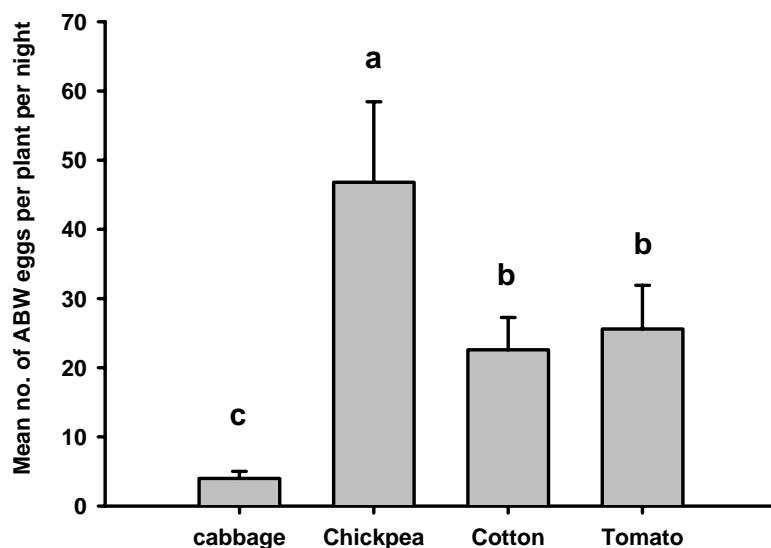


Figure 1: Oviposition preference of the American Bollworm on different host plants. Different letters indicate significant differences at  $P < 0.01$  with Bonferroni adjustment following ANOVA.

### 2. Performance of the American Bollworm larvae on different host plants

There were significant differences in larval weights when the larvae of *H. armigera* were reared on different host plants (df = 4; F= 86.25; P= 0.000; Fig.2). The average weight of the 20-day old larvae reared on wild cabbage plant, chickpea,



cotton, tomato, and artificial diet were 182.85 ( $\pm$  23.4), 79.47 ( $\pm$  14.24), 147.58 ( $\pm$  18.18), 18.34 ( $\pm$  3.74), and 419.54 ( $\pm$  16.4) mg, respectively.

The length of *H. armigera* larvae reared on different host plants were significantly different ( $df = 4$ ;  $F = 50.08$ ;  $P = 0.000$ ); (Fig.3). The average length of the 20-day old larvae reared on wild cabbage, chickpea, cotton, tomato plants, and artificial diet were 24.53 ( $\pm$  1.25), 17.6 ( $\pm$  1.51), 20.93 ( $\pm$  1.26), 8.8 ( $\pm$  0.91), and 32.87 ( $\pm$  1.26) cm, respectively.

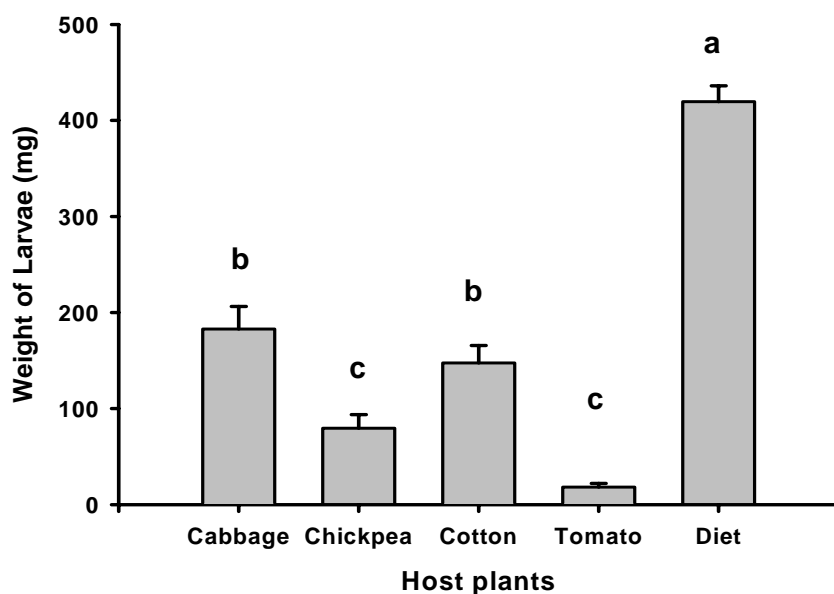


Figure 2: Weight of *H. armigera* larvae reared on different host plants. Different letters indicate significant differences at  $P < 0.01$  with Bonferroni adjustment following ANOVA.

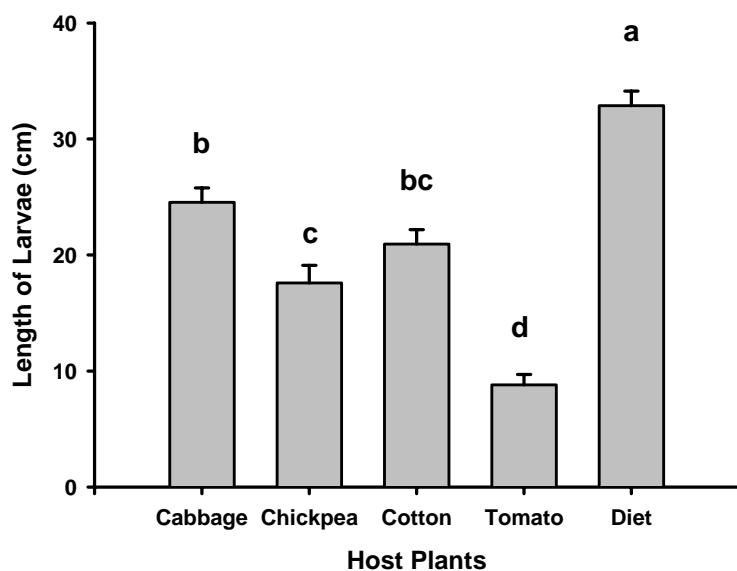


Figure 3: Length of *H. armigera* larvae reared on different host plants. Different letters indicate significant differences at  $P < 0.01$  with Bonferroni adjustment following ANOVA.

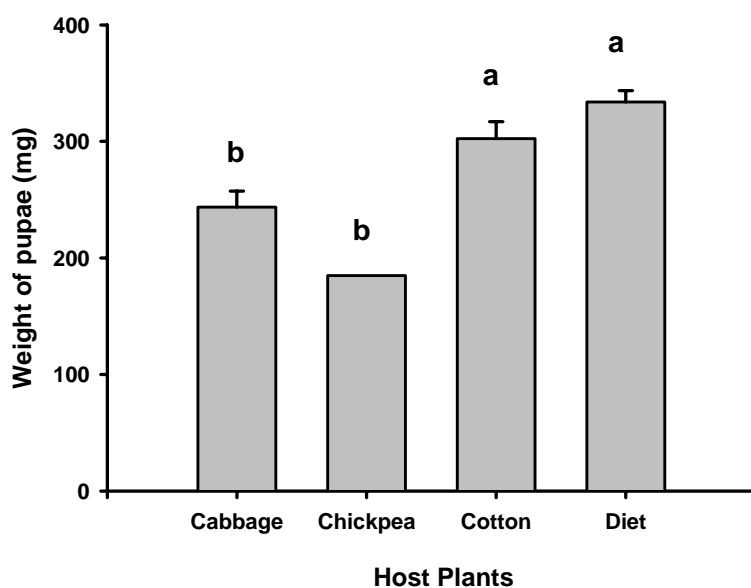


Figure 4: Weight of *H. armigera* larvae reared on different host plants. Different letters indicate significant differences at  $P < 0.01$  with Bonferroni adjustment following ANOVA.

The weight of *H. armigera* pupae was analysed at day 27. Host plants significantly influenced the weight ( $df = 4$ ;  $F = 21.55$ ;  $P = 0.000$ ; Fig.4). Weight of pupae was varying depend on the host plants; no pupae was found in tomatoes. The average weight of the pupae reared on wild cabbage plant, chickpea, cotton, and artificial diet were  $243.59 (\pm 13.77)$ ,  $184.9 (\pm 1.00)$ ,  $302.38 (\pm 14.54)$ , and  $333.88 (\pm 9.76)$  mg, respectively.

### **3. Predation efficiency of *Eocanthecona furcellata* on American Bollworm *Helicoverpa armigera* reared on different host plants (Cabbage, Cotton, Chickpea and Tomato)**

*E. furcellata* produced their proboscis after placed into the center of the plastic box and approached towards the prey while touching the bottom with the tip of their antennae. Most of *E. furcellata* (20-60 %) directly approached towards cotton plants preying on *H. armigera* larvae. After testing the prey with the tip of their antennae, *E. furcellata* tightened their antennae ( $180^\circ\text{C}$ ) and used the rostrum to suck hemolymph from the prey, leaving no body fluids behind. *E. furcellata* shared their prey with other *E. furcellata* and once seven *E. furcellata* were preying on one *H. armigera* larvae at the same time. Although they were also preying on the faeces of *H. armigera* larvae, only one *E. furcellata* selected the *H. armigera* larvae reared on artificial diet. The majority of the *E. furcellata* preferred to suck under side of the bollworm larvae. The predation rates were  $19.83 (\pm 2.88)$ ,  $18.33 (\pm 2.97)$ ,  $39.17 (\pm 3.58)$ ,  $14.17 (\pm 2.88)$ , and  $3.33 (\pm 1.42)$  % on the *H. armigera* larvae reared on wild cabbage plant, chickpea plant, cotton plant, tomato plant and artificial diet, respectively ( $df = 5$ ;  $F = 16.97$ ;  $P = 0.000$ ); (Fig.5). 9.17 % of *E. furcellata* did not find the prey or selected no prey in the experiments, which was recorded as no choice.

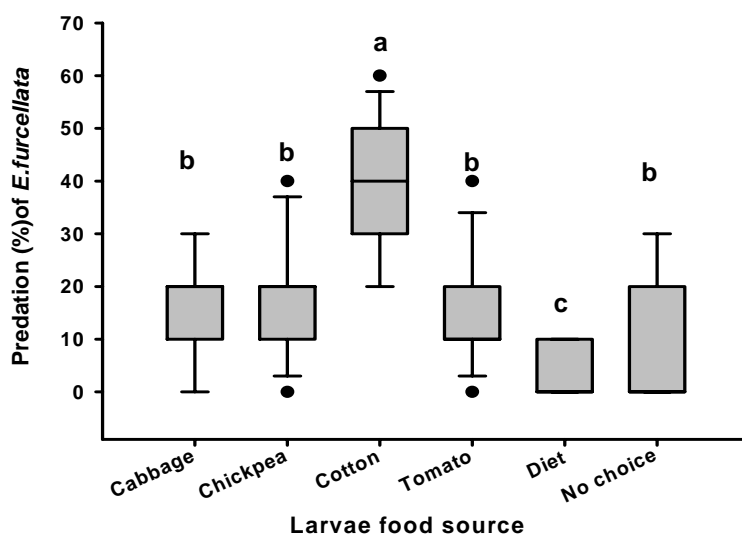


Figure 5: Predation % of *E. furcellata* (EO) on *H. armigera* larvae reared on different host plants. Different letters indicate significant differences at  $P < 0.01$  with Bonferroni adjustment following ANOVA.

#### 4. Prey preference of *Eocanthecona furcellata* on wrapped American Bollworm *Helicoverpa armigera* (ABW) reared on different host plants (Cabbage, Cotton and Chickpea)

When *H. armigera* larvae and their faeces were wrapped with Para film 'M', predation efficacy was significantly reduced on the larvae reared on cotton plant and cabbage plant ( $df = 6$ ;  $F = 11.75$ ;  $P = 0.000$ ); (Fig.6). The predation rates were  $14.44 (\pm 3.77)$ ,  $22.22 (\pm 4.94)$ , and  $24.58 (\pm 5.80)$  % on the *H. armigera* larvae reared on wild cabbage plant, chickpea plant and cotton plant, respectively.  $38.89 (\pm 8.07)$  % of *E. furcellata* were not searching for prey in the experiments and none of *E. furcellata* fed on wrapped faeces of larvae reared on wild cabbage, chickpea and cotton plants.

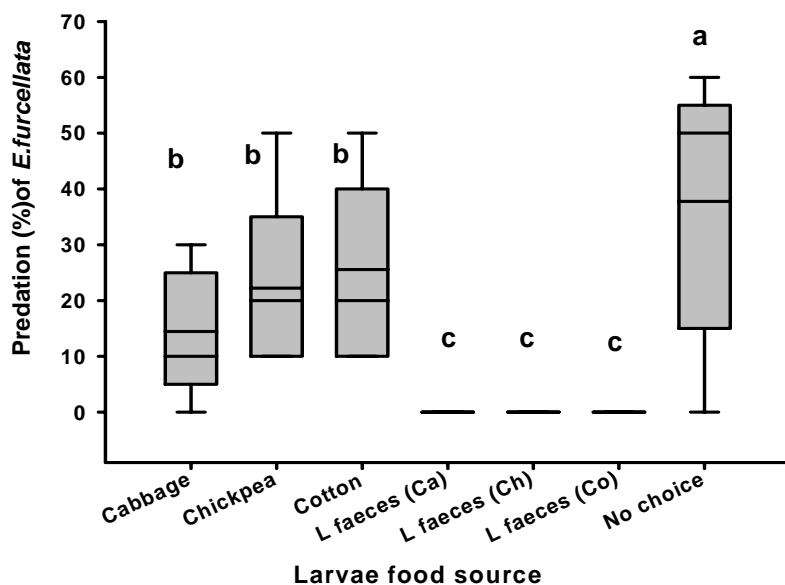


Figure 6: Predation of *E. furcellata* on the wrapped *H. armigera* larvae from different host plants. Different letters indicate significant differences at  $P < 0.01$  with Bonferroni adjustment after ANOVA.

## DISCUSSION

Host plant species significantly influenced the oviposition of females of *H. armigera* (Fig.1). 44.23 % of the females preferred to lay their eggs on chickpea plants. This host selection behavior of adult females of *H. armigera* is consistent to results reported previously by several authors (Parsons, 1940; Roome, 1975; Wardhaugh et.al., 1980; and Schneider et.al., 1986). They found that the host selection is strongly influenced by the flowering stage of their hosts. Tomato plants and cotton plants have long trichomes and even so 24.2 % and 21.39% of the eggs were laid on these plants while only 2.84 % were found on cabbage plants. These results are consistent with Shanower and Romeis (1999), reporting that reproductive structures, trichome exudates and enemy-free space contributed most to the oviposition preferences.

Host plants also affected the performance of *H. armigera* larvae. The weights of the 20-day old larvae ranged from 18.34 ( $\pm 3.74$ ) mg on tomato plants to 419.54 ( $\pm$

16.4) mg on artificial diet. Similar results were found by Lui (2004) with body weight of young last instar *H. armigera* larvae ranging from 176.7 mg on cotton to 132.5 mg on cherry tomatoes. Sharmad et al. (2005) also found that larvae of *H. armigera* weighed < 50 mg when reared on *Cicer pinnatifidum* because the wild relatives of chickpea showing high levels of antibiosis to *H. armigera*. Cotter and Edwards (2006) discuss that plants use a number of resistance mechanisms affecting insect feeding, including physical factors such as leaf toughness or trichome density, or chemical factors such as toxic allelochemicals and proteinase inhibitors. Moreover, Tan et al. (2001) and Subramanian (2006) proved the effect of host plant influence on the genetic variability of *H. armigera* populations.

Adult female of *H. armigera* preferred to lay their eggs on chickpea plants but the performance of the larvae was not better as compared to the larvae developing on cotton and cabbage plants. Several authors already discussed that females do not always oviposit on plant species on which larval performance is best (Thompson, 1988; Courtney & Kibota, 1990; Jallow et al., 1999; Jallow et al., 2001; and Jallow et al., 2003).

The predation efficacy of *E. furcellata* was tested with American Bollworm from four different host plants (cabbage, cotton, chickpea and tomato plants). The majority of *E. furcellata* (30-60 %) directly approached towards cotton plants sucking on *H. armigera* larvae. Most probably, *E. furcellata* responded to (E)- phytol, which is produced by larvae when feeding on the chlorophyll in their food plants. *E. furcellata* prefers to feed on larvae fed with a chlorophyll-rich diet than with a chlorophyll-poor diet (Yasuda, 1997, 1998a, 1998b). My results correspond to these findings that larvae reared on an artificial diet, basically made from bean flour, were inadequately attractive to *E. furcellata*. Henaut (2000) found that adults of the predatory bug *Orius majusculus* (Reuter), having no experience of aphid predation as nymphs, did not prey on pea aphids in the experimental arena. Therefore, nutrient content of prey and the standard diet used for rearing *E. furcellata* may affect the efficiency of the predator as a biological control agent.

When *H. armigera* larvae and their faeces were wrapped with Para film 'M', selection of prey was significantly reduced up to 20-40 % and the numbers of bugs not feeding or not searching for prey increased. They did not select larvae faeces from different host plant and predation on the larvae fed on cotton plants was reduced by half as compared with the first experiment. Yasuda (1998b) mentioned that about 90% of the (E)- phytol can be detected in the faeces of *S. litura*. *E. furcellata* fed the faeces of *H. armigera* in the first experiment but after covering with Para film 'M', none of *E. furcellata* adults was interested in faeces any more. Yasuda (1997 and 2000) proved that *E. furcellata* located their prey by chemical cues emanating from their prey. Para film 'M' seem to prevent the visual and olfactory cues from prey larvae; and their faeces; however, we may speculate the this predatory bug may detect the prey by vibrations caused by feeding. Vibrations produced by prey as when chewing on leaves is be an important cue used by the predatory stinkbug *Podisus maculiventris* (Say) to locate the prey (Pfannenstiel, 1995).

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## Chapter 2

### **Prey consumption and fitness of *Eocanthecona furcellata* Wolff (Hemiptera: Pentatomidae) on Diamondback Moth larvae at different temperature regimes and prey density**

#### **ABSTRACT**

The predatory bug (*Eocanthecona furcellata* (**EO**), native to Southeast Asia offers potential to be used as a biological control agent against lepidopteran pests. However, in order to establish mass rearing methods, the life history of *E. furcellata* has to be evaluated in detail. Temperature is the most important environmental factor affecting development of insects and prey consumption of the predator.

We investigated the effect of three temperature regimes and prey density temperatures on the development, prey consumption and predation rate of the bug using Diamondback Moth larvae (**DBM**) *Plutella xylostella* L. (Lepidoptera: Plutellidae) under laboratory conditions. We used 2<sup>nd</sup> instars of *E. furcellata* nymphs at three constants temperatures and eight different diamondback moth larval densities.

The maximum prey consumption per day and *E. furcellata* larvae was rather high, exceeding 9.65 ( $\pm$  0.29) larvae at 30°C in the 5th instar of *E. furcellata*. During the whole lifecycle (2<sup>nd</sup> instar to adult), *E. furcellata* consumed a minimum of 13.00 ( $\pm$ 1.08 diamondback moth larvae at 35°C to a maximum of 102.25  $\pm$  2.84 diamondback moth larvae at 25°C. The shortest nymphal developmental time (egg to adult) was found at 35°C daily offering 10 Diamondback moth larvae as a prey with 14.75 ( $\pm$  0.25) days, whereas the longest developmental time was 35.25 ( $\pm$  0.25) days at 25°C fed with only 1 diamondback moth larva.

