THE EFFECT OF INSECTICIDAL RESISTANCE OF DIAMONDBACK MOTH (*Plutella Xylostella*) AS A HOST ON DEVELOPMENT AND SURVIVAL OF ITS ENDOPARASITOID (*Diadegma Semiclausum*)

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ABSTRACT

***Diadegma semiclausum* Hellén (Hymenoptera: Ichneumonidae) is a larval endoparasitoid of *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), a major pest of crucifer crops. The aim of this study was to evaluate the effect of insecticidal tolerance status of this host on this parasitoid. Host larvae from Waite susceptible (WS), emamectin benzoate selected (EBS) and *Bacillus thuringiensis kurstaki* selected (*BtK*S) strains were used in this study. The tolerance in both the EBS and *BtK*S strains is associated with an inducible tolerance mechanism. Since developmental penalties may be associated with inducible tolerance in some insects, we measured developmental parameters of the host reared on free toxin cabbage. We measured the survival, developmental time, sex ratio and head capsule width of *D. semiclausum* reared on different host larvae without toxin. The insecticidal tolerance status of the EBS had no effect on any of the measured parameters. However, for the *BtK*S without toxin the developmental time of the *Diadegma* males was greater than the WS, but the head width for both sexes was not affected Therefore, this study shows the effect host status associated with *BtK*, compared to emamectin benzoate toxin, on developmental time and head capsule width on *D. semiclausum*.**

INTRODUCTION

The diamondback moth (DBM), *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) is one of the most serious, destructive pests of brassica production in tropical and sub-tropical environments worldwide (Furlong *et al.* 2008; Talekar & Shelton 1993; Talekar & Yang 1991).

***P. xylostella* has developed resistance against a wide range of the extensively used insecticides (Li *et al.* 2012; Sarfraz & Keddie 2005; Shelton *et al.* 2000), and it has been reported as the first insect pest that developed resistance to DDT (Ankersmit 1953; Metcalf 1980; Shelton *et al.* 1993). It has also evolved resistance against bio-pesticides such as *Bt* toxin (Tabashnik *et al.* 1990). Therefore, much attention should be applied to the use of Non- chemical approaches, such as biological control agents such as parasitoids to control this pest.**

***Diadegma semiclausum* Hellén (Hymenoptera: Ichneumonidae), is one of the most effective larval parasitoids of *P. xylostella* worldwide (Nakamura & Noda 2001; Talekar & Shelton 1993; Waterhouse & Norris 1987). *D. semiclausum* was introduced from the UK to New Zealand, then to Australia in 1936 (Furlong *et al.* 2004; Robertson 1948). This parasitoid is currently the dominant *P. xylostella* parasitoid in Australia and New Zealand and it plays a significant role in controlling the population of this pest (Wang & Keller 2005). It is considered to be an important factor, along with other control components**

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such as insecticides and bio-pesticides, to keep the population of this pest under the economic threshold level (Zalucki *et al.* 2012; Furlong *et al.* 2008; Furlong & Zalucki 2007). Importantly, several studies reported that *D. semiclausum* as a biological control agent has been used effectively to manage *P. xylostella* (Cameron *et al.* 1997; Tabashnik *et al*. 1990). In recent years, management of pesticide resistance in *P. xylostella* has appeared as one of the major concerns (Tabashnik *et al.* 1994).

IPM program is one of the most effective tactics that has been used in several countries to control pests. This tactic, often based on using biological control agents with compatible insecticides, can provide sustainable management with reliance on biological control and reduced use of broad spectrum pesticides (Amano & Haseeb 2001; Haseeb *et al*. 2000; Shelton *et al.* 2000; Waage 1999). For instance, in the Philippines, parasitoids are used as a main factor in the IPM to control *P. xylostella* effectively (Rejesus 2001, Ibrahim & Kim 2006). IPM practices can delay the development of *P. xylostella* resistance. However, combining a selective chemical control with a biological control agent has been considered as a complex and controversial issue (Devine & Furlong 2007).

We aimed to understand the interaction between the host status of three *P. xylostella* strains (susceptible, emamectin selected and *BtK* selected stains) and their effects on *D. semiclausum*. This will improve our understanding the interaction between the host and its parasitoids and the concept of IPM of this pest. A number of experiments were conducted to determine the influence of insecticidal tolerance of the host *P. xylostella* on development and survival of its endoparasitoid *D. semiclausum*. Insecticide and biopesticide, emamectin benzoate and *Bacillus thuringiensis kurstaki* (*BtK*) toxins, were used in this study.