For the extreme temperature regime experiment, *E. furcellata* was reared at constant temperatures (15, 20, 37, and 40°C, respectively) in climatic cabinets at 75% RH and 12:12 (L: D) photoperiod. The longest developmental time of EO from egg to adult was recorded at 20°C 116.0 ( $\pm$  1.14) days, while *E. furcellata* did not develop at 15°C and 40°C. The maximum prey consumption per day per adult *E.*

*furcellata* was very high and was on average 23.1 ( $\pm$  0.6) Diamondback moth larvae at 37°C. Individuals consumed up to 388.9 ( $\pm$  8.2) larvae at 37°C during the whole developmental time. *E. furcellata* females deposited on average 14.8 ( $\pm$  3.2) eggs per batch at 37°C, but these eggs failed to begin embryonic development. Therefore no viable offspring were recorded at the four tested extreme temperatures. Our data suggest that *E. furcellata* is adapted to climatic conditions prevailing in the tropical regions and may be used for biological control purposed only in areas with mean temperatures above 25°C.

Keywords: Biological control, *Eocanthecona furcellata*, Diamondback moth, Myanmar, temperature, prey density

## INTRODUCTION

The Diamondback moth (*Plutella xylostella* Linnaeus) (Lepidoptera: Plutellidae) (**DBM**) is a major pest of cabbage and cauliflower and severe infestations are regularly found in the Bago and Mandalay Division, the main vegetable growing area in Myanmar (Morris and Waterhouse, 2001). The diamondback moth is well known as a worldwide pest of cruciferous crops, such as broccoli and cabbage, and is often found on cruciferous weeds (Harcourt, 1957; Talekar & Shelton, 1993; and Voice & Chapman, 2000). Several Diamondback moth populations in the major production regions are known to be resistant to several insecticidal compounds, including resistance to *Bacillus thuringiensis* and insect growth regulators in Hawaii, India, Australia, New Zealand; South-East Asia, Japan, USA and Central America, respectively (Tabashnik *et al.*, 1987; Saxena *et al.*, 1989; Endersby & Ridland, 1994; Bell and Fenemore, 1990; Sun, 1992). In addition, insecticide applications increased the costs of production, have reduced the numbers of non-target arthropod predators, thereby reducing biodiversity, have increased the chances of consumers eating pesticide-contaminated products, and have contaminated soil and water. To avoid these consequences, biological control measures to control Diamondback moth populations are recommended.



The pentatomid predatory bug *Eocanthecona furcellata* (Wolff), preying on the larvae of *Spodoptera litura* and *Helicoverpa armigera*, was regularly found in cauliflower and cotton fields in Myanmar (Gillham, 1980; Nu Nu Yi and Win Kyi, 2000, and Khin, 2001). *E. furcellata* has been documented as a predator in Southeast Asia, Japan, India, and Taiwan, respectively, preying on larvae of Lepidoptera, Coleoptera and Heteroptera (Ahmad, 1996; Chu, 1975; Chang, 2002; Jakhmola, 1983; Prasad et. al., 1983). In Myanmar, *E. furcellata* has been reared on larvae of *Spodoptera litura* at room temperature (27-30°C). Chu (1975) proved that *E. furcellata* can be easily reared on larvae of *Pieris rapae* at 25°C in Taiwan and Yasuda and Wakamura (1992) investigated that *E. furcellata* can be maintain on frozen-preserved larvae of *Spodoptera litura* at 26°C in Japan. Ho et.al. (2003) used *Alphitobius sp.* (Coleoptera: Tenebrionidae) and *Chrysomya megacephala* (Fabricius) (Diptera: Callipaoridae) for rearing *E. furcellata* at (23 ± 3 °C) in China. Based on previous host range tests, *E. furcellata* accepted Diamondback moth as prey and diamondback moth larvae may thus be used for rearing *E. furcellata* under the laboratory conditions in Germany.

The ability of a natural enemy to adapt to different environmental conditions is an essential prerequisite for its successful utilization in a biological control program. Among other environmental conditions, temperature is considered to be a key factor affecting the biology and ecology of both harmful and beneficial insects (Hassell, 1985). Constant temperatures affected the development and survival of the predatory bug *Podisus maculiventris* (Say) (De Clercq and Degheele, 1992; Mohaghegh et. al., 2001 and Legaspi, 2004). Temperature also influenced the metabolism, reproduction and longevity of the predatory bug *Podius nigrispinus* (Dallas) (De Clercq and Degheele, 1990, 1992; Torres et.al., 1998 and Medeiros et.al., 2003a,b). Therefore, the optimal temperature for *E. furcellata* rearing and low and high temperature thresholds for releasing the predator in the field needs to be evaluated.

Prey density can also influence the biology and efficacy of predators (Solomon 1949). It is important to understand the influence of temperature and prey density

with regard to *E. furcellata* when considering this species for a biological control program to control the Diamondback moth. Data on the effect of temperature and prey density on *E. furcellata* fed with Diamondback moths are not available.

Taking this into consideration, prey consumption and fitness of the pentatomid predator *Eocanthecona furcellata* was studied at three constant temperatures (25°C, 30°C and 35°C) using eight different prey densities and at four extreme temperatures.

## **MATERIAL AND METHODS**

### **Laboratory rearing**

*Eocanthecona furcellata* (Wolff) (Hemiptera: Pentatomidae) **(EO)** eggs and adults were originally collected in November 2004 from cotton fields in Myanmar. They were released in rearing cages (75 x 55 x 75 cm) and fed with Diamondback moth **(DBM)** at room temperature ( $22 \pm 1$  °C) under laboratory conditions at the Entomology Section, Georg-August University, Goettingen, Germany.

A stock culture of the Diamondback moth larvae was reared on cabbage plants in a rearing cage (75 x 55 x 75 cm) at room temperature ( $22 \pm 1$ °C) under laboratory condition and last instar larvae were used as a prey for the experiments.

### **Effect of different temperature regimes and prey density on the development time, prey consumption and predation rate**

The development time of eggs and development time, prey consumption, and predation rate of nymphs were observed in climatic exposure test cabinets with constant temperatures at 25°C, 30°C, and 35°C, respectively. The photoperiod in all experiments was 12:12 (L: D) h and the relative humidity (RH) was 75%. Newly laid *E. furcellata* eggs were collected from the laboratory colony and placed in 9 cm Ø plastic Petri dishes and transferred to the climatic exposure test cabinets and incubation periods and hatching nymphs were recorded.

For the nymphal development studies, newly hatched 2<sup>nd</sup> instars of *E. furcellata* nymphs were collected from the laboratory colony and used for each treatment; three temperatures (25°C, 30°C, and 35°C), eight different densities of prey (1, 2, 3, 4, 5, 6, 8, and 10 diamondback moth larvae, respectively) in four replications were used. For the tests Diamondback moth larvae were placed in 9 cm Ø plastic Petri dishes and one *E. furcellata* nymph was placed in the center of each arena. A moistened cotton wool was also placed in all experimental Petri dishes to keep the temperature and humidity fixed. These Petri dishes were then transferred to the climatic exposure test cabinets with constant temperatures at 25°C, 30°C, and 35°C, respectively. Larvae consumed per day, larvae still alive and molting date were recorded for this experiment till adult stage of *E. furcellata*. Killed Diamondback moth larvae were replaced daily throughout the experiments to maintain the tested prey densities constant.

#### **Effect of high constant temperatures on the development, mortality and prey consumption of *Eocanthecona furcellata***

These experiments aimed at understanding the effect of four extreme temperatures (15°C, 20°C, 37°C, and 40°C, respectively) on prey consumption, survivorship and longevity of the predatory bug *E. furcellata*. 2<sup>nd</sup> instars of *E. furcellata* nymphs were used for each treatment at four temperatures and ten replications. Diamondback moth larvae were used as prey for the predator in this experiment. Diamondback moth larvae were placed in 9 cm Ø plastic Petri dishes and one *E. furcellata* nymph was placed in the center of each arena. A moistened cotton wool was also placed in all experimental Petri dishes to keep the temperature and humidity constant. These Petri dishes were then transferred to growth chambers with constant temperatures at 15°C, 20°C, 37°C, and 40°C, respectively. All cabinets were set at 75% relative humidity (RH) and 12:12 (L: D) photoperiod. Daily consumed larvae, larvae still alive and data on molting were recorded for these experiments until all *E. furcellata* specimens were dead.

## Statistical analyses

Analysis of variance (ANOVA) was used to determine statistical differences in the development data among the temperature and densities tested (SPSS Inc., 2004). Means of treatments were separated by Bonferroni adjustment.

## RESULT

### **Effect of different temperature regimes and prey density on the development time, prey consumption and predation rate of *Eocanthecona furcellata***

Temperature significantly affected *Eocanthecona furcellata* egg development (df = 2; F= 1.047; P= 0.000), and first instar nymph development (df = 2; F= 2.094; P= 0.000) (Fig. 1; Appendix 1). The time required for egg development decreased with increasing temperatures up to 35°C, and ranged from 10.84 ( $\pm$  0.07) days at 25°C to 4.81 ( $\pm$  0.07) days at 35°C.

Total development, measured in days (egg to adult), decreased from 31.00 ( $\pm$  0.00) days at 25°C to 19.25 ( $\pm$  0.25) days at 30°C and 14.75 ( $\pm$  0.25) days at 35°C, when 10 Diamondback moth larvae were offered as a prey (Fig. 2). The 5<sup>th</sup> instar nymph of *E. furcellata* had the longest development time at all temperature. The development time of *E. furcellata* nymphs was also significantly different at different prey densities (Appendix 1). Nymph developmental times decreased when increasing prey densities up to 10 DBM larvae. The interaction of temperature and prey densities also affected the longevity of *E. furcellata* (Appendix 1). The shortest nymph development time 14.75 ( $\pm$  0.25 days) was found at 35°C when 10 Diamondback moth larvae were offered per day, whereas the longest was 35.25 ( $\pm$  0.25) days at 25°C fed with only 1 Diamondback moth larva.

Daily prey consumption of *E. furcellata* on Diamondback moth larvae gradually increased with increasing prey densities (Fig. 3). The highest numbers of daily prey consumption per 5<sup>th</sup> instar nymphs of *E. furcellata* were found at 30°C (9.65  $\pm$  0.15 larvae) when 10 Diamondback moth larvae were offered; a decreasing prey consuming rate was found at the higher temperature regime (7.43  $\pm$  0.08 larvae at

35°C) and at the lower temperature regime ( $8.46 \pm 0.27$  larvae at 25°C) (Appendix.2). Total prey consumed by *E. furcellata* nymph was minimum 18.25 ( $\pm 0.25$ ), 15.25 ( $\pm 0.48$ ) and 13.00 ( $\pm 1.08$ ) larvae and maximum 102.3 ( $\pm 2.84$ ), 70.50 ( $\pm 3.18$ ) and 65.25 ( $\pm 2.43$ ) larvae at 25°C, 30°C and 35°C, respectively. Daily prey consumption rates were significantly different between the three temperatures tested and the eight different prey densities (Table.1).

Predation rates of *E. furcellata* on 10 diamondback moth larvae increased with nymphal instar and at 25°C, the predation rates were 40.83 ( $\pm 1.59$ ), 42.5 ( $\pm 4.38$ ), 60.0 ( $\pm 3.85$ ) and 84.64 ( $\pm 2.69$ ) % for 2<sup>nd</sup>, 3<sup>rd</sup>, 4<sup>th</sup>, and 5<sup>th</sup> instars, respectively. Nymphs preyed on 58.75 ( $\pm 4.47$ ), 65.63 ( $\pm 4.83$ ), 74.41 ( $\pm 8.32$ ), and 96.46 ( $\pm 4.46$ ) % ; and 65.5 ( $\pm 4.79$ ), 66.25 ( $\pm 2.39$ ), 67.0 ( $\pm 2.89$ ) and 74.25 ( $\pm 0.75$ ) % for 2<sup>nd</sup>, 3<sup>rd</sup>, 4<sup>th</sup>, and 5<sup>th</sup> instars at 30°C and 35°C, respectively (Fig. 4).

The predation rates of *E. furcellata* decreased with increasing prey densities and an optimal predation rate was found at 30°C (Fig. 5; Appendix 3). Mean predation rates were 97.67( $\pm 1.34$ ), 97.67( $\pm 1.34$ ), 86.79 ( $\pm 1.28$ ), 87.72 ( $\pm 3.72$ ), 84.14 ( $\pm 0.71$ ), 83.78 ( $\pm 2.00$ ), 64.57 ( $\pm 1.64$ ), 66.5 ( $\pm 1.63$ ), and 63.91 ( $\pm 1.77$ ) % for preying on 1, 2, 3, 4, 5, 6, 8, and 10 larvae of DBM at 25°C and 100.0 ( $\pm 0.00$ ), 91.55 ( $\pm 2.49$ ), 90.31 ( $\pm 4.08$ ), 86.82 ( $\pm 1.40$ ), 86.86 ( $\pm 1.33$ ), 88.80 ( $\pm 3.51$ ), 71.28 ( $\pm 2.13$ ), and 76.31 ( $\pm 3.58$ ) at 30°C; and 96.13 ( $\pm 2.25$ ), 87.23 ( $\pm 3.48$ ), 97.73 ( $\pm 2.27$ ), 93.58 ( $\pm 1.73$ ), 96.46 ( $\pm 2.05$ ), 64.77 ( $\pm 2.78$ ), 71.04 ( $\pm 2.59$ ), and 74.55 ( $\pm 1.41$ ) at 35°C. Regarding the predation rates of *E. furcellata*, the effects of temperature and prey density as well as their interaction were significant (Appendix 3 and Table 1).

Statistical analyses revealed that temperature and prey density as well as their interaction significantly affected mean adult weight (df = 2; F= 5.952; P= 0.004); (df = 7; F= 17.15; P= 0.000) (Table 1). Adult weight of *E. furcellata* increased with increasing prey densities (Fig. 6) and *E. furcellata* attained significantly lower weight in lowest prey treatment (1 Diamondback moth larvae) at all temperatures; 42.15 ( $\pm 2.82$ ), 40.35 ( $\pm 4.01$ ), and 32.25 ( $\pm 2.45$ ) mg at 25°C, 30°C and 35°C, respectively. Maximum adult weight was 77.97 ( $\pm 3.33$ ), and 82.00 ( $\pm 7.64$ ) mg for

10 Diamondback moth larvae at 30°C and 35°C, and 87.85 ( $\pm$  13.03) mg for 8 Diamondback moth larvae at 25°C. The gender of *E. furcellata* also effected adult weight at the three tested temperatures, with the females being heavier and bigger than males.

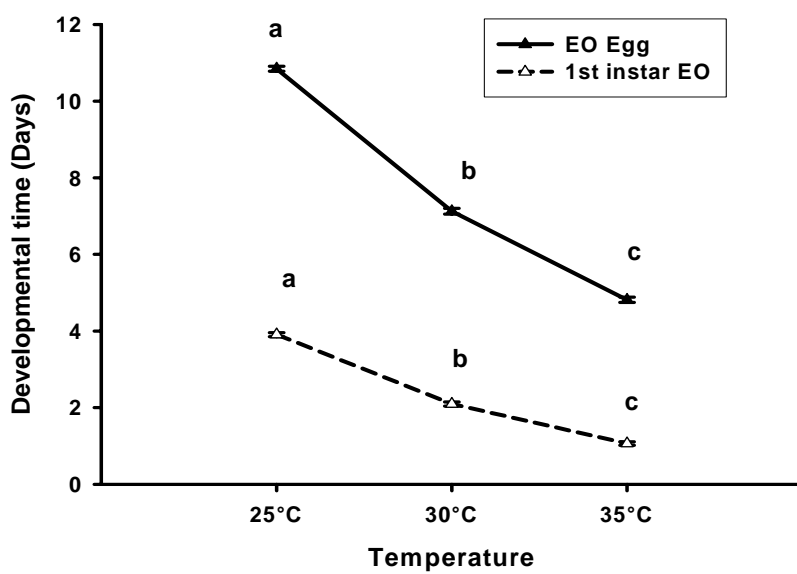


Figure 1: Developmental time [days] of *E. furcellata* eggs and 1<sup>st</sup> instars at different temperatures. Different letters indicate significant differences at  $P < 0.01$  with Bonferroni adjustment following ANOVA.

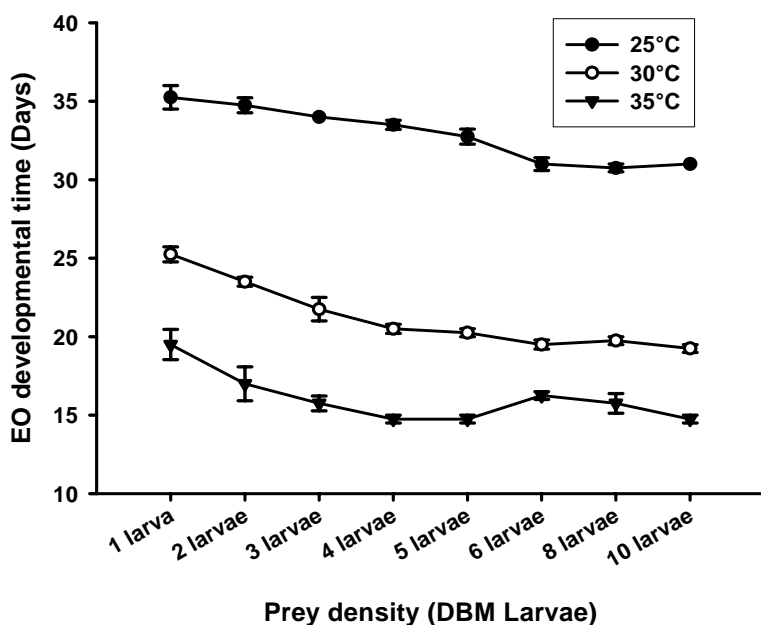


Figure 2: Developmental time [mean  $\pm$ SE] of *E. furcellata* (EO) at different temperatures when offered different prey densities

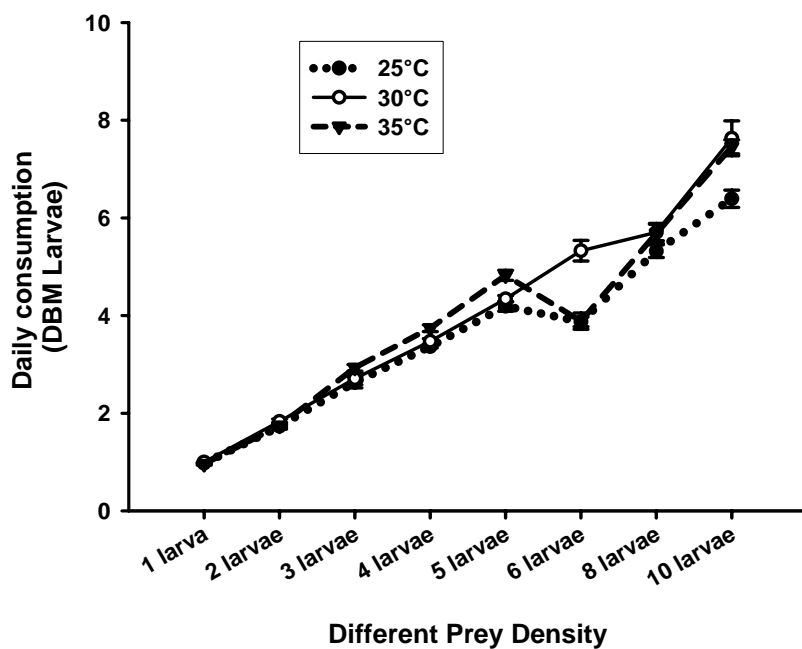


Figure 3: Daily prey consumption [mean  $\pm$ SE] of *E. furcellata* at different temperatures when offered different prey densities

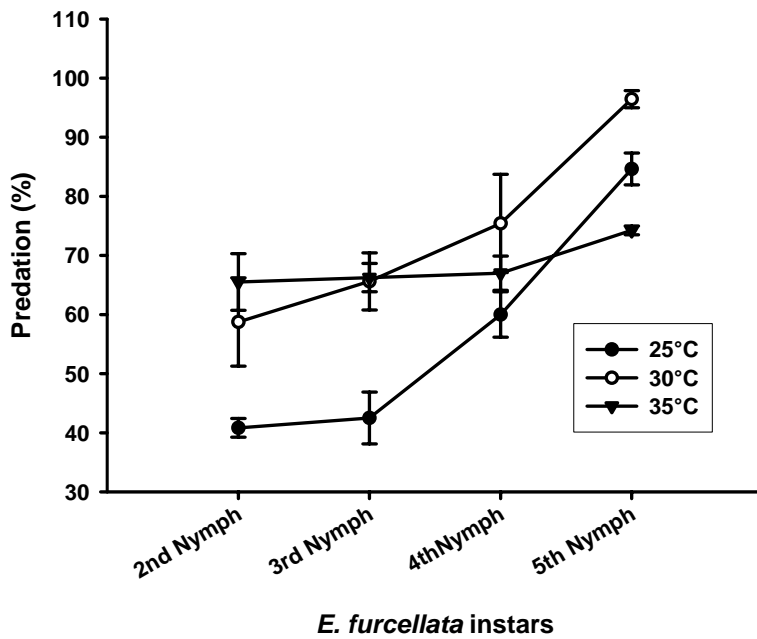


Figure 4: Predation rates [mean±SE] of the four instars of *E. furcellata* on 10 Diamondback moth larvae at different temperatures

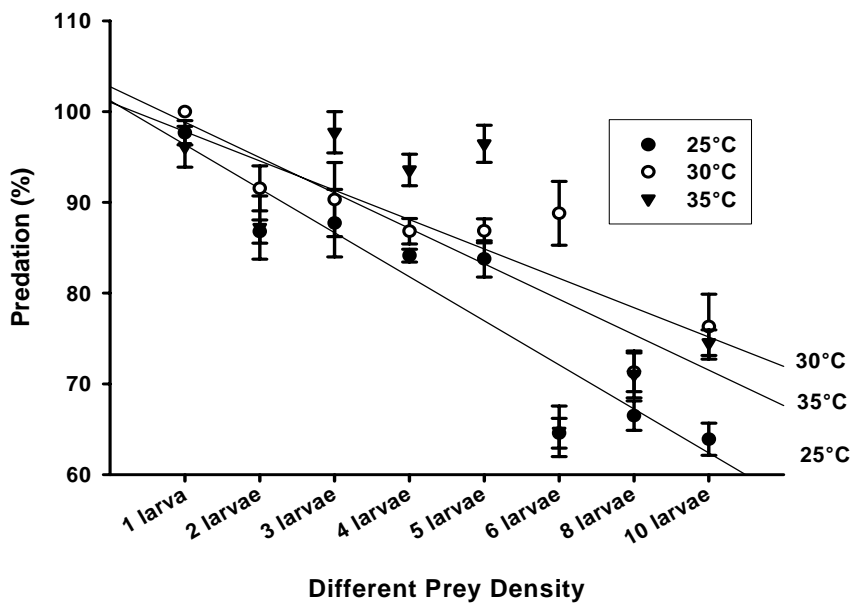


Figure 5: Mean Predation rate [mean±SE] of *E. furcellata* at different temperatures offered with different prey density



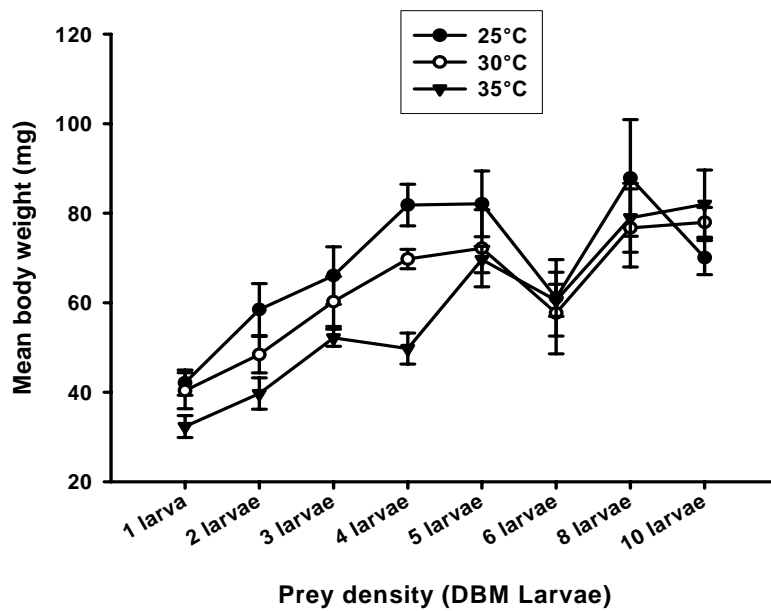


Figure 6: Adult body weight [mean $\pm$ SE] of *E. furcellata* at different temperatures when offered different prey densities of Diamondback moth larvae

Source	Sum-of-Squares	df	Mean-Square	F-ratio	P*
<b>MEAN LIFEDAYS (Egg to Adult)</b>					
TEMPERATURE	4747.89	2	2373.948	2579.989	0.000
DENSITY	247.823	7	35.403	38.476	0.000
TEMPERATURE* DENSITY	42.771	14	3.055	3.320	0.000
Error	66.250	72	0.920		
<b>MEAN DAILY CONSUMPTION</b>					
TEMPERATURE	3.44	2	1.721	24.317	0.000
DENSITY	340.59	7	48.656	687.432	0.000
TEMPERATURE* DENSITY	7.52	14	0.537	7.585	0.000
Error	5.10	72	0.071		
<b>MEAN PREDATION (%)</b>					
TEMPERATURE	916.350	2	458.175	20.746	0.000
DENSITY	9695.712	7	1385.102	62.717	0.000
TEMPERATURE* DENSITY	1894.891	14	135.349	6.129	0.000
Error	1590.104	72	22.085		
<b>MEAN ADULT WEIGHT</b>					
TEMPERATURE	1788.418	2	894.209	5.952	0.004
DENSITY	18039.902	7	2577.129	17.154	0.000
TEMPERATURE* DENSITY	2564.825	14	183.202	1.219	0.281
Error	10817.140	72	150.238		

\* Significance level within the same rows by ANOVA

Table1. ANOVA of the effects of temperature and prey density on predation and development parameters of *Eocanthecona furcellata*

### **Effect of high constant temperatures on the development, mortality and prey consumption of *Eocanthecona furcellata***

Nymphal development of *E. furcellata* was significantly affected by the tested extreme temperatures ( $df = 3$ ;  $F = 10.73$ ;  $P = 0.000$ ). The 2<sup>nd</sup> instars *E. furcellata* survived for 12.6 ( $\pm 0.40$ ) days at 15°C and *E. furcellata* did only survive for one day at 40°C and all *E. furcellata* died during moulting during the 2<sup>nd</sup> instar to the 3<sup>rd</sup> instar at both temperatures (Fig. 7). 50 % and 90 % of the predator nymphs were able to develop successfully and reach the adult stage (2<sup>nd</sup> instar to adult) on average after 26.0 ( $\pm 3.26$ ) and 8.45 ( $\pm 0.31$ ) days at 20°C and 37°C. Adults were able to survive for 69.2 ( $\pm 1.24$ ) days at 20°C and 12.67 ( $\pm 0.58$ ) days at 37°C (Fig. 8 and 9). *E. furcellata* was able to survive at these two constant temperatures; however *E. furcellata* was unable to lay eggs at 20°C and even though *E. furcellata* females deposited on average 14.8 ( $\pm 3.2$ ) eggs per batch at 37°C, these eggs failed to begin embryonic development (Fig. 10). Thus, no viable offspring was recorded at the four tested extreme temperatures.

Regarding the average daily prey consumption of *E. furcellata* on Diamondback moth larvae, the effect of temperature and nymph instars were significant ( $df = 3$ ;  $F = 55.79$ ;  $P = 0.000$ ) and ( $df = 4$ ;  $F = 20.34$ ;  $P = 0.000$ ); (Fig. 11). At the lower temperature (15°C) and the higher temperature (40°C), 2<sup>nd</sup> instar *E. furcellata* nymph consumed 0.355 ( $\pm 0.32$ ) and 0.30 ( $\pm 0.18$ ) larvae per day and all 2<sup>nd</sup> instar nymphs used at 15°C and 40°C died even moulting to 3<sup>th</sup> nymph instar. At 20°C, *E. furcellata* nymph daily consumed 2.35 ( $\pm 0.11$ ), 2.26 ( $\pm 0.20$ ), 2.62 ( $\pm 0.23$ ), 3.96 ( $\pm 0.29$ ), and 2.35 ( $\pm 0.18$ ) Diamondback moth larvae for 1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup>, 4<sup>th</sup>, 5<sup>th</sup> instar *E. furcellata* nymph and adult ( $df = 4$ ;  $F = 10.39$ ;  $P = 0.000$ ). A slightly higher consumption was found when tested 5<sup>th</sup> instar nymphs at 20°C. The daily prey consumption by *E. furcellata* at 37°C gradually increased with *E. furcellata* instars, where it was 5.90 ( $\pm 0.53$ ) Diamondback moth larvae for 2<sup>nd</sup> instar *E. furcellata* nymph and reached up to 23.09 ( $\pm 0.61$ ) Diamondback moth larvae for adult *E. furcellata* ( $df = 4$ ;  $F = 163.13$ ;  $P = 0.000$ ); (Fig. 11). During their entire lifespan, one

*E. furcellata* was able to consume up to 260.00 ( $\pm$  16.56) larvae at 20°C and 388.89 ( $\pm$  8.20) larvae at 37°C (Fig. 12).

Adult weight of *E. furcellata* was significantly affected by temperature and gender (df = 1; F= 14.52; P= 0.002); and (df = 1; F= 14.52; P= 0.036); (Fig. 13). Female weight of *E. furcellata* (87.0 ( $\pm$  8.20) and 118.96 ( $\pm$  3.25) mg) was heavier than that of the male (68.28 ( $\pm$  1.32) and 76.3 ( $\pm$  8.70) mg) at 20°C and 37°C.

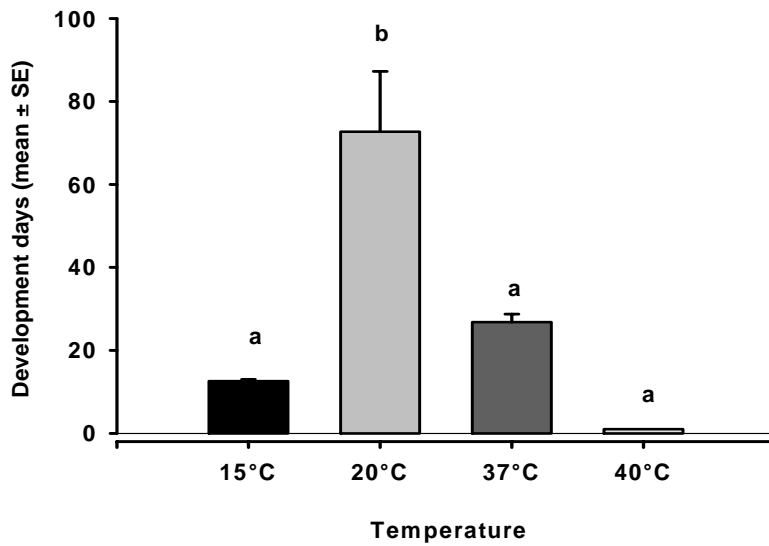


Figure 7: Longevity of *Eocanthecona furcellata* at different temperatures. Different letters indicate significant differences at P < 0.01 with Bonferroni adjustment after ANOVA.

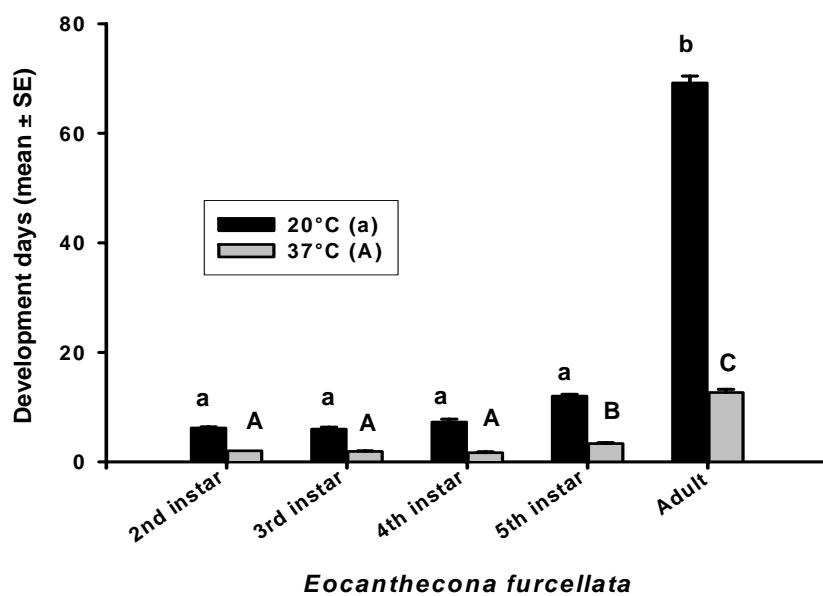


Figure 8: Developmental time of *Eocanthecona furcellata* from the 2<sup>nd</sup> instar to adult at different temperatures. Different letters indicate significant differences at  $P < 0.01$  with Bonferroni adjustment after ANOVA.

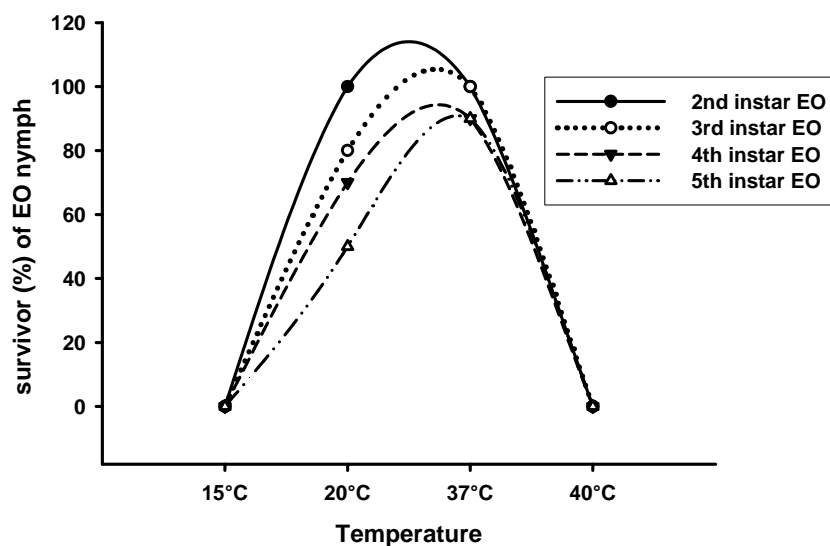


Figure 9: Percent surviving *E. furcellata* (EO) from the 2<sup>nd</sup> instar to 5<sup>th</sup> instar at different temperatures.

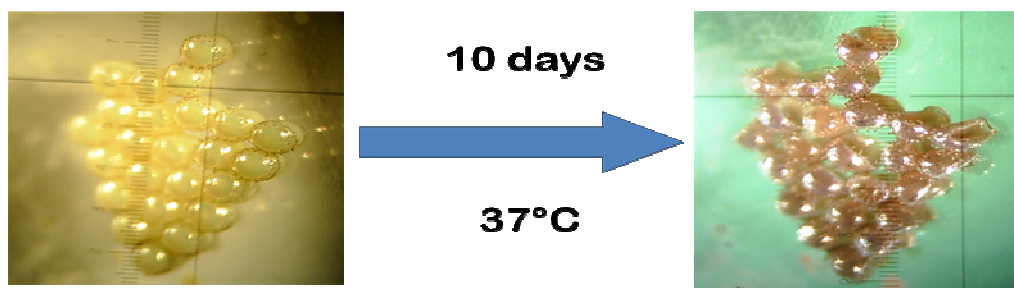


Figure 10: *Eocanthecona furcellata* eggs that failed to begin embryonic development at 37°C

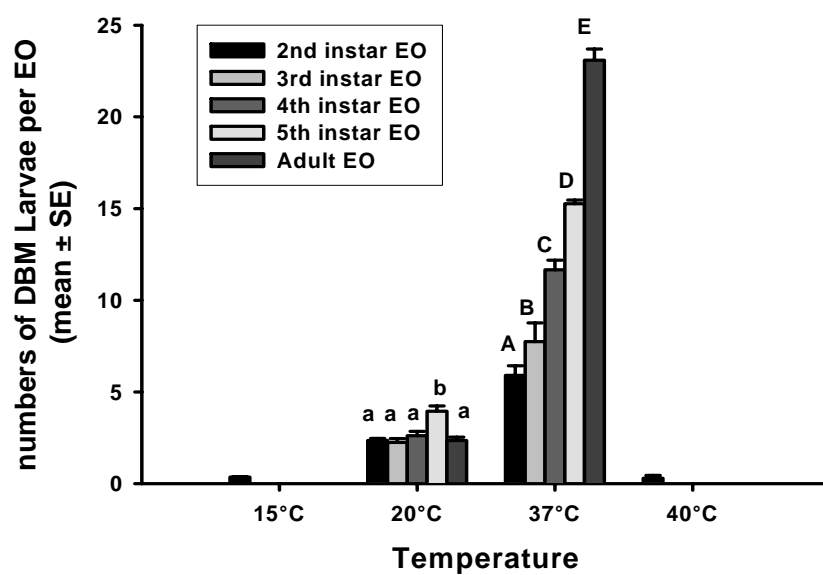


Figure 11: Daily consumption of 2<sup>nd</sup> instar *E. furcellata* (EO) nymphs on Diamondback moth larvae at different temperatures. Different letters indicate significant differences at  $P < 0.01$  with Bonferroni adjustment after ANOVA.