MATERIALS AND METHODS

Insect populations

A susceptible laboratory population of *P. xylostella* (Waite susceptible strain) (WS) has been maintained on cabbage seeding leaves, *Brassica oleracea* L. Variety *capitata* (Green Coronet), in the laboratory at 24±0.5ºC and a photoperiod of 14:10 hr (L: D) in a separately caged laboratory culture at the South Australian Research and Development Institute (SARDI) Entomology Unit at Waite Campus, South Australia, without exposure to any insecticides for approximately 18 years (≈210 generations). Cabbage seeds were placed in plastic trays (40 cm× 30cm) with 5cm depth of soil. These were transferred to plastic pots (14cm x 11cm) with compost and vermiculite (3:1 ratio). To ensure larvae were constantly available in adequate number for rearing and experiments, extra cabbage leaves were added periodically to the culture when required. A thin layer of honey on masking tape and a 10% honey solution with 0.1% sorbic acid were provided as food source for the adults (Rahman *et al.* 2010). This WS strain was used as the reference strain for comparison with emamectin benzoate selected and *BtK* selected *P. xylostella* strains.

Laboratory-selected emamectin benzoate (EBS) and *Bacillus thuringiensis kurstaki* (*BtK*S) tolerant *P. xylostella* strains were used during this project. In brief, *P. xylostella* collected from the field (Queensland, Australia) during 2006-2007, with a relatively high level of tolerance to emamectin benzoate and no detectable tolerance to *BtK* formulation, was exposed each generation to a sublethal concentration of emamectin benzoate (0.022 mg ai L^{-1}, ~1/1000th of registered rate) and *BtK* formulation (25 µg/ml, ~1/40th of registered rate), and maintained at the same level for five and seven generations (F_5, F_7) respectively.

The emamectin benzoate selecting concentration was doubled to 0.044 mg ai L^{-1} at F_6 and to 0.088 mg ai L^{-1} at F_{13}. The insects from the EBS strain that were used in these experiments were generation 52 (F_{52}). The *Bt*K concentration was doubled to 50µg/ml at F_8, to 100µg/ml at F_{22}, to 1000µg/ml at F_{50}, and to 2000 mgL^{-1} at generation 55 (F_{55}). The insects from the *BtK*S strain that were used in these experiments were generation 56 (F_{56}).

Diadegma semiclausum population

A laboratory population of *Diadegma semiclausum* (Waite strain) was maintained on Waite susceptible *P. xylostella* larvae in the laboratory at 23±2ºC (14:10 hr, L: D photoperiod) in plastic cages (45cm height x 45cm diameter), covered by terylene voile fabric for ventilation. Cabbage plants (6-8 leaf stage) were infested with *P. xylostella* larvae at weekly intervals, to allow staggered emergence of adults wasps to be used in the experiments. Each cage contained approximately 75 to 100 wasps. Parasitoid adults were provided with a food source of 10% honey solution containing 0.1% sorbic acid in a small container fitted with a dental wick (T. Cooper, personal communication). The newly emerged *D. semiclausum* adults were divided into two groups, one for renewing the cultures and the second for the experiments. The wasps (males and females) were kept together for two day to ensure mating.

Insecticides

Commercial formulations of emamectin benzoate (Proclaim® containing 44 g ai./L) and *Bacillus thuringiensis kurstaki* (*BtK*) toxins (Dipel®, a mixture of five bacterial protein toxins Cry1Aa, Cry2Aa, Cry1Ab, Cry2Ab, Cry1Ac, and spores) were obtained from Syngenta Crop Protection (Greenshoro, NC, USA) and Sumitomo Chemical Australia Pty Ltd respectively, and used for the study.

Determination of current levels of tolerance

Dose-response bioassays for the susceptible and selected strains of *P. xylostella* were performed on insecticide-coated leaf discs to determine the current level of tolerance according to the procedures of Rahman *et al.* (2010). In brief, eight-week old washed cabbage leaves from a toxin-free glass house were used for preparing 85mm diameter leaf discs. Agar media was poured into a 90mm diameter Petri dish and, while it was still soft, the leaf discs were placed in each dish facing upwards and gently pressed into the agar. Ten 2nd to 3rd instar *P. xylostella* larvae were randomly placed in each Petri dish. A defined amount of toxin was sprayed in each Petri dish by Potter Spray Tower (PST). The six concentrations of emamectin benzoate (Proclaim®) toxin (0.176, 0.088, 0.044, 0.022, 0.011 and 0.005 mg ai./L^{-1}) plus 220, 110, 22 and 2.2 mg ai./L^{-1} for the selected emamectin benzoate strain. There were 7 concentrations of *Bt*K toxin (500, 250, 125, 50, 25, 12.5 and 5 mg/L^{-1}) for susceptible strain plus 5000, 2500 mg/L^{-1} for the selected *BtK* strain. The concentrations were made by serial dilution using distilled water. MilliQ water was used as a control. Each concentration had four replicates (four Petri dishes per one concentration, 10 larvae per dish). To perform the application, 7 ml of the toxins were applied to the larvae. After the application, the Petri dishes were covered with plastic film secured by a rubber band to keep the larvae inside, with 100 to 150 micro holes for ventilation. The samples were held under controlled conditions in an incubator at 23 °C and 14: 10 hr (L:D photoperiod). The larval mortality rates were assessed after 48 and 72 hr feeding for *Bt*K formulation and emamectin benzoate respectively. The larvae were classified dead if they did not move when

prodded with a brush. The current level of susceptibility was determined for both susceptible and selected *P. xylostella* populations.

Egg size, developmental time and pupa weight of the *Plutella xylostella* different strains

The purpose of this study was to determine egg size, developmental time of the immature stages, and the pupal weight of the WS, EBS and *BtK*S strains. Nine young cabbage plants (6-8 leaves) were grown on black plastic pots were placed and distributed into nine cages. Each cage had 20 pairs of adults (2 days post-emergence) of one of the three strains of *P. xylostella* strains (susceptible, *BtK*S and EBS) which have been taken from the main culture with an aspirator at growth chamber conditions (23±2^{o}C, 14:10 hr, L:D photoperiod). Each treatment was replicated 3X. After 4 hr, the adults were removed from the cages. One cabbage leaf from each plant was removed and 60 eggs sizes were measured using a computer software program (Olympus Soft Imaging Solution GmbH, analySIS® Five) with a dissecting microscope. Other leaves with eggs (30 eggs/plant) were observed daily by using a hand lens and the time of egg hatching, larval and pupal development period of *P. xylostella* was recorded for each replicate group. In addition, the weights of 3 day old pupae were measured by using a Mettler AE 160 scale (Sartorius Inc., Edgewood, NY, USA).

The same method above was repeated with cabbages treated with low concentrations of toxin. 0.0000044 mg ai/L-1 and1 mg/L-1 (EB and *BtK* respectively) were used for WS strain, while 0.0044 mg ai/L-1 and 100 mg/L-1 (EB and *BtK* respectively) were used for EBS and *BtK*S respectively.

Parasitism rate

An experiment was conducted to determine the successful parasitism of *D. semiclausum* with 2nd instar susceptible and EBS *P. xylostella* larvae (5 days old) by using two mated females *D. semiclausum*, which were transferred from the oviposition cage to parasitize 20 *P. xylostella* larvae from each strain. The larvae that had visually been observed to have been stung were dissected under the microscope 4 days after the stinging event. The results indicated that all the dissected larvae had *D. semiclausum* larvae. This provided us with an indicator how we could observe the parasitism visually and how we considered the stung larvae were parasitized.

Effect of host strain (WS, EBS and *BtK*S larvae) on the development time, sex ratio and head capsule width of the parasitoid *D. semiclausum*

Three replicates of the three *P. xylostella* strains were used in this experiment. Each replicate had 20 *P. xylostella* larvae as hosts for the *D. semiclausum*. To obtain stung *P. xylostella* larvae, two mated *D. semiclausum* females (5-7 days old) were placed with 10 larvae (6-8 days old) from each strain in each of the three plastic containers (8 cm high, 7 cm in diameter). There were evidence that parasitism had occurred on visual observation of ovipositional stinging and the response of host larvae. The parasitized larvae were removed directly after stinging to avoid any superparasitism and maintained individually in a cage (35cm high, 25cm in diameter) provisioned with fresh young cabbage plants (6-8 leaves). To avoid any bias, the same wasp adults were used for each replicate of the different *P. xylostella* strains. New cabbage plants were added when necessary to sustain the development of the *P. xylostella* larvae. The developmental time (stinging to adults' emergence), sex ratio and the head

capsule width of the adults of *D. semiclausum* were measured. The latter was measured using the same software program referred to above. The developmental time data were recorded every two hours from the time of first observed emergence on the day for 48 hours. The emergent wasps were directly placed into small labeled tubes (2ml) and frozen at -20ºC for later measurement of the head capsule width. This study was repeated twice.

Statistical analysis

Probit analysis of dose-response data of bioassays was carried out using the POLO program (LeOra Software 1997). Abbotts formula was used to correct the larval mortality rate (Abbot 1925) for each data analysis. To analyse treatment effect on developmental parameters (size, weight and developmental time), analysis of a one-way analysis of variance (ANOVA) was performed.

A chi-square test of independence was used to analyse the *D. semiclausum* survivorship and sex ratio data. General analysis of variance was used to analyse the *D. semiclausum* developmental time and head capsule width data. The statistical program Genstat (14th edition) was used to analyse these data (VSN International Ltd.).