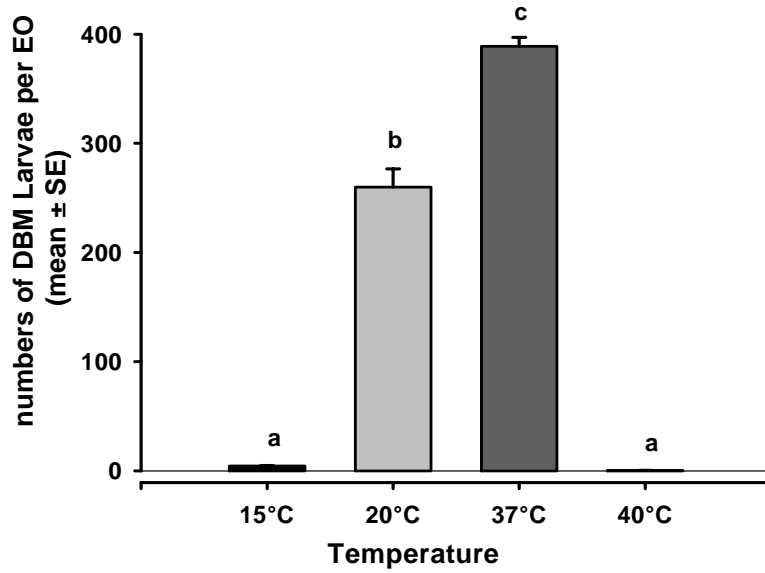


Figure 12: Total consumption of 2<sup>nd</sup> instar *E. furcellata* nymph on Diamondback moth (**DBM**) larvae at different temperatures. Different letters indicate significant differences at  $P < 0.01$  with Bonferroni adjustment after ANOVA.

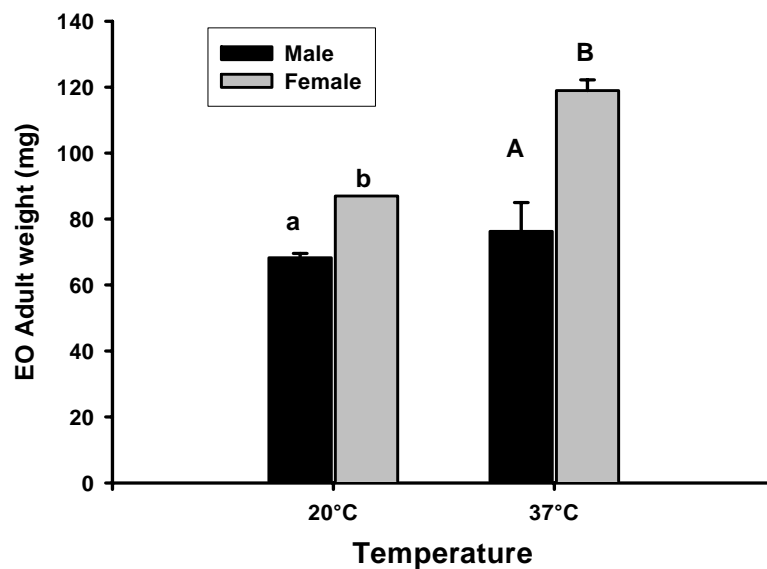


Figure 13: Adult weight of *Eocanthecona furcellata* (**EO**) developing at different temperatures. Different letters indicate significant differences at  $P < 0.01$  with Bonferroni adjustment after ANOVA.

## DISCUSSION

The results of the experiments indicate that *E. furcellata* successfully fed and completed its development at the temperatures (25°C, 30°C, and 35°C) tested irrespective to the prey density tests. The average developmental period was significantly shorter at higher temperatures (35°C). These results correspond to Chang (2001) and also agreed with the results of previous studies on *Podisus maculiventris* (De Clercq and Degheele, 1992; Mohaghegh et. al., 2001 and Legaspi, 2004); on *Podisus nigrispinus* (De Clercq and Degheele, 1992 and Medeiros et. al., 2003); on *Rhyzobius lophanthae* (Stathas, 2000); and on *Scymnus levaiillanti* (Uygun and Atlihan, 2000). Nymph developmental times decreased when increasing the prey densities up to 10 Diamondback moth larvae. The interaction of temperature and prey densities also affected the longevity of *E. furcellata*. The shortest nymphal developmental time (egg to adult) was found doubling at 35°C when 10 Diamondback moth larvae were offered daily and the longest was at 25°C fed with only 1 Diamondback moth larva. Adults were able to survive longer when temperatures were lower. The total developmental time of *E. furcellata* (egg to mortality of adults) was two times longer at 25°C than at 35°C. When *E. furcellata* had sufficient prey, they may produce up to 11 generations per year. This is an important result with regard to potential biological control measures in hot countries like Myanmar.

Successful biological control of a pest is based on the fact that the natural enemy kills a sufficient number of preys to keep its density at low levels. Daily prey consumption of *E. furcellata* on Diamondback moth larvae increased gradually with increasing prey density. The highest amount of daily prey consumption per 5<sup>th</sup> instar nymph of *E. furcellata* was found at 30°C when we offered 10 Diamondback moth larvae. Similar results were found by Saleh et.al. (2003) for prey consumption of *Dicyphus tamaninii* Wagner (Het., Miridae) with *Aphis gossypii* Glover at 30°C. Prey consumption decreased at higher temperature (35°C) where *E. furcellata* was only able to consume a minimum of Diamondback moth larvae, whereas the maximum (about 10 times) was found at 25°C. This indicates that *E. furcellata* is



adapted to tropical temperature regimes, as compared to the prey consumption in the predatory stink bugs *P. bidens* and *P. maculiventris* which was highest at 23°C (Mahdian et. al., 2006)

Functional responses describe the trend in which the number of prey consumed per predator changes with changes in prey density (Solomon, 1949). Predation rates of *E. furcellata* on Diamondback moth larvae increased with nymphal instars; the highest predation rate was recorded for the 5<sup>th</sup> nymphal instar of *E. furcellata* at all tested temperatures. The most favorable predation rate was found at 30°C with regard to the 4<sup>th</sup> and 5<sup>th</sup> nymphal instars of *E. furcellata* and daily prey consumption of 4<sup>th</sup> & 5<sup>th</sup> instars of *E. furcellata* nymphs is regarded sufficient for mass releasing purposes in biological control strategies. High predation rates of *P. bidens* were found at a wide range of temperatures (Mahdian et. al.,2006). Therefore it is suggested that this species may be a valuable asset for the biological control of Diamondback moth, provided that obstacles to its mass production can be overcome.

Statistical analyses revealed that temperature and prey density as well as their interactions were significantly effecting adult weight. The adult weight of *E. furcellata* increased when increasing the prey density. The gender also affected the adult weight at three tested temperature, with the female being heavier and bigger than males.

The result of the experiments conducted on the effect of extreme temperatures on the development of *E. furcellata* showed that 50 % and 90 % from 2<sup>nd</sup> instar developed to adult the stage at constant temperatures of 20°C and 37°C, respectively. *E. furcellata* was able to survive at these two constant temperatures; however *E. furcellata* was unable to lay eggs at 20°C and even though they laid eggs at 37°C, no larvae hatched to nymphs. The 2<sup>nd</sup> instars *E. furcellata* survived for 12.6 ( $\pm$  0.40) days at 15°C and *E. furcellata* did only survive for one day at 40°C and all *E. furcellata* died during moulting from 2<sup>nd</sup> instar to 3<sup>rd</sup> instar nymphal stage at both temperatures. Therefore no viable offspring was recorded in the four tested

extreme temperatures. We regard the low temperature threshold for *E. furcellata* at 15°C and high temperature threshold at 40°C.

Adult weight of *E. furcellata* was significantly affected by temperature and gender; female weight of *E. furcellata* was heavier than male weight at 20°C and 37°C. Effects of extreme temperatures can lead to failure of an IPM strategy that may be quite effective in a narrow temperature range (Horn, 1998). Biological control agents, especially arthropod natural enemies, often exhibit temperature optima different from those of their prey, and may become ineffective at higher or lower temperatures.

As a result *E. furcellata* seems to be adapted to climatic conditions prevailing in the tropical regions. Given these preferences *E. furcellata* may be used for biological control purposes only in areas with mean temperatures of above 25°C.

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### Chapter 3

#### Prey searching and feeding behavior of *Eocanthecona furcellata* Wolff (Hemiptera: Pentatomidae on different prey items, host plant species and plant status

##### ABSTRACT

The predatory stink bug *Eocanthecona furcellata* Wolff (Hemiptera: Pentatomidae) (**EO**), native to Southeast Asia offers potential to be used as a biological control agent against lepidopteran pests. However, in order to establish the biocontrol agent the prey searching and feeding behavior of **EO** has to be evaluated in detail. We investigated the effect of two different prey items (American bollworm **ABW** and Diamondback moth (**DBM**), feeding on different host plants (cotton and cabbage plants) subject to different treatments under laboratory condition and green house conditions.

Searching time and prey consumption time of **EO** was significantly longer with regard to **DBM** ( $70.78 \pm 2.84$  minutes) as compared to **ABW** ( $36.75 \pm 3.96$  minutes) on cabbage plants. Under laboratory conditions, **EO** was able in a second to locate the prey items and needed  $36.73 \pm 2.57$  minutes for consuming **DBM** as compared to  $15.1 \pm 0.98$  minutes to consume **ABW**.

When **ABW** larvae were offered on cotton leaves **EO** needed less time ( $24.2 \pm 1.91$  minutes) to locate the prey items than on cabbage leaves ( $36.75 \pm 3.961$  minutes). Mean prey consumption time of **EO** on **ABW** larvae was not different between both plant species tested; however prey consumption under laboratory conditions was three times faster than on the plants.

**EO** visits on cotton and cabbage plants were distinctly shorter as compared to insect infested plants and visiting time of **EO** on **ABW** infested cotton plants were half than on **DBM** infested cabbage plants. **EO** was attracted more by **ABW** than **DBM** and movement of **EO** was quicker on cotton plants.

In a Y-olfactometer **EO** was attracted more by **ABW** than **DBM** larvae; however searching time of **EO** was not significantly different with regard to both larval species.

The results are discussed with regard to the potential of the predatory bug to be used as a biocontrol agents in cotton fields.

Keywords: Biological control, Cotton plant, *Eocanthecona furcellata*, Diamondback Moth, Myanmar

## INTRODUCTION

The American bollworm (*Helicoverpa armigera* Hübner)(**ABW**) is the most severe pest of cotton and is found in all agricultural regions and on all important crops in Myanmar (MCSE 1999, Morris and Waterhouse 2001). Due to its highly polyphagous feeding behaviour the pest has a wide distribution in the world (Commonwealth 1968; Hill 1975; Reed and Pawar 1982). The management of **ABW** is difficult and multiple insecticide applications have led to high levels of resistance to major groups of active compounds (Fitt 1989; Armes *et al.* 1996). To solve this problem, one promising approach could be to manage **ABW** by releasing natural enemies.

The Diamondback moth (*Plutella xylostella* Linnaeus)(**DBM**) is a major pest of vegetable brassicas in Myanmar (Morris and Waterhouse 2001). **DBM** populations in the major production regions are resistant to organophosphate, carbamate, pyrethroid and organochlorine insecticides in Hawaii (Tabashnik *et al.* 1987); India (Saxena *et al.* 1989); Australia (Endersby & Ridland 1994); New Zealand (Bell and Fenemore 1990) and in South-East Asia, Japan, USA and Central America (see Sun 1992 for a review). This problem has prompted a decision to search for biological control options in controlling **DBM** in Myanmar.

The predatory bug (*Eocanthecona furcellata* Wolff)(**EO**) was found preying on **ABW** larvae in cotton and cauliflower fields in Myanmar (Gillham, 1980; Nu Nu Yi and Win Kyi, 2000, and Khin, 2001). **EO** was also found in Southeast Asia preying on other larvae of Lepidoptera, Coleoptera and Heteroptera (Ahmad 1996; Chu



1975; Chang 2002; Jakhmola 1983; Prasad et. al. 1983). A laboratory evaluation of the efficacy of **EO** on lepidopteran pest resulted in a positive decision to release **EO** as a biocotrol agent to control **ABW** and **DBM** in Myanmar (Khin and Vidal 2006, and Khin and Vidal 2007a, Khin and Vidal 2007b). Prey location behavior of *Eocanthecona furcellata* was already studied by Yasuda & Wakamura (1996) and Yasuda (2000). Bell (1990) found that the searching behavior of insects depended on the biological characteristics of the insect, internal factors and external environmental factors. Therefore, the predation behavior **EO** may change under field conditions and more studies are needed to understand prey searching and feeding behavior of the predatory bug **EO** with regard to specific plant conditions. This study aims at testing the impact of two different prey items, two different host plant species ; and three different conditions of these plants on the behaviour of **EO**. Furthermore, searching and feeding behavior of **EO** was compared using either greenhouse conditions or Petri dishes.

## **MATERIAL AND METHODS**

### **Culture of insects**

Eggs and adults of the pentatomid *Eocanthecona furcellata* (Wolff) (Hemiptera: Pentatomidae) (**EO**) were originally collected in November 2004 in cotton fields in Myanmar. They were released in rearing cages (75 x 55 x 75 cm ) and reared on Diamondback moth infested cabbage plants because of the high egg laying capacity of **DBM** adults. Eggs were transferred to Germany and **EO** was maintained at room temperature ( $22 \pm 1$  °C) under laboratory conditions at the Entomology Section, Georg-August University, Goettingen, Germany. 5<sup>th</sup> instars Nymph of **EO** were collected randomly from the rearing cage and individuals were maintained in Petri-dish and starved 24 hours before starting the experiments.

American bollworm (*Helicoverpa armigera* Hübner) (Lepidoptera: Noctuidae) (**ABW**) eggs were obtained from Bayer AG, Germany, and hatched larvae were reared on cabbage plants under laboratory condition and 6.00 ( $\pm 1.00$ ) mg larvae were used for the experiments.

A stock culture of Diamondback moth (*Plutella xylostella* Linnaeus)(**DBM**) larvae was maintained on cabbage plant in rearing cages at room temperature ( $22 \pm 1$  °C) under laboratory conditions and 6.00 ( $\pm 1.00$ ) mg larvae were used for the experiments.

### **Culture of plants**

Cotton (*Gossypium hirsutum* cv. MCU 9) and cabbage (*Brassica oleracea* var. *viridis*) were grown in controlled greenhouse conditions; plants were grown in 13 cm diameter pots (Sand: Clay 50: 50) for this experiments. Four- to six-weeks old plants, with four fully expanded true leaves were used in the experiments. Seeds of *G. hirsutum* cv. MCU 9 were provided from Myanmar and seeds of *B. oleracea* var. *viridis* from Germany.

### **Effect of different preys**

Cabbage plants (*Brassica oleracea* var. *viridis*) at the four true leaf stages were used as food sources for **ABW** and **DBM** larvae, respectively. 5<sup>th</sup> instar larvae of starved **EO** nymphs were used in this experiment. Each treatment was replicated ten times. Each larva of **DBM** and **ABW** were weighed and 6.00 ( $\pm 1.00$ ) mg larvae were placed on each leaf of 10 cabbage plants and also placed in 10 Petri-dishes for each insect. Larvae were allowed to settle on the leaves for 15 minutes. A single predator was released into the arena or on the plants and the searching time (including waiting time) and the time to consume the prey was recorded for each individual.

### **Effect of different host plants**

Cotton plants (*Gossypium hirsutum* L.) and cabbage plants (*Brassica oleracea* var. *viridis*) at the four leaf stage were used as food sources for **ABW** larvae. 5<sup>th</sup> instars of starved **EO** nymphs were used in this treatment; these were offered two host plants and Petri-dishes and each treatment was replicated ten times. Each larva of **ABW** was weighed and 6.00 ( $\pm 1.00$ ) mg larvae were placed on each leaf of both host plants and allowed to settle on the leaves for 15 minutes. A single

predator was released into the arena and the searching time (including waiting) and the time to consume the prey of **EO** was recorded for each individual.

### **Effect of different host plant conditions**

30 Cotton plants and 30 cabbage plants at the four leaf stage were used for this experiment; 10 of each host plants were used as control plants, another 10 of each host plants were cut with a pair of scissors and used as wounded plants, and the remaining 10 cotton and cabbage plants were used as insect infected plants. **ABW** larvae were placed on each leaf of these plants and were allowed to settle for 15 minutes on the leaves. Thereafter **ABW** larvae were removed from the plants and the plants were used for the experiment. A single 5<sup>th</sup> instar nymph of **EO** was released into the arena and the visiting time of **EO** for each **ABW** individual was recorded.

### **Prey selecting behavior of *Eocanthecona furcellata* towards American bollworm and Diamondback moth larvae in an Y-olfactometer**

This experiment aimed at understanding the host selection behavior of **EO** towards larvae of **ABW** and **DBM** using a Y-shaped dual tube olfactometer (Fig. 17) at room temperature 22 ( $\pm 1$ ) °C with twenty replications. Both **ABW** and **DBM** larvae were reared on cabbage plants and ten 5<sup>th</sup> instar **EO** nymphs were randomly selected from a rearing cage and starved for 24 hours. **ABW** and **DBM** larvae were placed in the alternative odor source tubes and a single **EO** was released into the starting point of the olfactometer and collected in the observation arena. If **EO** did not move within 5 minutes we regarded this as no choice; **EO** was then removed and replaced with another **EO**. We studied searching time (from starting point to observation area) percentage of **EO** either selecting **DBM** or **ABW**..

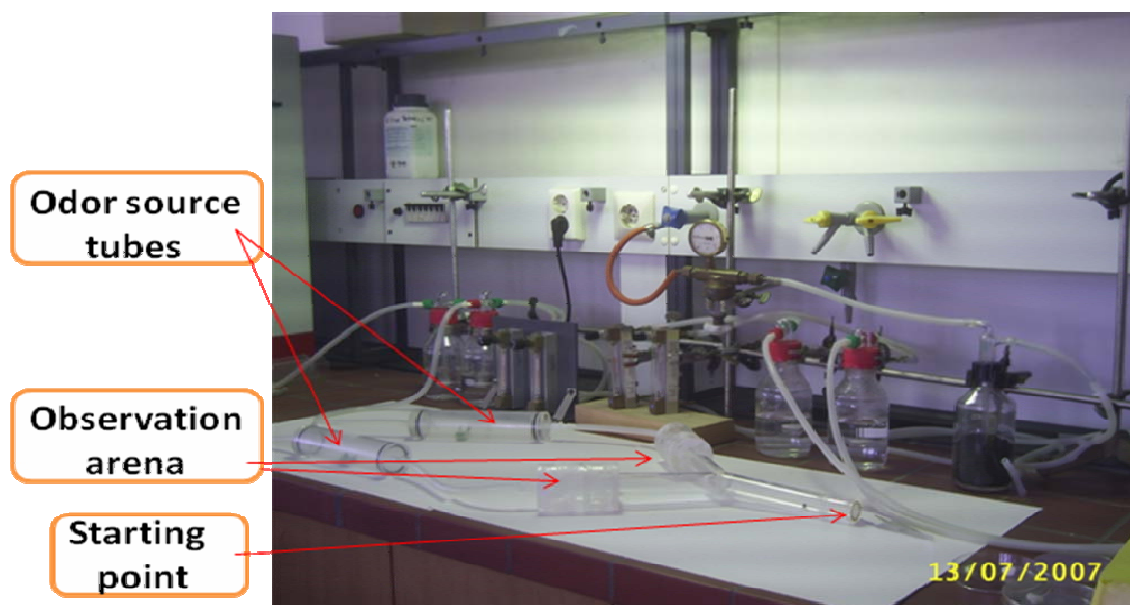


Figure 1: Y-shape dual tube Olfactometer

### Statistical analyses

Data were analyzed using Analysis of variance (ANOVA) by SYSTAT 11 for Windows (SPSS Inc., 2004). We used a one-way analysis of variance (ANOVA) with a Fisher's Least-Significant-Different Test (LSD) to compare the individual samples of searching time and consuming time of **EO** for different preys, different host plants and different conditions against each other in pair wise comparisons.