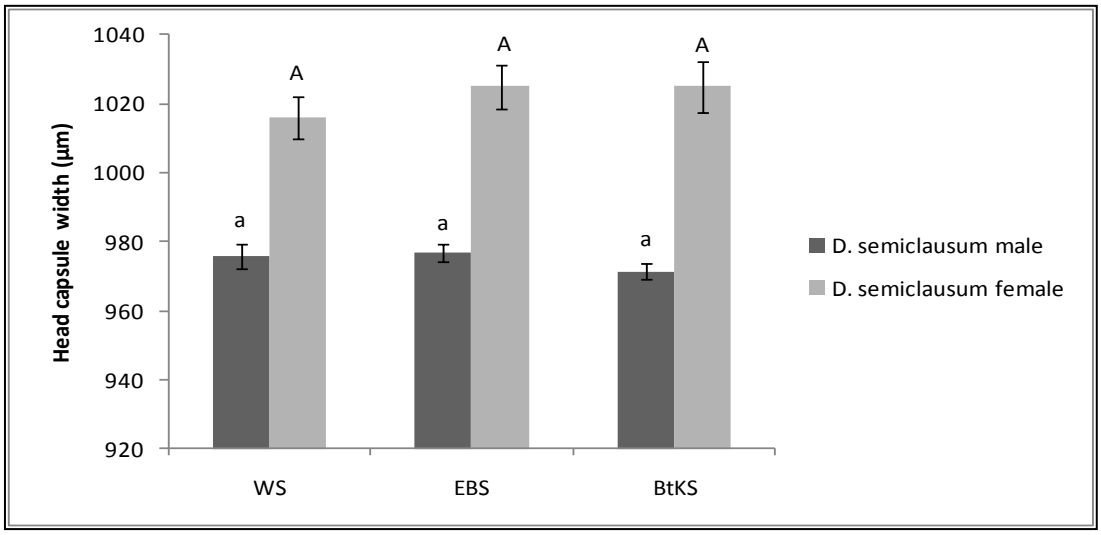

Fig. 6: **The average head capsule width of *D. semiclausum* emerged from three *P. xylostella* strains (susceptible (WS), *BtK* selected (*BtK*S) and emamectin benzoate selected (EBS) fed on toxin-free plants) (n = 60). Susceptible strain was used as a control for comparison. Means (± SE) with the columns followed by the same letters show no significant difference at $p > 0.05$ (general analysis of variance).**

RESULTS AND DISCUSSION

Bioassay experiments

Four bioassays were performed using 2nd to 3rd instar larvae to determine the susceptibility level of susceptible and selected populations of *P. xylostella*. The full dose bioassays showed that the LC_{05} and LC_{50} of the susceptible *P. xylostella* population exposed to emamectin benzoate toxin were 0.0079 and 0.024 mg ai L^{-1} respectively, while the LC_{05} and LC_{50} values of the same population exposed of *BtK* toxin were 4.24 and 25.21 mg/L^{-1} respectively (see table 1). The LC_{05} doses were used to treat the cabbage plant that were used to rear *P. xylostella* parasitised larvae for *BtK* or EBS *P. xylostella*.

Developmental time, egg size and pupal weight of the three *Plutella xylostella* strains

The objective of this experiment was to determine whether there were significant developmental differences between the three *P. xylostella* strains. After analysis, the outcomes revealed that there was no significant difference between the susceptible control (WS) and the selected populations in the developmental time of the egg stage ($p > 0.1$; F = 2.5). Similarly, no statistically significant differences were recorded in the developmental time of the pupal stage between these populations ($p > 0.3$; F = 0.72). However, the larval developmental time of the EBS strain was significantly greater than the susceptible control, and the larval developmental time of the *BtK* selected strain was significantly greater than that of the EBS ($p < 0.001$; F = 24.62) (Fig. 1).