## RESULTS

### Effect of different preys

When **EO** larvae were released onto the plants, 90 % of them were able to search for and capture the prey larvae on the first leaf of the plants. Thereafter **EO** moved upwards on the plant. However, **EO** searching time for **DBM** larvae on the first leaf was significantly different ( $df = 3$ ;  $F = 7.119$ ;  $P = 0.001$ ) as compared to the other leaves on the cabbage plants; **EO** found the 1<sup>st</sup> **DBM** larva within 32.10 ( $\pm 4.21$ ) minutes but 2<sup>nd</sup> to 4<sup>th</sup> **DBM** larvae were caught in 89.00 ( $\pm 2.59$ ) minutes. **EO** searching for **DBM** larvae was significantly different ( $df = 1$ ;  $F = 127.981$ ;  $P = 0.000$ ) when comparing **DBM** larvae placed on host plants and offered in Petri dishes. **EO**

needed only 1.30 ( $\pm$  0.058) seconds to locate **DBM** in Petri-dishes although the searching time of **EO** within the **DBM** larvae in the Petri dishes was not significantly different ( $df = 3$ ;  $F = 0.480$ ;  $P = 0.698$ ) (Fig. 1).

On the other hand, searching behavior of **EO** when exposed to **ABW** larvae was not statistically different on the plant ( $df = 3$ ;  $F = 0.913$ ;  $P = 0.446$ ) and in a Petri dishes ( $df = 3$ ;  $F = 0.025$ ;  $P = 0.889$ ). However **EO** searching for **ABW** larvae was significantly different ( $df = 1$ ;  $F = 241.768$ ;  $P = 0.000$ ) between **ABW** larvae placed on host plants as compared to larvae offered in Petri dishes. On cabbage plants, **ABW** larvae were found in 36.75 ( $\pm$  3.961) minutes whereas **EO** took only 1.13 ( $\pm$  0.108) seconds in Petri-dishes for searching each **ABW** larva (Fig. 2).

Prey consumption time of **EO** larvae was significantly different on plants as compared to Petri-dishes with regard to both prey larvae; for **DBM** ( $df = 1$ ;  $F = 99.868$ ;  $P = 0.000$ ) and for **ABW** ( $df = 1$ ;  $F = 282.276$ ;  $P = 0.000$ ) (Fig. 3 and 4). No significant differences could be observed within larvae when offered **ABW** larvae on the plant ( $df = 3$ ;  $F = 0.707$ ;  $P = 0.556$ ) or in Petri dishes ( $df = 3$ ;  $F = 0.619$ ;  $P = 0.607$ ) and also offered **DBM** larvae on the plant ( $df = 3$ ;  $F = 2.644$ ;  $P = 0.065$ ) although prey consumption time of **EO** for 1<sup>st</sup> **DBM** larva was significantly longer than other **DBM** larvae in Petri dishes ( $df = 3$ ;  $F = 4.638$ ;  $P = 0.008$ ).

Prey searching time of **EO** for **DBM** larvae on the plants was two times longer than searching for **ABW** larvae ( $df = 3$ ;  $F = 97.229$ ;  $P = 0.000$ ) (Fig. 5). **EO** needed only half time for consuming **DBM** and one third for **ABW** when prey was offered in Petri-dishes ( $df = 3$ ;  $F = 88.574$ ;  $P = 0.000$ ) (Fig. 6).

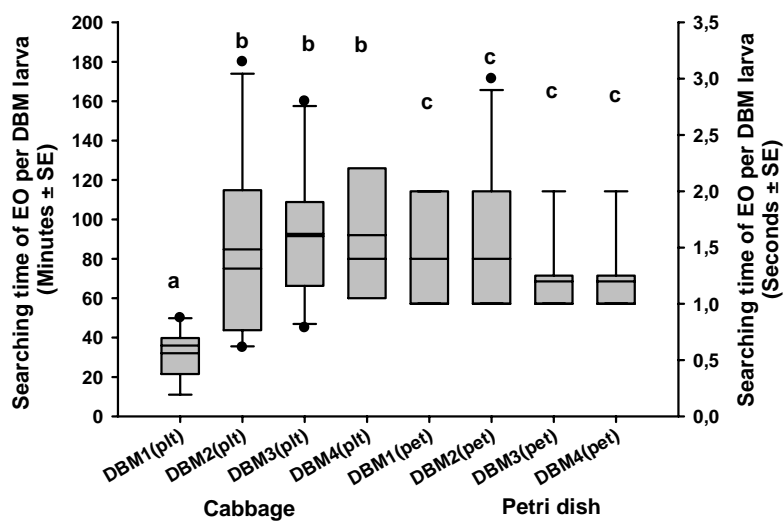


Figure 1: Searching time of **EO** for **DBM** larvae either placed on different leaves on the cabbage plants (plt – in minutes) or offered in Petri dishes (pet – in seconds). Same letters indicate no significant differences at  $P < 0.01$  for Fisher's LSD test after ANOVA.

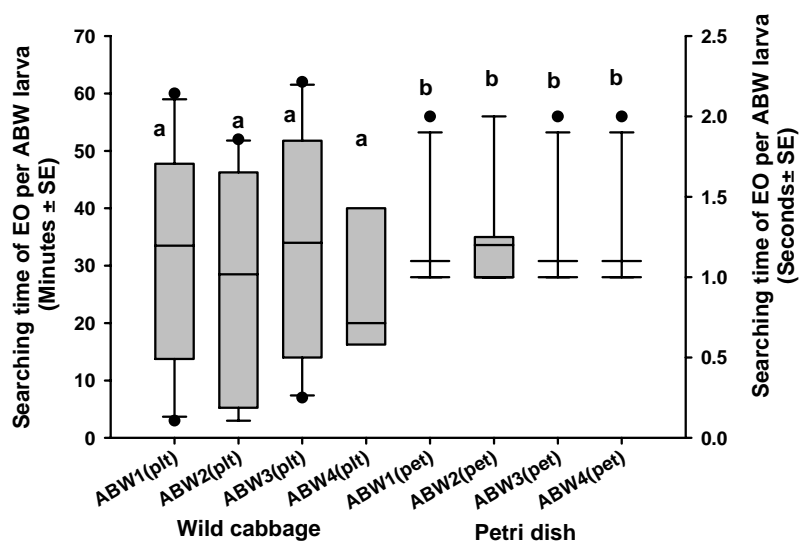


Figure 2: Searching time of **EO** for **ABW** larvae either placed on different leaves on the cabbage plants or offered in a Petri dish. Same letters indicate no significant differences at  $P < 0.01$  for Fisher's PLSD test after ANOVA.

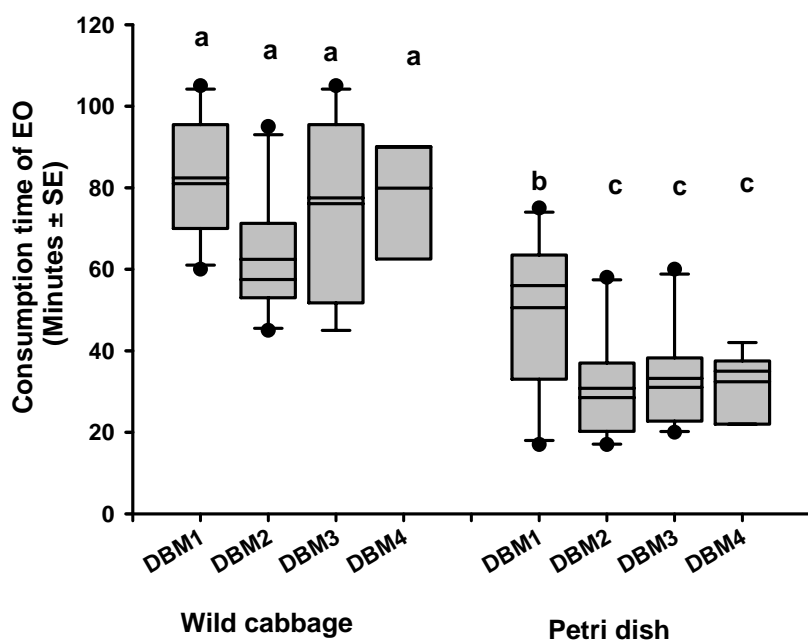


Figure 3: Consumption time of **EO** when offered **DBM** larvae either placed on different leaves on the cabbage plants or in a Petri dish. Same letters indicate no significant differences and different letters indicate significant differences at  $P < 0.01$  for Fisher's LSD test after ANOVA.

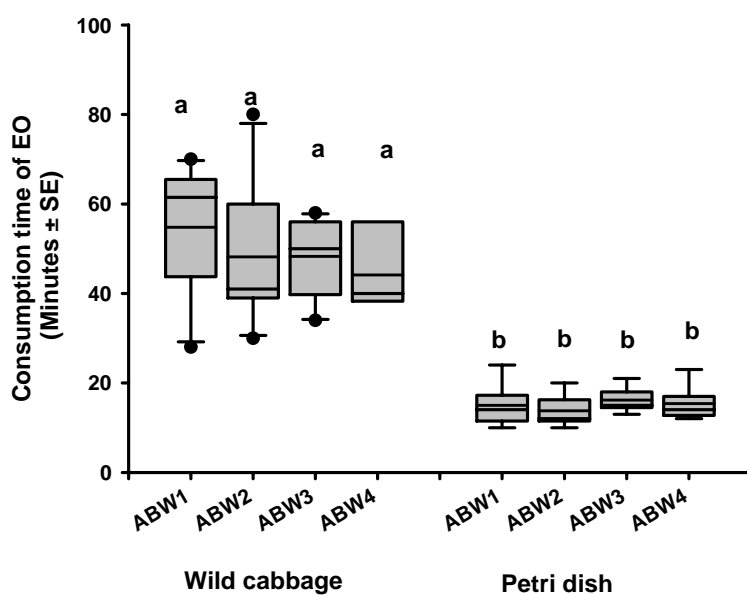


Figure 4: Consumption time of **EO** when offered **ABW** larvae either placed on different leaves on the cabbage plants or in a Petri dish. Same letters indicate no significant differences at  $P < 0.01$  for Fisher's LSD test after ANOVA.

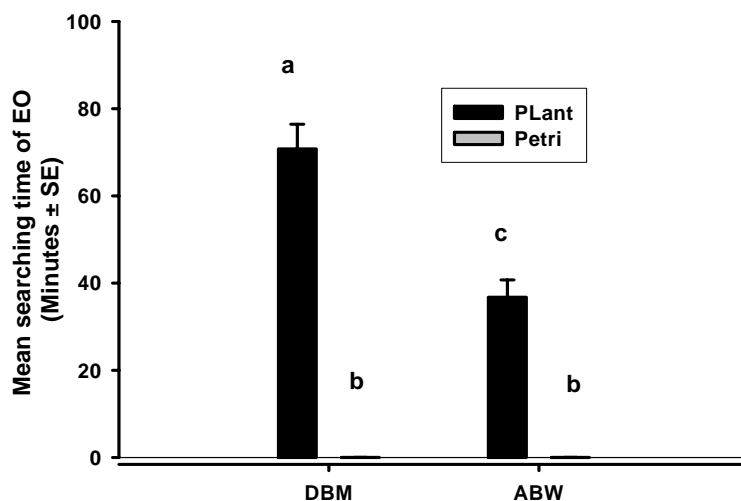


Figure 5: Mean searching time of **EO** when offered **DBM** larvae or **ABW** larvae on the plants and in a Petri dish. Different letters indicate significant differences at  $P < 0.01$  for Fisher's LSD test after ANOVA.

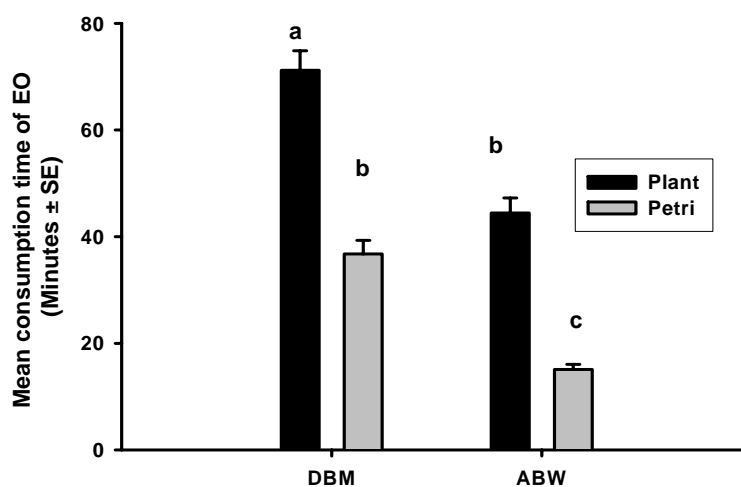


Figure 6: Mean consumption time of **EO** when offered **DBM** larvae or **ABW** larvae on the plants and in a Petri dish. Different letters indicate significant differences at  $P < 0.01$  for Fisher's LSD test after ANOVA.



### Effect of different host plants

Mean prey searching time of **EO** for **ABW** larvae was significantly different between the host plants ( $df = 2$ ;  $F = 54.089$ ;  $P = 0.000$ ) (Fig. 7). When **ABW** larvae were offered on cotton leaves **EO** was able to locate them more easily than on cabbage leaves ( $24.2 \pm 1.91$  and  $36.75 \pm 3.961$  minutes); however, in Petri dishes **EO** was able to catch them within 1-3 seconds.

Mean prey consumption time of **EO** for **ABW** larvae was not different between the **ABW** larvae offered on cotton plants and cabbage plants but in the Petri dishes consumption time of **EO** for **ABW** larvae was about three times quicker than on the plants ( $df = 2$ ;  $F = 52.933$ ;  $P = 0.000$ ) (Fig. 8).

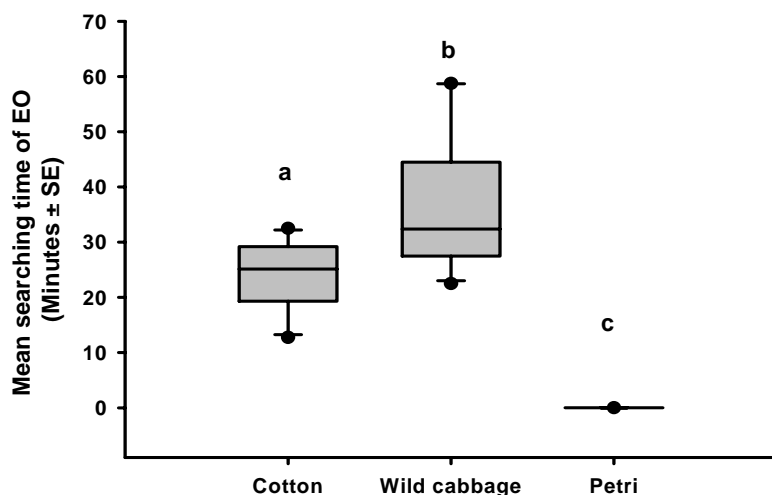


Figure 7: Mean searching time of **EO** when offered **ABW** larvae on the cotton plants, wild cabbage plants and in Petri dishes. Different letters indicate significant differences at  $P < 0.01$  for Fisher's LSD test after ANOVA.

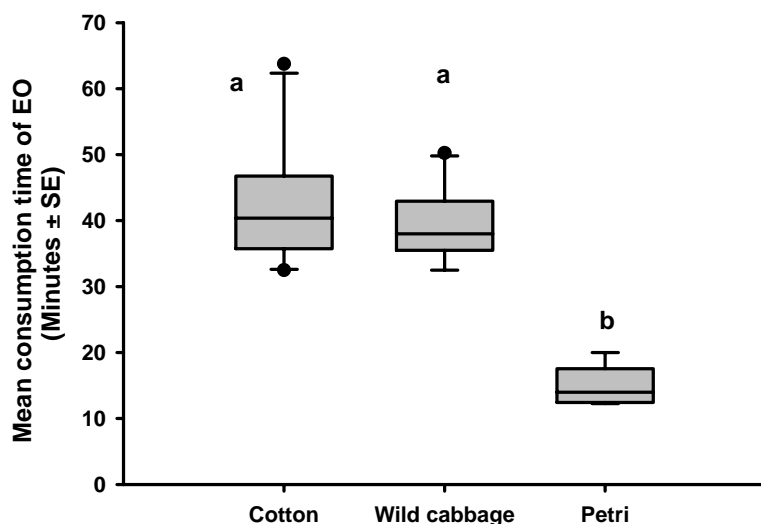


Figure 8: Mean consumption time of **EO** when offered **ABW** larvae on the cotton plants, wild cabbage plants and in Petri dishes. Different letters indicate significant differences at  $P < 0.01$  for Fisher's LSD test after ANOVA.

### Effect of different conditions

Visiting time of **EO** on host plants differing in their status was significantly different ( $df = 2$ ;  $F = 115.64$ ;  $P = 0.000$ ) (Fig. 9). **EO** visits on control cotton and cabbage plants were distinctly shorter ( $5.8 \pm 1.618$  and  $10.0 \pm 1.606$  minutes) as compared to insect infested plants and visiting time of **EO** on **ABW** infested cotton plants was reduced by half than on **DBM** infested cabbage plants ( $5.8 \pm 1.618$  and  $10.0 \pm 1.606$  minutes).

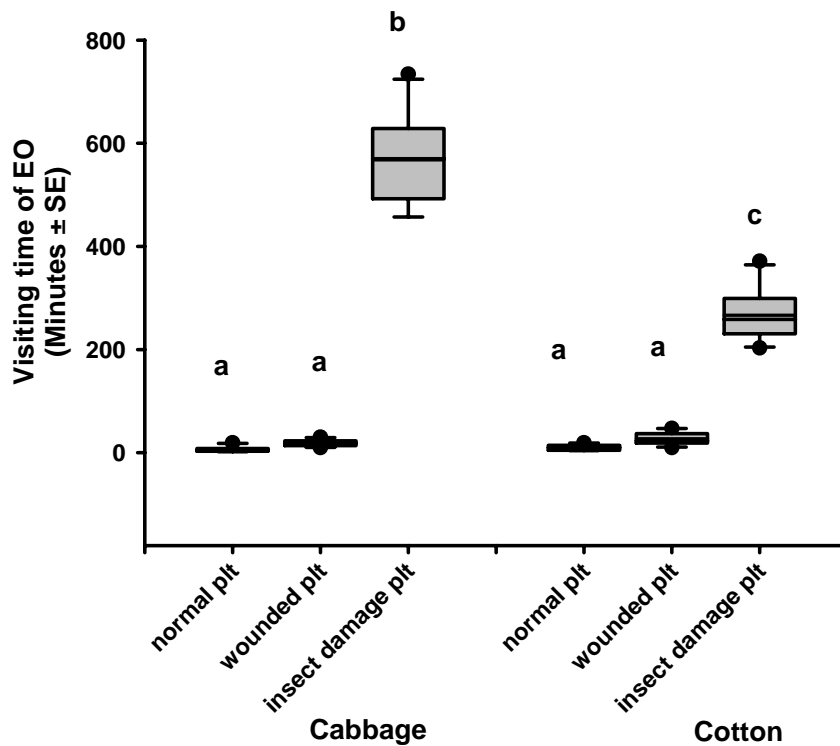


Figure 9: Visiting time of **EO** on different host plants (wild cabbage, cotton) and also on different conditions (control,, wounded and insect damage plants). Same letters indicate no significant differences at  $P < 0.01$  for Fisher's LSD test after ANOVA.