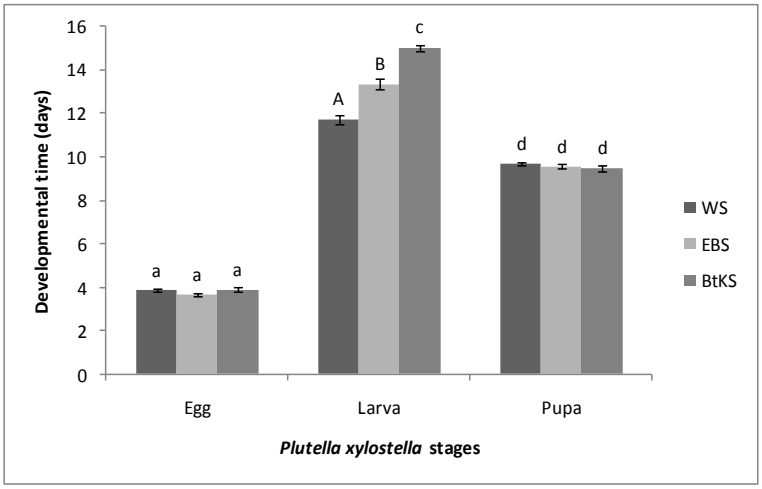

Fig. 1: **Average of development time in days of the immature stages for three *P. xylostella* strains (susceptible (WS), emamectin benzoate selected (EBS) and *BtK* selected (*BtK*S)), under growth chamber conditions (23 ± 2 °C). Means (± SE) with columns followed by the same letters show no significant difference according to one- way ANOVA at $p > 0.05$. The data presented in this figure are the average of three replicates and the bars represent standard errors of means.**

In terms of 2hr observation, when the larvae of the three *P. xylostella* strains reared on toxin free cabbages, the developmental time of *P. xylostella* larvae was longer in both EBS and *BtK*S compared to the WS ($P < 0.03$; F = 4.44, $P < 0.001$; F = 97.84 respectively). However, the developmental time of the WS larvae reared on plants treated with EB (0.0000044 mg ail/L^{-1}) or *BtK* (1 mg/L^{-1}) was shorter than the WS larvae reared on toxin free plants ($p < 0.003$).

Similarly, the larval developmental time of *BtK*S was shorter, when the larvae reared on plants treated with a low concentration of *BtK* (100 mg/L^{-1}) compared to the larvae that reared on toxin free (control) ($p < 0.004$; F = 8.76). (see Fig. 1). The three strains were similar in terms of their egg size ($p > 0.1$; F = 1.86, $P > 0.3$; F = 0.74 respectively). However, the mean egg size of the EBS strain was significantly smaller than that of the *BtK*S *P. xylostella* ($p < 0.03$; F = 4.36) (Fig. 2). In terms of pupal weight, there was no significant difference between EBS *P .xylostella* and the susceptible control ($p > 0.07$; F = 0.09), whereas the pupal weight of the *BtK*S strain was greater than the pupal weight of both the susceptible control and emamectin benzoate selected strain ($p < 0.001$; F = 140.78) (Fig. 3).

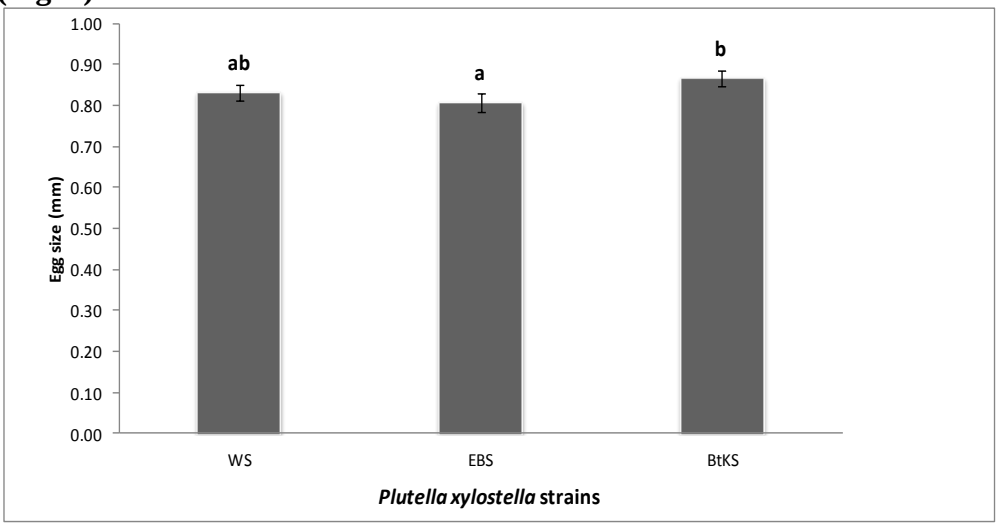

Fig. 2: **The egg size (mm) of susceptible (WS), emamectin benzoate selected (EBS) and *BtK* selected (*BtK*S) *P. xylostella* strains at one day old under growth chamber conditions, (n = 60). Means (± SE) with columns followed by the same letters are not significant according to one- way ANOVA at $p > 0.05$. The data were means of three replicates. The bars are standard errors of means**