### Observing of host choice behavior of *Eocanthecona furcellata* (EO) towards American bollworm (ABW) and Diamondback moth (DBM) larvae in Olfactometer

**EO** was attracted by both **ABW** and **DBM** larvae ( $43.75 \pm 5.58$  versus  $30 \pm 5.16$  % respectively) and  $25 \pm 4.87$  % of **EO** were did not leave the starting point ( $df = 2$ ;  $F = 3.471$ ;  $P = 0.033$ ); (Fig.10). Searching time of **EO** was not significantly different on both prey items ( $3.33 \pm 0.78$  minutes for DBM and  $3.12 \pm 0.47$  minutes for **ABW**;  $df = 1$ ;  $F = 0.06$ ;  $P = 0.807$ ; Fig.11).

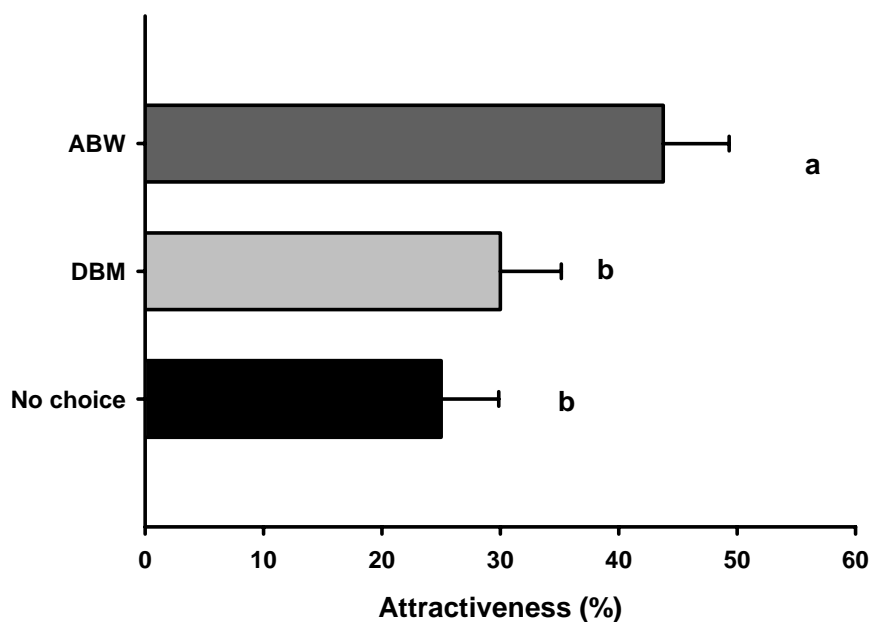


Figure 10: Responses of **EO** to odours of **ABW** and **DBM** larvae. Different letters indicate significant differences at  $P < 0.05$  with Fisher's LSD test after ANOVA.

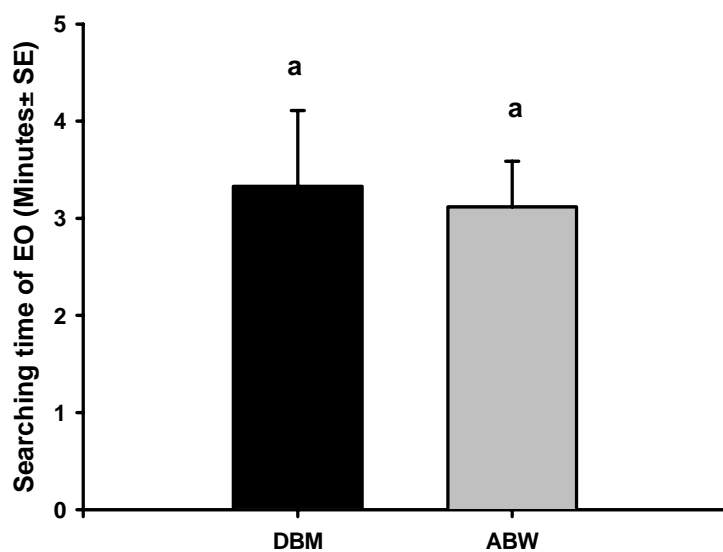


Figure 11: Searching time of **EO** to odours of **ABW** and **DBM** larvae. Different letters indicate significant differences at  $P < 0.01$  with Fisher's LSD test after ANOVA.

## DISCUSSION

This study demonstrates that prey species may influence the searching behavior of a predator bug. **EO** was not able to easily find **DBM** larvae because after placing the larva of **DBM** on the upper surface of each leaf, all larvae moved towards the lower surface of the leaf and started to feed there. On the other hand the green colour of **DBM** larvae may camouflage them given the colour of the leaves. However, we never observed dropping off the leaf when a predator approached near the **DBM** larva. On the other hand **EO** waited a maximum of 15 minutes at that place until **DBM** climbed up again and then preyed on the larva. In contrast, only 2-3 minutes waiting time was needed for catching brown coloured **ABW** larvae that fed on the upper surface of the cabbage plant leaves. Drop off behaviour of **ABW** larvae was recorded in many studies (Awan 1985; Johnson and Zalucki 2005; Terry *et. al.* 1989) but larvae never displayed this behaviour in our experiments. **EO** approaching behaviour was studied by Yasuda (1997, 1998a, b, 2000). He proved that **EO** used 2 different chemical cues: *n*-Pentadecane (C15) as a long-range attractant and (*E*)-phytol as a short distance attractant as a kairomone to locate larvae of several lepidopterans.

Our results also indicate that the searching behavior of **EO** significantly differed between host plants. Searching time of **EO** on cotton plants was lower than on cabbage plants, because **EO** walked slower on cotton leaves and spent more time on the upper part of the cotton plant but searching was more repeated on the whole plants as compared to cabbage plants. The influence of the host plants on the behaviour of predators was found in other studies as well (Coll *et. al.* 1997; Grosman *et. al.* 2005; Guershon and Gerling 2006; Yang 2000). Prey consumption time of predator **EO** for **ABW** larvae increased on cotton plants as compared to cabbage plants was probably influenced by the prey's host plants (Bergman and Tingey 1979). Prey searching time of **EO** was on average only 1-3 seconds in the Petri dishes but 24 minutes on cotton plants and 37 minutes on cabbage plants for each **ABW** larvae and prey consumption time of **EO** on the plants was about 3 times longer than in Petri dishes. These results may help to calculate the numbers of predator to be released in the field.

Mechanically damaged plants were more attractive than control plants of both species but did not significantly differ from normal plants. **EO** was strongly attracted by insect infested plants and these results are supported by findings of Yasuda (1997) who showed that free phytol is produced by insect damaged plants attracting the predatory stink bug **EO**. These results are also consistent with Krips *et. al.* (2001), Takabayashi *et. al.* (1991) and Venzon *et. al.* (1999). They reported that insect induced plants were attracted for predators.

Prey selection of **EO** was studied in the Y-Olfactometer and the results indicate that **EO** was attracted more by **ABW** than by **DBM** larvae; however, searching time of **EO** was not significantly different between both larvae.

Results from our experiments indicate that the predatory bug *E. furcellata* seems to be a good predator to be included into existing biological control programs for the American bollworm on cotton plants. However, it may be useful to consider the surrounding vegetation for releasing **EO** as a biocontrol agent to control **ABW** in Myanmar.

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## GENERAL DISCUSSION

American bollworm is the most severe pest of economically important crops like cotton, chickpea, pigeonpea, and maize in Myanmar. Even though chemical insecticides were widely applied to control this pest, yield loss on seed cotton was not uncommon. Yield reduction may be as high as 90 percent in some cases (Myat Htwe and Mya Maung, 1992). Biological control is recognized as one of the best alternatives to the use of chemical insecticides for controlling insect pests. Pest control with natural enemies has been increasing due to environmental, economical, social and ecological problems with insecticides and Heteropteran predators are important biological control agents on Lepidopteran pests. The predatory pentatomid bug *Eocanthecona furcellata* (Wolff) (**EO**) is regarded a potential biological control agent against lepidopteran pests in Southeast Asia.

I am interesting to get the answer of this question. Can predatory bug *E. furcellata* be a potential biocontrol agent to control American bollworm?

The main aim of my study is to observe the ecology and biology of *Eocanthecona furcellata* by:

1. Prey preferences and predation efficacy of *Eocanthecona furcellata*
2. Prey consumption and fitness of *Eocanthecona furcellata* Wolff (Hemiptera: Pentatomidae) on Diamondback moth larvae at different temperature regimes and prey density
3. Prey searching and feeding behavior of *Eocanthecona furcellata* Wolff (Hemiptera: Pentatomidae) on different preys (American bollworm and Diamondback moth), different host plants (cotton and wild cabbage) and different conditions (normal plant, wounded plant, and insect infected plant)

### **Prey preferences and predation efficacy of *Eocanthecona furcellata***

The significant differences were found in larval weight when the larvae of *H. armigera* reared on different host plants. The smallest larvae got from tomato plant and the biggest from artificial diet. Similar result was found by Lui (2004) that body weight of young last instar **ABW** larvae ranged from 176.7 mg on cotton to 132.5 mg on cherry tomato. Sharmad (2005) also found that larvae of *H. armigera* weighed < 50 mg when reared on *Cicer pinnatifidum* because the wild relatives of chickpea showing high levels of antibiosis to *H. armigera*. Moreover, Subramanian (2006) proved that the effect of host plant influenced on the genetic variability of *H. armigera* population.

Predation efficacy of **EO** was tested with American Bollworm from four different host plants (Cabbage, Cotton, Chickpea and Tomato). Majority of **EO** approached towards cotton plants eating **ABW** larvae. Predatory **EO** response to (*E*)- phytol, which is produced by larvae from chlorophyll in food plant and **EO** more prefer to feed on the larvae with chlorophyll-rich diet than on chlorophyll-poor diet (Yasuda, 1997, 1998a, 1998b), My results correspond to their finding that larvae reared with artificial diet, which basically made from bean flour, were inadequately attractive to **EO** in my experiment. Henaut (2000) found that the predatory bug *Orius majusculus* (Reuter) adults that had no experience of aphid predation as nymphs did not prey on pea aphids in the experimental arena. Therefore, prey nutrient and the standard diet used for rearing **EO** may affect the efficiency of the predator as an agent of biological control.

When **ABW** larvae and their faeces were wrapped with Para film 'M', the prey selecting efficacy was significantly reduced up to 20-40 %. Most of the predators were walking around in the plastic box and when they touched the prey with the tip of their antennae, they extended the rostrum and then approached and sucked the body fluids of **ABW** larvae. They didn't select all larvae faeces from different host plant and predation on the larvae reared on cotton plants reduced about half percent compare with the first experiment. Yasuda (1997) described that about

90% of the (*E*)- phytol can detect in the faeces of *S. litura*. **EO** fed the faeces of **ABW** in the first experiment but after covering with Para film 'M', none of **EO** interested in faeces. Para film 'M' seen to prevent the visual and olfactory cue from prey larvae and their faeces but predatory bug can detect the prey by vibration. Vibrations produced by prey as they chew on leaves may be an important cue used by the predatory stinkbug *Podisus maculiventris* (Say) to locate the prey (Pfannenstiel, 1995).

### **Prey consumption and fitness of *Eocanthecona furcellata* Wolff (Hemiptera: Pentatomidae) on Diamondback moth larvae at different temperature regimes and prey density**

The result of the experiment showed that **EO** was successfully feed and complete its development at the temperatures (25°C, 30°C, and 35°C) with regard to all prey density tests. The average developmental period was significantly shorter at higher temperature 35°C. These results correspond to Chang (2001) and also agreed with the results of some previous studies on *Podisus maculiventris* (Legaspi, 2004; Mohaghegh et. al., 2001); on *Podisus nigrispinus* (Medeiros et. al., 2003); on *Rhyzobius lophanthae* (Stathas, 2000); and on *Scymnus levaiillanti* (Uygun and Atlihan, 2000). Nymph developmental times decreased with rising the prey densities up to 10 **DBM** larvae. The interaction of temperature and prey densities also affect on the longevity of predator **EO**. The shortest Nymph developmental time (egg to adult) was found double at 35°C when daily offered 10 **DBM** larvae and the longest was at 25°C fed with only 1 **DBM** larva. Adult are able to survive longer when temperatures are lower. The total developmental time of **EO** (egg to mortality of adults) takes two times longer at 25°C than at 35°C. If **EO** has enough prey, they may have up to 11 generations per year. This is a promising result for biological control in hot countries like Myanmar.

Successful biological control of a pest is based on the face that the natural enemy kills a sufficient number of a prey to keep its density at low level. Daily prey consumption of **EO** on **DBM** larvae increased gradually with increasing prey density. The highest amount of daily prey consumption per 5<sup>th</sup> instar Nymph of **EO**

was found at 30°C when we offered 10 **DBM** larvae. Similar result was found by Saleh et. al.(2003) for prey consumption by *Dicyphus tamaninii* Wagner (Het., Miridae) with *Aphis gossypii* Glover at 30°C. Decreasing of prey consuming was found at higher temperature 35°C and **EO** can consume a minimum of **DBM** larvae at 35°C to maximum **DBM** larvae (about 10 time) at 25°C. But prey consumption was high at 23°C for predatory stink bug *P. bidens* and *P. maculiventris* (Mahdian et. al.,2006) Functional responses describe the trend in which the number of prey consumed per predator changes with changes in prey density (Solomon, 1949).

Predation rates of **EO** on **DBM** larvae increased with nymph instars and the highest amount of predation rate was recorded from 5<sup>th</sup> Nymph of **EO** at all tested temperatures. The most favorable predation rate were found at 30°C on 4<sup>th</sup> and 5<sup>th</sup> instars **EO** nymph and daily prey consumption of 4<sup>th</sup> & 5<sup>th</sup> instars of **EO** nymphs is also high enough for mass releasing purposes in biological control strategies. High predation rates of *P. bidens* was found at a wide range of temperatures (Mahdian et. al.,2006). Therefore it suggests that the species may be a valuable asset for the biological control of Diamondback moth, provided that obstacles to its mass production can be overcome.

Statistical analyses revealed that temperature and prey density as well as their interaction were significantly affected on adult weight. The gender also significantly affect on adult weight at three tested temperature, with the female being heavier and bigger than males.

The result of the experiments conducted on the effect of extreme temperatures on the development of **EO** showed that, the predator bug was able to develop 50 % and 90 % from 2<sup>nd</sup> instar to adult stage at the constant temperatures 20°C and 37°C. **EO** was able to survive at these two constant temperatures; however **EO** was unable to lay the eggs at 20°C and even though they could lay the eggs at 37°C, no eggs was able to hatch to nymphs. The 2<sup>nd</sup> instars **EO** survived for 12.6 ( $\pm 0.40$ ) days at 15°C and **EO** did only survive for one day at 40°C and all **EO** died during moulting the stage of the 2<sup>nd</sup> instars to the 3<sup>rd</sup> instar nymphs at both

temperatures. Therefore no viable offspring were recorded in four tested extreme temperatures. Low temperature threshold for **EO** is 15°C and high temperature threshold is 40°C.

The prey consumption by **EO** during its development and adulthood, by feeding on **DBM** as prey, was considerably temperature dependent. 2<sup>nd</sup> instar **EO** nymph consumed about 1/3 larvae per day at 15°C and 40°C while 2 and 6 larvae at 20° and 37°C. The daily prey consumption by **EO** at 37°C increased gradually with **EO** instars, where it was 5.90 ( $\pm$  0.53) **DBM** larvae for 2<sup>nd</sup> instar **EO** nymph and reached up to 23.09 ( $\pm$  0.61) **DBM** larvae for adult **EO** but adult longevity last only 12.67 ( $\pm$  0.58) days even though adult can survive 69.2( $\pm$  1.24) days at 20°C. Until their entire lifespan, one **EO** was able to consume up to 260.00 ( $\pm$  16.56) larvae at 20°C and 388.89 ( $\pm$  8.20) larvae at 37°C. Adult weight of **EO** was significantly affected by temperature and gender; female weight of **EO** was heavier than male weight at 20°C and 37°C. Effects of extreme temperatures can lead to failure of an IPM strategy that may be quite effective in a narrow temperature range (Horn, 1998). Biological control agents, especially arthropod natural enemies, often exhibit temperature optima different from those of their prey, and may become ineffective at higher or lower temperatures.

As a result **EO** seems to be adapted to climatic conditions prevailing in the tropical regions. Given these preferences **EO** may be used for biological control purposes only in areas with mean temperatures of above 25°C.

### **Prey searching and feeding behavior of *Eocanthecona furcellata* Wolff (Hemiptera: Pentatomidae on different prey items, host plant species and plant status**

This study demonstrates the prey species can influence the searching behavior of predator bug. **EO** could not find easily for **DBM** larvae because of their feeding habit on the lower surface of the leaf. On the other hand green colour **DBM** larvae could confuse with leaves colour and dropping off behavior was found when predator approached near the **DBM** larva but **EO** waited maximum 15 minutes



from that place until **DBM** climbing up and then caught the larva. In contrast, only 2-3 minutes waiting time was needed for catching brown colour **ABW** larvae that fed on the upper surface of the wild cabbage. Drop off behaviour of **ABW** larvae was recorded in many studies (Awan 1985; Johnson and Zalucki 2005; Johnson *et. al.* 2007; Terry *et. al.* 1989) but drop off behaviour of **ABW** larvae was not found for in this experiment. EO approaching was studied by Yasuda in 1997, 1998 and 2000, and he prove that EO used 2 different chemical cue (*n*-Pentadecane (C15) as a long-range attractant for bug and (*E*)-phytol as a short distance) as kairomones to locate larvae of several lepidopterans.