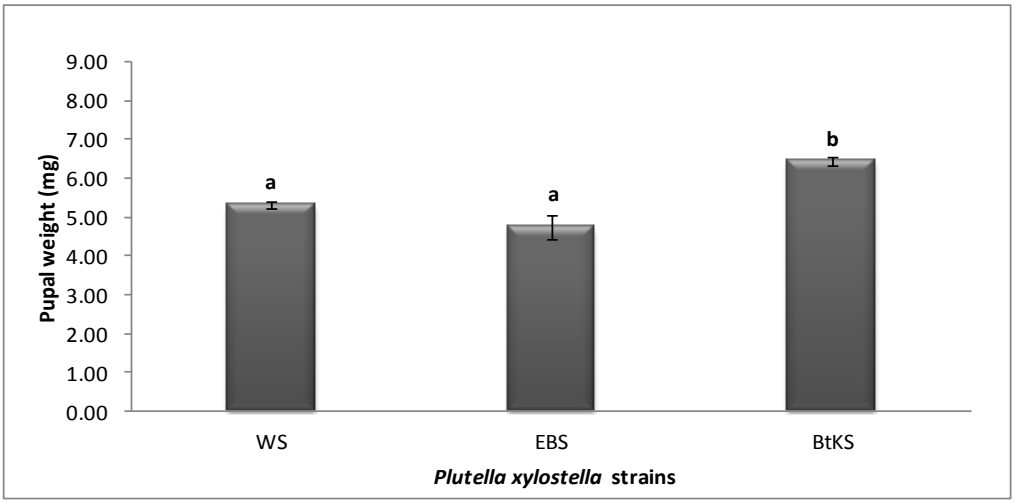

Fig. 3: **The pupal weight of Waite susceptible (WS), emamectin benzoate selected (EBS) and *BtK* selected (*BtK*S) *P. xylostella* strains at three day old under growth chamber conditions, (n = 60). Means (± SE) with columns followed by the same letters are not significant according to one- way ANOVA at $p > 0.05$. The data were means of three replicates. The bars are standard errors of means.**

Survival and developmental time of *Diadegma semiclausum*

There was no significant difference in percentage of the adult *D. semiclausum* that emerged from the stung larval hosts between the control (96.6%), *BtK*S (98.3%) and EBS (95%) strains ($p > 0.6$). Therefore, the survival of the parasitoid was not affected when parasitized larvae fed on untreated cabbage for both selected strains (EBS, *BtK*S) compared to the control (susceptible strain).

Generally, for the given treatment, the developmental time of *D. semiclausum* females was longer than the males, and hence the male emerged earlier than the females. In the first developmental time experiment, in which the emergence of *D. semiclausum* adults was recorded at 24 hr intervals, there was no significant difference in developmental rate of *D. semiclausum* reared on the three different *P. xylostella* strains without toxin ($p>0.2$; F=1.29) (Fig. 4). When this experiment was repeated and the emergence of *D. semiclausum* adults was recorded at 2 hr intervals following the emergence of the first wasps, there were no significant differences in developmental time of the female *D. semiclausum*; however the developmental time of the male *D. semiclausum* reared on the *BtK*S strain was significant longer than those reared on the control and EBS strains ($p < 0.001$; F = 84.27) (Fig. 5).

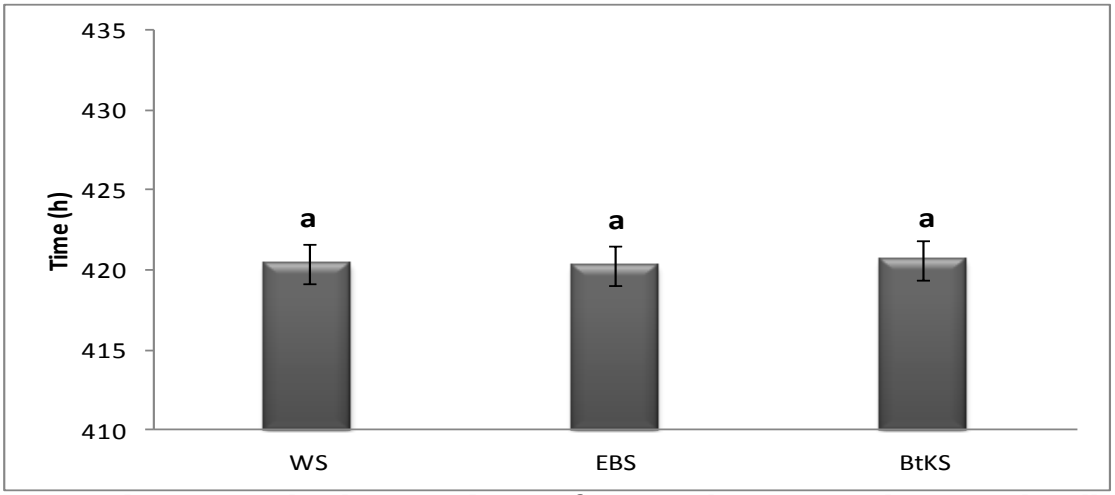

Fig. 4: **The average developmental time of *D. semiclausum* into three *P. xylostella* strains (susceptible (WS), emamectin benzoate selected (EBS) and *BtK* selected (*BtK*S) fed on toxin-free plants) from egg to adults (n= 60). Susceptible strain was used as reference for comparison. Means (± SE) with the columns followed by the same letters show no significant difference at $p > 0.05$ (general analysis of variance). The observations were taken every 24 hr.**