The results indicated that searching behavior of predator **EO** was a significant difference between host plants. Searching time of predator **EO** on cotton plants was lower than on wild cabbage plants, even **EO** walked slower on cotton leaves and spent more time on the upper part of the cotton plant but more repeated searching on the whole plants was found on wild cabbage. Many study also proved that predator behavior was influenced by the host plants (Coll *et. al.* 1997; Guershon and Gerling 2006; Grosman *et. al.* 2005; Yang 2000). Prey consumption time of predator **EO** for **ABW** larvae was longer on cotton plants than wild cabbage plants and it may be influenced by the prey's host plants (Bergman and Tingey 1979; Carter *et. al.* 1984; Kareiva and Sahakian 1990; Rapusas *et. al.* 1996). Prey searching time of predator **EO** was only average 1-3 seconds in the Petri dishes but 24 minutes in cotton plant and 37 minutes in wild cabbage plants for each **ABW** larvae and prey consumption time of **EO** on the plants was about 3 times longer than in Petri dished. This result could help to predict the releasing population of predator in the field.

Mechanically damaged plants were more attractive than normal plants of both species but not statistically different between normal and damaged plants. Predator bug **EO** was strongly attracted by insect infested plants and these results are support to the finding of Yasuda (1997) that free phytol produced by insect damaged plants attracted the predatory stink bug *E. furcellata*. These results also consistent with Krips *et. al.* (2001), Takabayashi *et. al.* (1991) and Venzon *et. al.* (1999). They report that insect induced plants were attracted by predator.

Prey selection of EO was studied in the Olfactometer and the results indicate that **EO** was more attractive by **ABW** than **DBM** larvae and the searching time of **EO** was not significantly different on both larvae. It may be helpful for consideration of the surrounding vegetation for releasing **EO** as biocontrol agent to control **ABW** in Myanmar.

Results from these experiments indicated that the predatory bug *E. furcellata* seems to be a good predator to incorporate into an existing biological control program for American bollworm on cotton plant.

## CONCLUSION

1. The highest numbers of *H. armigera* eggs was recorded on chickpea plants, followed by tomato, cotton and cabbage plants.
2. The best performance of *H. armigera* larvae was recorded on artificial diet followed by wild cabbage, cotton, chickpea and tomato plants.
3. American Bollworm reared on cotton plant was the optimal prey for predatory **EO** compare with ABW reared on chickpea plant, wild cabbage plant and tomato plant. **EO** selected **ABW** larvae even larvae were wrapped with Para film 'M'.
4. The optimal temperature for rearing and releasing for predatory bug **EO** was 30°C and the highest predation rate and reliable developmental time of **EO** was found at this temperature.
5. No viable offspring were recorded in four tested extreme temperatures; 15°C, 20°C, 37°C, and 40°C.
6. Prey searching and consumption behaviour of predatory bug **EO** on **ABW** larvae was higher than **DBM** larvae in green house condition
7. Predatory bug **EO** can easily find their prey on cotton plant than wild cabbage plant.
8. Predator bug **EO** was strongly attracted by insect infested plants compare to normal plants and mechanically damaged plants.
9. The result of Olfactometer proved that **EO** was more attractive by **ABW** and the searching time of **EO** was not significantly different on both larvae of **ABW** and **DBM**.

Based on these finding, predatory stink bug *Eocanthecona furcellata* can be used to release the predatory bug *Eocanthecona furcellata* in cotton fields as a biocontrol agent for controlling *Helicoverpa armigera* in Myanmar.

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## SUMMARY

The American Cotton Bollworm (*Helicoverpa armigera* Hübner)(**ABW**) is one of the most serious pests of cultivated crops, especially in cotton in Myanmar. Insecticide applications to control this insect pest are too expensive for smallholder farmers; moreover residues are frequently washed from the plants by continuously light rainfalls in Myanmar. An alternative approach for controlling the herbivorous pests aims at enhancing the abundance of predators, naturally occurring in this crop. A number of promising natural enemies were identified in the cotton agro-ecosystems, and among them the predatory bug *Eocanthecona furcellata* (Wolff)(**EO**) holds potential for controlling the American Cotton Bollworm.

Diamondback moth (*Plutella xylostella* L.)(**DBM**) is most damaging vegetable pest not only in Myanmar than also in most tropical countries, where cabbage plants and ideal temperatures for high **DBM** populations prevail throughout the year. Due to frequent insecticide applications development of resistance towards specific compounds, including *Bacillus thuringiensis*, has been reported in several regions. We wanted to experienced a predatory bug (*Eocanthecona furcellata* Wolff), native to Myanmar and commonly found in the field, for its effectiveness to prey on **DBM**. Therefore, this study aimed at testing the effectiveness of biological control measures by:

1. Testing the effect of host plants on the oviposition preference and larvae performance of American Bollworm *Helicoverpa armigera*

Chickpea, cotton and tomato, and wild cabbage plants were used for ovipositing preference and larvae performance of American Bollworm *Helicoverpa armigera*. The highest numbers of *H. armigera* eggs was recorded on chickpea plants followed by tomato, cotton and cabbage plants. The best performance of *H. armigera* larvae was recorded on artificial diet followed by wild cabbage, cotton, chickpea and tomato plants.

2. Testing the predation efficacy of *E. furcellata* on American Bollworm *Helicoverpa armigera*

Predation efficacy of **EO** was tested with American Bollworm from four different host plants (Cabbage, Cotton, Chickpea and Tomato) and larvae reared on artificial diet. Majority of **EO** (30-60 %) directly approached towards cotton plants eating **ABW** larvae. When **ABW** larvae and their faeces were wrapped with Para film 'M', the prey selecting efficacy was reduced up to 20-40 %.

3. Evaluating the effect of temperature and prey density on the predation efficacy of *E. furcellata*

Developmental time and predation efficacy were tested with three different temperatures and eight prey densities; and four extreme temperatures. Developmental time was significantly shorter in high temperature (35°C) but the highest predation rate of **EO** was found at 30°C. Developmental time increased with increasing prey density but prey consumption percent reduced with increasing prey density. Predation rate increased with nymph instars of **EO** and the highest predation efficacy was found on 5<sup>th</sup> instar **EO** nymph at 30°C. **EO** could not survive at two extreme temperatures (15°C and 40°C) and **EO** was able to survive at these two constant temperatures; however **EO** was unable to lay the eggs at 20°C and even though they could lay the eggs at 37°C, no eggs was able to hatch to nymphs. Therefore no viable offspring were recorded in four tested extreme temperatures.

4. Testing the prey searching and feeding behavior of *Eocanthecona furcellata* under laboratory and greenhouse conditions

Prey searching and feeding behavior of **EO** was tested with two different preys (American bollworm **ABW** and Diamondback moth (**DBM**), two different host plants (cotton plant and wild cabbage plant) and three different conditions (normal plant, wounded plant, and insect infected plant) under laboratory condition and greenhouse conditions. Searching time and prey consumption time of **EO** was significantly lower on **ABW** larvae than on **DBM** larvae. Searching time of **EO** on

ABW larvae on the wild cabbage plant was significantly longer than on cotton plant but prey consumption of **EO** on **ABW** larvae was not significant on both host plants. Visiting time of predatory bug on the insect infested plant was significantly longer than on normal plant and mechanically wounded plant.

5. Observing of host choice behavior of *E. furcellata* towards **ABW** and **DBM** larvae in Olfactometer

Host choice behavior of **EO** was studied in Olfactometer and the attractiveness % of ABW and DBM were 43% and 30%. The searching time of **EO** was not significantly different in both larvae.

Appendix1. Developmental times (days) of *Eocanthecona furcellata* (EO) nymphs offered with eight different prey density and reared at three different temperatures

Tem:	Functions	EO Instar	Prey Density ( DBM larvae)								df	F- ratio	P *
			1	2	3	4	5	6	8	10			
25°C	Develop- mental Time (Days) (mean±SE)	<b>egg</b>	10.84 ± 0.07										
		<b>1st</b>	3.91 ± 0.05										
		<b>2<sup>nd</sup></b>	6.00 ±0.41a	5.25 ±0.25a	5.25 ±0.25a	5.25 ±0.25a	3.50 ±0.29b	2.75 ±0.25b	3.00 ±0.00b	3.00 ±0.00b	7	27.43	0.000
		<b>3<sup>rd</sup></b>	4.00 ±0.58a	4.00 ±0.41a	3.25 ±0.25a	3.25 ±0.48a	4.00 ±0.00a	3.25 ±0.25a	2.75 ±0.25a	3.00 ±0.00a	7	2.143	0.078
		<b>4<sup>th</sup></b>	4.00 ±0.00a	4.00 ±0.00a	4.50 ±0.29a	4.00 ±0.00a	4.25 ±0.25a	3.00 ±0.00b	3.00 ±0.00b	3.00 ±0.00b	7	21.00	0.000
		<b>5<sup>th</sup></b>	6.25 ±0.25a	6.25 ±0.25a	6.50 ±0.29a	6.00 ±0.00a	6.00 ±0.00a	6.00 ±0.00b	7.00 ±0.00b	7.00 ±0.00b	7	12.18	0.000
		<b>Total Life day</b>	35.25 ±0.75a	34.75 ±0.48a	34.00 ±0.00a	33.50 ±0.29a	32.75 ±0.48ab	31.00 ±0.41b	30.75 ±0.25b	31.00 ±0.00b	7	19.18	0.000
30°C	Develop- mental Time (Days) (mean±SE)	<b>egg</b>	7.12± 0.08										
		<b>1st</b>	2.09 ± 0.52										
		<b>2<sup>nd</sup></b>	2.00 ±0.00a	1.75 ±0.25a	1.00 ±0.00ab	1.00 ±0.00ab	1.00 ±0.00ab	1.50 ±0.29a	1.75 ±0.25a	1.75 ±0.25a	7	4.978	0.001
		<b>3<sup>rd</sup></b>	2.25 ±0.25a	2.25 ±0.25a	2.75 ±0.25ab	2.25 ±0.25a	2.50 ±0.29a	1.50 ±0.29a	1.25±0. 25ab	1.25 ±0.25b	7	5.011	0.001
		<b>4<sup>th</sup></b>	4.00 ±0.00a	3.25 ±0.48a	3.00 ±0.41a	2.75 ±0.25a	2.50 ±0.29a	2.50 ±0.29a	2.75 ±0.25a	2.75 ±0.25a	7	2.619	0.037
		<b>5<sup>th</sup></b>	7.00 ±0.41a	6.25 ±0.25a	5.00 ±0.41ab	4.50 ±0.29b	4.25 ±0.25b	4.00 ±0.00b	4.00 ±0.00b	3.50 ±0.29b	7	18.91	0.000
		<b>Total Life day</b>	25.25 ±0.48a	23.50 ±0.29a	21.75 ±0.75ab	20.50 ±0.29b	20.25 ±0.25b	19.50 ±0.29b	19.75 ±0.25b	19.25 ±0.25b	7	29.92	0.000
35°C	Develop - mental Time (Days) (mean±SE)	<b>egg</b>	4.813 ± 0.07										
		<b>1st</b>	1.062 ± 0.04										
		<b>2<sup>nd</sup></b>	1.00 ±0.00a	1.00 ±0.00a	1.00 ±0.00a	1.00 ±0.00a	1.00 ±0.00a	1.00±0. 00a	1.25±0. 25a	1.00 ±0.00a	7	1.000	0.455
		<b>3<sup>rd</sup></b>	2.50 ±0.29a	2.25 ±0.25a	2.00 ±0.41a	2.00 ±0.00a	1.25 ±0.25a	2.00±0. 00a	1.50±0. 29a	1.50 ±0.29a	7	2.637	0.036
		<b>4<sup>th</sup></b>	3.25 ±0.25a	3.00 ±0.41a	2.50 ±0.29a	2.00 ±0.00a	2.00 ±0.00a	2.50±0. 29a	2.75±0. 48a	2.00±0. 00a	7	2.971	0.022
		<b>5<sup>th</sup></b>	6.75 ±0.48a	4.75 ±0.48a	4.25 ±0.25b	3.75 ±0.25b	4.50 ±0.29b	4.75 ±0.25b	4.25 ±0.25b	4.25 ±0.25b	7	7.683	0.000
		<b>Total Life day</b>	19.50 ±0.96a	17.00 ±1.08a	15.75 ±0.48ab	14.75 ±0.25ab	14.75 ±0.25ab	16.25 ±0.25ab	15.75 ±0.63ab	14.75 ±0.25ab	7	6.990	0.000

\* Significance level within the same rows by ANOVA. Means (± SE) in a row followed by the same letters indicate no significant differences and different letters indicate significant differences between prey densities (ANOVA, Bonferroi adjustment)

Appendix 2. Daily prey consumption (DBM larvae) of *Eocanthecona furcellata* (EO) nymphs offered with eight different prey density and reared at three different temperatures

Tem:	Function s of EO	EO Instar	Prey Density ( DBM larvae)								df	F- rati o	P
			1	2	3	4	5	6	8	10			
25°C	Daily prey consumption (DBM larvae)	2 <sup>nd</sup>	0.96 ±0.04a	1.58 ±0.09a	2.46 ±0.21ab	3.18 ±0.18b	4.04 ±0.22b	2.46 ±0.31ab	3.08 ±0.16b	4.08 ±0.16bc	7	34.47	0.00
		3 <sup>rd</sup>	0.95 ±0.05a	1.72 ±0.14a	2.83 ±0.17ab	3.94 ±0.06ab	4.88 ±0.07c	2.31 ±0.14ab	3.54 ±0.33bc	4.25 ±0.44bc	7	37.97	0.00
		4 <sup>th</sup>	1.00 ±0.00a	1.63 ±0.07a	2.80 ±0.14ab	3.25 ±0.14ab	3.91 ±0.32ab	3.92 ±0.34ab	6.00 ±0.62c	6.00 ±0.38c	7	33.02	0.00
		5 <sup>th</sup>	1.00 ±0.00a	1.93 ±0.07b	2.54 ±0.18b	3.29 ±0.22bc	4.00 ±0.15c	5.14 ±0.10d	6.71 ±0.13e	8.46 ±0.27f	7	241.1	0.00
		Mean con.	0.98 ±0.01a	1.74 ±0.03b	2.63 ±0.11b	3.37 ±0.03b	4.19 ±0.10b	3.87 ±0.10c	5.32 ±0.13c	6.39 ±0.18c	7	312.7	0.00
		Total con.	18.25 ±0.25a	31.25 ±0.63b	47.00 ±1.47c	62.25 ±0.85	74.25 ±1.44e	62.00 ±2.42d	83.75 ±2.02f	102.3 ±2.84g	7	259.1	0.00
30°C	Daily prey consumption (DBM larvae)	2 <sup>nd</sup>	1.00 ±0.00a	1.38 ±0.24a	2.25 ±0.48a	3.25 ±0.25ab	3.75 ±0.25ab	5.63 ±0.24c	4.00 ±0.20ab	5.88 ±0.75c	7	24.37	0.00
		3 <sup>rd</sup>	1.00 ±0.00a	1.92 ±0.08a	3.00 ±0.00a	3.63 ±0.24ab	4.33 ±0.30bc	5.50 ±0.50cd	5.13 ±0.52c	5.88 ±0.92d	7	15.99	0.00
		4 <sup>th</sup>	1.00 ±0.00a	2.00 ±0.00a	2.56 ±0.36a	3.92 ±0.08ab	5.00 ±0.00c	5.17 ±0.17c	5.88 ±0.18c	7.54 ±0.83d	7	42.86	0.00
		5 <sup>th</sup>	1.00 ±0.00a	1.88 ±0.08a	2.68 ±0.11ab	3.16 ±0.10bc	4.13 ±0.07d	5.31 ±0.43e	6.50 ±0.20f	9.65 ±0.15g	7	227.2	0.00
		Mean con.	1.00 ±0.00a	1.83 ±0.05a	2.71 ±0.12b	3.47 ±0.06b	4.34 ±0.07c	5.33 ±0.21d	5.70 ±0.17d	7.63 ±0.36e	7	169.5	0.00
		Total con.	15.25 ±0.48a	24.75 ±1.11a	32.00 ±3.19b	36.50 ±1.50bc	44.50 ±1.04cd	50.75 ±3.33de	55.50 ±1.04e	70.50 ±3.18f	7	68.09	0.00
35°C	Daily prey consumption (DBM larvae)	2 <sup>nd</sup>	1.00 ±0.00a	1.25 ±0.25a	3.00 ±0.00b	3.75 ±0.25bc	5.00 ±0.00c	4.75 ±0.48c	6.13 ±0.31d	6.75 ±0.48d	7	52.34	0.00
		3 <sup>rd</sup>	1.00 ±0.00a	2.00 ±0.00a	3.00 ±0.00ab	3.88 ±0.13b	4.75 ±0.25bc	3.38 ±0.24b	5.88 ±0.77d	6.63 ±0.24e	7	36.23	0.00
		4 <sup>th</sup>	1.00 ±0.00a	1.79 ±0.13a	3.00 ±0.00ab	4.00 ±0.00c	5.00 ±0.00d	2.92 ±0.34ab	4.75 ±0.75d	8.50 ±0.29e	7	55.31	0.00
		5 <sup>th</sup>	0.92 ±0.04a	1.70 ±0.12a	2.85 ±0.15ab	3.52 ±0.22bc	4.71 ±0.18c	4.50 ±0.58c	6.36 ±0.39d	7.43 ±0.08d	7	63.81	0.00
		Mean con.	0.96 ±0.02a	1.74 ±0.07b	2.93 ±0.07c	3.74 ±0.07d	4.82 ±0.10e	3.89 ±0.17d	5.68 ±0.21f	7.45 ±0.14g	7	304.7	0.00
		Total con.	13.00 ±1.08a	19.00 ±1.22a	28.50 ±0.87b	32.75 ±1.11b	42.25 ±1.89c	39.75 ±1.25bc	55.25 ±3.47d	65.25 ±2.43e	7	88.88	0.00

\* Significance level within the same rows by ANOVA

Means (± SE) in a row followed by the same letters indicate no significant differences and different letters indicate significant differences between prey densities (ANOVA, Bonferroi adjustment)

Appendix 3. Consumption (%) of *Eocanthecona furcellata* (EO) nymphs fed with eight different prey density and reared at three different temperatures

Tem:	Function s of EO	EO Instar	Prey Density (DBM larvae)								df	F- ratio	P*
			1	2	3	4	5	6	8	10			
25°C	Predation (%)	2 <sup>nd</sup>	96.43 ±3.57a	78.75 ±4.27a	81.94 ±7.03a	79.58 ±4.43a	80.83 ±4.33a	40.97 ±5.24b	38.54 ±1.99b	40.83 ±1.60b	7	28.34	0.000
		3 <sup>rd</sup>	95.00 ±5.00a	86.04 ±7.00a	94.44 ±5.56a	98.44 ±1.56a	97.50 ±1.44a	38.54 ±2.29b	44.27 ±4.11b	42.50 ±4.38b	7	40.05	0.000
		4 <sup>th</sup>	100.0 ±0.00a	81.25 ±3.61a	89.17 ±3.08a	81.25 ±3.61a	78.25 ±6.30a	65.28 ±5.73b	75.00 ±7.80b	60.00 ±3.85b	7	7.111	0.000
		5 <sup>th</sup>	100.0 ±0.00a	96.43 ±3.57a	84.72 ±6.16a	82.29 ±5.48a	80.00 ±3.1ab	85.71 ±1.68a	83.93 ±1.63a	84.64 ±2.70a	7	3.899	0.006
		Mean	97.67 ±1.34a	86.79 ±1.28b	87.72 ±3.72b	84.14 ±0.71b	83.78 ±2.00b	64.57 ±1.64c	66.50 ±1.63c	63.91 ±1.77c	7	42.47	0.000
30°C	Predation (%)	2 <sup>nd</sup>	100.0 ±0.00a	68.75 ±11.9a	75.00 ±15.9a	81.25 ±6.25a	75.00 ±5.00a	93.75 ±3.99a	50.00 ±2.5ab	58.75 ±7.5ab	7	4.112	0.004
		3 <sup>rd</sup>	100.0 ±0.00a	95.83 ±4.17a	100.0 ±0.00a	90.63 ±5.9ab	86.67 ±6.1ab	91.67 ±8.33a	64.06 ±6.44b	65.63 ±4.83b	7	7.057	0.000
		4 <sup>th</sup>	100.0 ±0.00a	100.0 ±0.00a	95.42 ±2.67a	97.92 ±2.08a	100.0 ±0.00a	86.11 ±2.78a	73.43 ±2.31b	75.42 ±8.32b	7	10.80	0.000
		5 <sup>th</sup>	100.0 ±0.00a	93.75 ±3.99a	89.17 ±3.70a	79.06 ±2.5ab	82.50 ±1.4ab	88.54 ±7.09a	81.25 ±2.6ab	96.46 ±1.46a	7	4.733	0.002
		Mean	100.0± 0.00a	91.55 ±2.49a	90.31 ±4.08a	86.82 ±1.4ab	86.86 ±1.3ab	88.80 ±3.51a	71.28 ±2.13c	76.31 ±3.58c	7	11.45	0.000
35°C	Predation (%)	2 <sup>nd</sup>	100.0 ±0.00a	62.50 ±12.5b	100.0 ±0.00a	93.75 ±6.25a	100.0 ±0.00a	79.17 ±7.9ab	76.56 ±3.9ab	65.50 ±4.79b	7	6.416	0.000
		3 <sup>rd</sup>	100.0 ±0.00a	100.0 ±0.00a	100.0 ±0.00a	96.88 ±3.13a	95.00 ±5.00a	56.25 ±3.99b	73.44 ±9.67b	66.25 ±2.39b	7	16.92	0.000
		4 <sup>th</sup>	100.0 ±0.00a	89.58 ±6.25a	100.0 ±0.00a	100.0 ±0.00a	100.0 ±0.00a	48.61 ±5.73b	59.38 ±9.38b	67.00 ±2.89b	7	19.73	0.000
		5 <sup>th</sup>	92.26 ±4.49a	85.00 ±6.12a	95.00 ±5.00a	88.02 ±5.54a	94.25 ±3.61a	75.00 ±9.67a	79.53 ±4.88a	74.25 ±0.75a	7	2.300	0.061
		Mean	96.13 ±2.25a	87.23 ±3.48a	97.73 ±2.27a	93.58 ±1.73a	96.46 ±2.05a	64.77 ±2.78b	71.04 ±2.59b	74.55 ±1.41b	7	30.04	0.000

\* Significance level within the same rows by ANOVA

Means (± SE) in a row followed by the same letters indicate no significant differences and different letters indicate significant differences between prey densities (ANOVA, Bonferroi adjustment)

## Appendix 4

**Potential for biocontrol of the Diamondback Moth in Myanmar by using a predatory bug**

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The Diamondback Moth (**DBM**) is most damaging vegetable pest not only in Myanmar than also in most tropical countries, where cabbage plants and ideal temperatures for high DBM populations prevail throughout the year. Due to frequent insecticide applications development of resistance towards specific compounds, including *Bacillus thuringiensis*, has been reported in several regions. We tested a predatory bug (*Eocanthecona furcellata* – **EO**), native to Myanmar and commonly found in the field, for its effectiveness to prey on **DBM**.

We used 2<sup>nd</sup> instars of **EO** nymphs and 5 different **DBM** larval densities. **DBM** larvae were placed in 9 cm Ø plastic petri dishes and one **EO** nymph was placed in the center of each arena; these were then kept at a constant temperature (30° C, 75% RH and 12:12 L:D) photoperiod in climate cabinets. Larvae consumed per day, larvae still alive and molting date were recorded to adult stage of **EO**.

The maximum prey consumption per day per **EO** larvae was surprisingly high and exceeded 9.65 ( $\pm$  0.29) larvae at 30°C in the 5<sup>th</sup> instar of **EO**. During the whole lifecycle (2<sup>nd</sup> instars to adult), **EO** was able to consume between 25.50  $\pm$  2.89 (minimum) and 70.5  $\pm$  6.35 (maximum) **DBM** larvae. **EO** larvae did feed on different lepidopteran species; however, they refused to feed on aphids.

Base on these preliminary data we recommend that the predatory bug *Eocanthecona furcellata* should be tested under field conditions as a biocontrol agent for controlling diamondback moth in Myanmar. Additional research is now done to understand the host spectrum and the ecology of this species.

**Keywords:** biological control, Diamondback Moth, *Eocanthecona furcellata*, Myanmar, predatory bug,

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# Potential for Biocontrol of Diamondback Moth in Myanmar by using a predatory bug

Khin Thein Nyunt and Stefan Vidal

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Entomology Section, Germany



## Introduction

- Diamondback Moth (DBM) is most damaging vegetable pest not only in Myanmar than also in most tropical countries
- cabbage plants and ideal temperatures for high DBM populations prevail throughout the year
- Due to frequent insecticide applications development of resistance including *B.thuringiensis*

## Hypothesis

- We tested a predator bug (*Eocanthecona furcellata*- EO), native to Myanmar and commonly found in the field, for its effectiveness to prey on Diamondback Moth

## Methods

- ➔ 2nd instars of EO nymphs and 5 different DBM larval densities(2,4,6,8,10) were used for this experiment.
- ➔ DBM larvae were placed in 9 cm Ø plastic petri dishes and one EO nymph was placed in the centre of each arena; these were then kept at a constant temperature (30\_ C, 75% RH and 12:12 L:D) photoperiod in climate cabinets.
- ➔ Larvae consumed per day, larvae still alive and molting date were recorded to adult stage of EO.

## Results

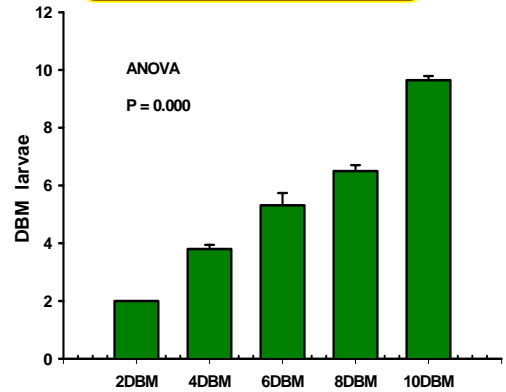


Fig 1. Amount of DBM larvae daily consumed by *E.furcellata*

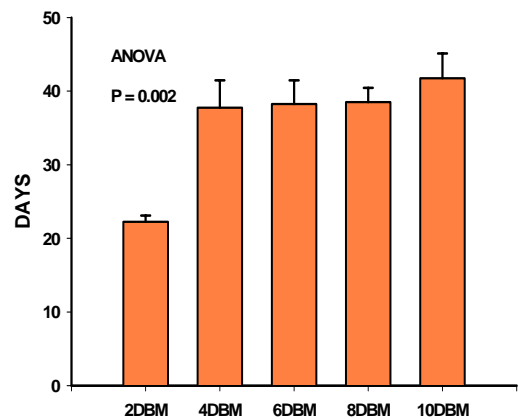


Fig 2. Logevity of *Eocanthecona furcellata*

## Conclusion

Base on these preliminary data we recommend that the predatory bug *Eocanthecona furcellata* should be tested under field conditions as a biocontrol agent for controlling diamondback moth in Myanmar



## Appendix 5

**Predatory potential of the pentatomid stink bug *Eocanthecona furcellata* at different temperature regimes****KHIN THEIN NYUNT, STEFAN VIDAL****Georg-August University, [Department of Crop Sciences](#), Agricultural Entomology Section, Goettingen (Email: [knyunt@gwdg.de](mailto:knyunt@gwdg.de))**

The predatory stink bug *Eocanthecona furcellata* Wolff (Hemiptera: Pentatomidae - **EO**), native to Southeast Asia offers potential to be used as a biological control agent against lepidopteran pests. However, in order to establish mass rearing methods the life history of **EO** has to be evaluated in detail. We investigated the effect of extreme temperature regimes on the development, fecundity and predation rate of the bug using Diamondback Moth larvae (DBM) *Plutella xylostella* L. (Lepidoptera: Yponomeutidae) under laboratory conditions. **EOs** were reared at constant temperatures (15, 20, 37, and 40°C, respectively) in climatic cabinets at 75% RH and 12:12 (L:D) photoperiod. 2<sup>nd</sup> instars of **EO** nymphs were used in the experiments and DBM larvae consumed per day, **EO** larvae still alive, molting date and oviposition dates of adults were recorded.

The longest developmental time of **EO** from egg to adult was obtained at 20°C (116.00 ± 1.14) days, while **EO** did not develop at 40°C. The maximum prey consumption per day per adult **EO** was very high and was on average 23.1 ± 0.6 DBM larvae during the whole lifecycle (2<sup>nd</sup> instars to adult). One individual consumed up to 388.9 ± 8.2 DBM larvae at 37°C. **EO** females deposited on average 14.8 ± 3.2 eggs per batch at 37°C, but these eggs failed to begin embryonic development.

Our data suggest that **EO** is adapted to climatic conditions prevailing in the tropical regions and may be used for biological control purposed only in areas with mean temperatures above 25°C.

**Key words:** biological control, development, extreme temperatures, fecundity

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*Eocanthecona furcellata* (Wolff)

## Introduction

- The predatory stink bug *Eocanthecona furcellata* Wolff (Hemiptera: Pentatomidae – **EO**), native to South-east Asia, offers potential to be used as a biological control agent against lepidopteran pests
- The life history of **EO** has to be evaluated in detail for establishing mass rearing methods

## Hypothesis

- We investigated the effect of extreme temperature regimes on the development, fecundity and predation rate of the bug using Diamondback Moth larvae (**DBM**) *Plutella xylostella* L. (Lepidoptera: Yponomeutidae) under laboratory conditions

## Methods

- We used 2nd instars of **EO** nymphs in the experiments
- DBM larvae were placed in 9 cm Ø Petri-dishes; one **EO** nymph was placed in the centre of each arena; these were then kept at constant temperatures (15, 20, 37, and 40°C, respectively) in climatic cabinets at 75% RH and 12:12 (L:D) photoperiod
- **DBM** larvae consumed per day, **EO** larvae still alive, moulting dates and oviposition dates of the adults were recorded

## Results

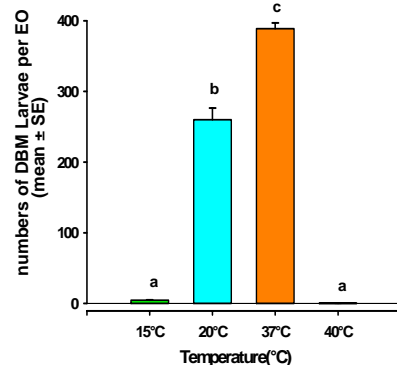


Fig. 1. Effect of temperatur on total prey consumption of EO significantly differed at  $P=0.01$  ( Bonferroni)

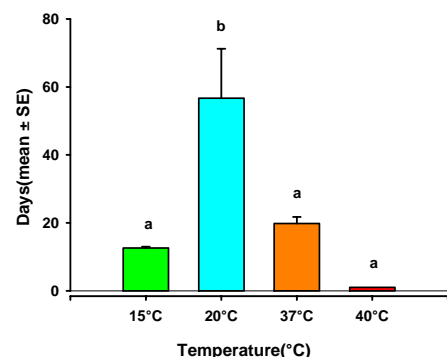


Fig. 2. Effect of temperatur on longevity of EO (from 2<sup>nd</sup> instar to death) significantly differed at  $P=0.01$  ( Bonferroni)

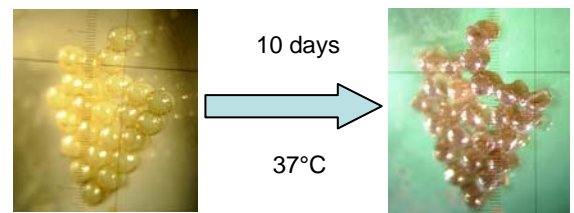


Fig. 3. EO eggs failed to begin embryonic development at 37°C

## Conclusion

- **EO** is unable to produce viable offspring at constant temperatures below 20°C or above 37°C
- **EO** seems to be adapted to climatic conditions prevailing in the tropical regions
- Thus it may be used for biological control purposed only in areas with mean temperatures of above 25°C

## Acknowledgement

Special thanks to the Gottlieb Daimler and Karl Benz Foundation for supporting this work

## Appendix 6

**Predation efficiency of *Eocanthecona furcellata* on *Helicoverpa armigera* larvae reared on different host plants**

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Germany

The predatory pentatomid bug *Eocanthecona furcellata* (Wolff) (**EO**) is regarded a potential biological control agent against lepidopteran pests in Southeast Asia. We investigated the predation efficiency of **EO** with regard to the noctuid *Helicoverpa armigera* (Hübner) (**ABW**), which is a highly polyphagous agricultural pest, especially in cotton, chickpea and tomato in Myanmar. Specifically, we tested the influence of larval feeding reared on different host plants (cotton, cabbage, chickpea and tomato plants) or artificial diet on bug predation.

In each experiment ten males and females **EO** adults were used, which were starved for 24 hours before the experiment. **ABW** larvae were fixed with tape and placed randomly in small plastic boxes before transferring ten **EO** adults to the center of the arena. In a second series **ABW** larvae and their faeces were wrapped with Para film and also tested the same way. Movement of **EO** was recorded at room temperature.

**EO** adults preferred to prey on **ABW** larvae reared on cotton plants (42 %); **ABW** larvae from cabbage, chickpea and tomato plants were accepted less as prey. **ABW** larvae fed on artificial diet were not accepted as prey (1%). 13% of **EO** were not actively searching for hosts; however in the experiment with wrapped **ABW** larvae 38% were not active, and predation on **ABW** larvae from cotton was reduced to 25%. Adding faeces to the larvae did not result in higher predation rates by **EO**.

Base on these data we recommend to release the predatory bug *Eocanthecona furcellata* in cotton fields as a biocontrol agent for controlling *Helicoverpa armigera* in Myanmar.

**Keywords:** biological control agent, cabbage, chickpea, cotton, *Eocanthecona furcellata*, *Helicoverpa armigera*, Myanmar, predatory bug,

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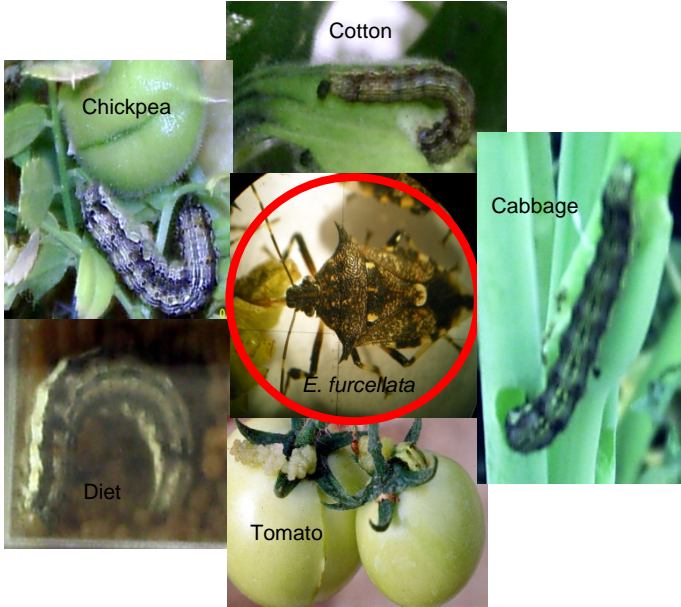
Contact Address: Khin Thein Nyunt, Georg-August-University Göttingen, Department of Crop Sciences, Entomological Section, Grisebachstr.6, 37077 Göttingen, Germany, e-mail: [knyunt@gwdg.de](mailto:knyunt@gwdg.de)

# Predation efficiency of *Eocanthecona furcellata* on *Helicoverpa armigera* larvae reared on different host plants

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## Results

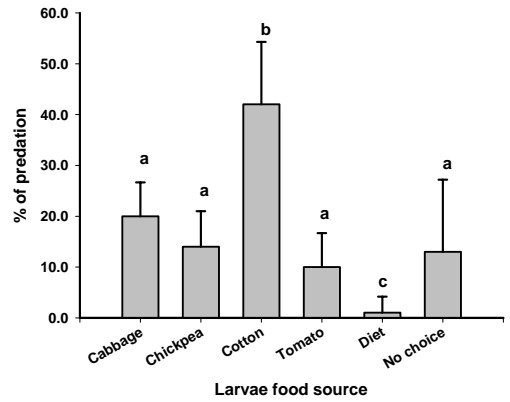


Fig.1 Food choice of *E.furcellata* on the larvae of *H. armigera* (unwrapped) ANOVA, P = 0.01

## Introduction

- The predatory pentatomid bug *Eocanthecona furcellata* (Wolff) (EO) is regarded a potential biological control agent against lepidopteran pests in Southeast Asia
- The noctuid *Helicoverpa armigera* (Hübner) (ABW) is a highly polyphagous agricultural pest, especially in cotton, chickpea and tomato in Myanmar

## Hypothesis

- Moth larval feeding on different host plants (cotton, cabbage chickpea or tomato plants) or an artificial diet will influence acceptance of the larvae by the bug EO

## Methods

- We used ten starved male and female EO adults per experiment
- ABW larvae were fixed with tape and placed randomly in small plastic boxes; ten EO adults were transferred in the centre of each arena
- In a second series ABW larvae and their faeces were wrapped with Para film and tested as mentioned above
- Prey selection behavior of EO adults was recorded at room temperature

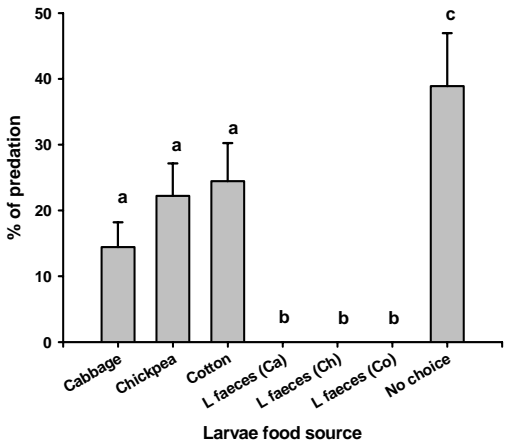


Fig. 2 Food choice of *E.furcellata* on the larvae of *H. armigera* (wrapped) ANOVA, P = 0.01

## Conclusion

- EO adults preferred to prey on ABW larvae reared on cotton plants in both experiments
- Based on these data we recommend to release the predatory bug *Eocanthecona furcellata* in cotton fields as a biocontrol agent for controlling *Helicoverpa armigera* in Myanmar

## Acknowledgement

- Special thanks to the Gottlieb Daimler and Karl Benz Foundation for supporting this work