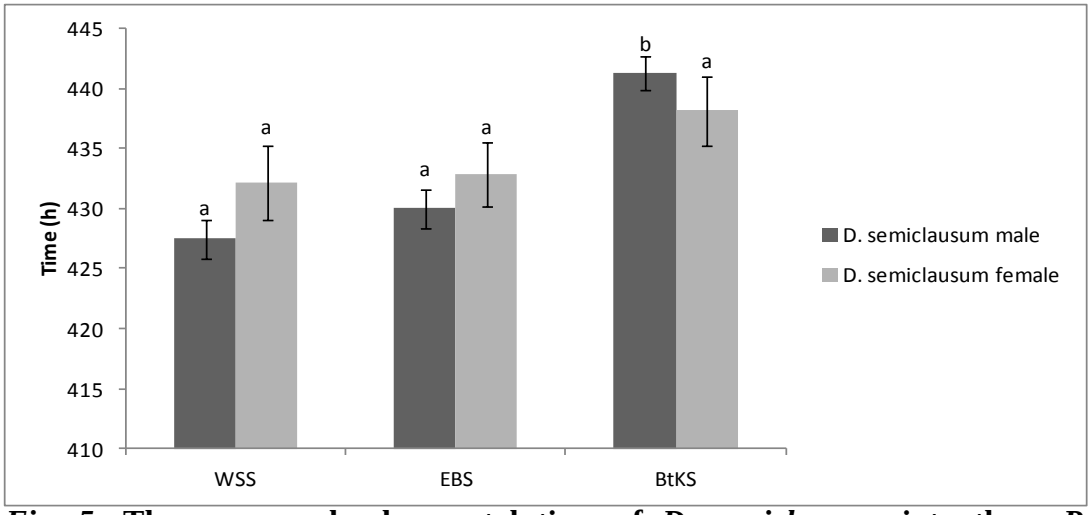

Fig. 5: **The average developmental time of *D. semiclausum* into three *P. xylostella* strains (susceptible (WS), *BtK* selected (*BtK*S) and emamectin benzoate selected (EBS) fed on toxin-free plants) from egg to adults (n = 60). Susceptible strain was used as reference for comparison. Means (± SE) with the columns followed by the same letters show no significant difference according to general analysis of variance at $p > 0.05$ (general analysis of variance). The observations were taken every 2 hr.**

Width Head capsule of *Diadegma semiclausum* adults

The head capsule widths of males and females were analysed individually since males were generally smaller than females. Irrespective of sex, no significant difference was recorded in the width of the head capsules among the *D. semiclausum* adults emerged from the three *P. xylostella* strains when the hosts were reared or fed on toxin free condition ($p > 0.22$; F = 1.59) (Fig. 6).

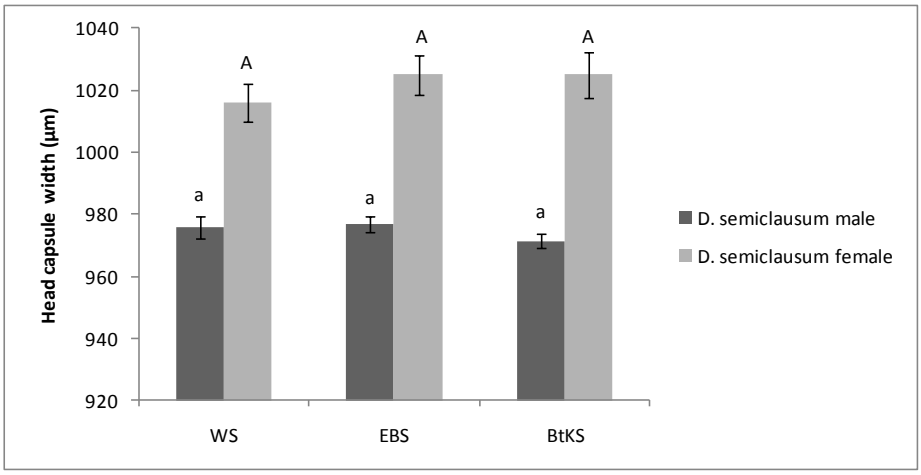

Fig. 6*:* The average head capsule width of *D. semiclausum* emerged from three *P. xylostella* strains (susceptible (WS), *BtK* selected (*BtK*S) and emamectin benzoate selected (EBS) fed on toxin-free plants) (n = 60). Susceptible strain was used as a control for comparison. Means (± SE) with the columns followed by the same letters show no significant difference at $p > 0.05$ (general analysis of variance).

Sex ratio of *Diadegma semiclausum*

Generally, the sex ratio of the *D. semiclausum* progeny was male dominated in all replicates of treatments compared to the susceptible control treatment, the proportion was approximately 75% males. There is no statistically

significant difference in sex ratio between any of the treatments compared to the susceptible control ($p > 0.6$). Table (2) provides a summary of the sex ratio of *D. semiclausum* that emerged from three *P. xylostella* strains when the larvae were reared on toxin free condition.

Providing sustainable methods based on IPM practices to control *P. xylostella* requires better understanding of the interaction between the different components of the IPM system. Understanding the interaction between insecticidal treatment and biological control agents is particularly important, including the effect of host's insecticidal tolerance status on their parasitoids' survival and development. In this study, we investigated the effect on the parasitoid *Diadegma semiclausum* of tolerance to the chemical pesticide emamectin benzoate and a commercial formulation of *Bacillus thuringiensis kurstaki* (*BtK*) in the host *P. xylostella*. Rahman *et al.* (2010) and Rahman and Baker (unpublished data) have reported that the emamectin benzoate (EBS) and *BtK* selected (*BtK*S) strains respectively used in this study have novel inducible mechanisms contributing to the observed tolerance.

When the three *P. xylostella* strains [susceptible (WS), EBS and *BtK*S] were reared on toxin free cabbages, no difference was found in the egg development period between these three strains. Similarly, no difference was recorded in the developmental time of the pupal stage between the EBS and *BtK*S strains compared to WS control. However, the developmental time of *P. xylostella* larvae was longer ($p < 0.001$) in *BtK*S strain compared to both the WS and EBS strains. Likewise, the time required to develop EBS larvae was significantly longer ($p < 0.001$) than the WS strain. Also, the egg size and pupal weight of the *BtK*S strain was significantly greater than those of the EBS and WS strains. The results of developmental time for the susceptible immature stages were similar to what was reported by Ahmad *et al.* (2008) and Campos (2008), who recorded the time of development for each stage of *P. xylostella* on cabbage crop.

These results demonstrate that there are fitness effects as a result of the insecticidal tolerance of the EBS and *BtK*S strains, but these effects were substantially broader in the *BtKS* strain. Since the EBS strain is at a higher level of the tolerance than the *BtK*S strain (respective RRs at the LC_{50} of 165 and 78 fold) (table 1), it is anticipated that this population may have already overcome the fitness cost associated with the tolerance. The main question in this study was to determine whether the EBS and the *BtK*S strains do effect the development of *D. semiclausum* differently.

Table 1 **: Toxicity to emamectin benzoate (EB) and a commercial formulation of *Bacillus thuringiensis kurstaki* (*BtK*) against 2nd to early 3 instar larvae of Waite susceptible (WS), EB selected (EBS) and *BtK* selected (*BtK*S) laboratory *P. xylostella* strains. N) the number of larvae used in each bioassay**

Insect strains	LC_{05}	95%CL	RR*	LC_{50}	95%CL	RR	Slope+/-S.E	N
Bioassays with emamectin benzoate (mg aiL^{-1})								
WS	0.0079	0.004-0.011	1	0.024	0.01-0.03	1	3.43+/-0.41	280
EBS	0.66	0.308-1.042	8.4	3.96	2.86-5.32	165	2.08+/-0.21	400
Bioassays with commercial formulation of *BtK* (mg L^{-1})								
WS	4.24	2.43-6.23	1	25.21	20.0-31.2	1	2.12+/-0.22	320
*BtK*S	96.6	28.95-191.6	22.8	1978.5	1199.1-4021.7	78.4	1.25+/-0.13	400

***Resistance ratio.**

***Table 2*: The survival and sex ratio of *D. semiclausum* emerged from Waite susceptible (WS), emamectin benzoate selected (EB) and *Bacillus thuringiensis kurstaki* selected (*BtK*S) laboratory *P. xylostella* host reared on cabbages. The data presented here are from the average of three replicates (n=60). For a particular strain, the means in a column followed by the same letters show no significant difference ($p > 0.05$) (Chi-square test of independence)**

Insect strain	Treatment	Survival (%)	Female/ Male
WS	Control	96.6a	1: 3.1a
	EB	87a	1: 2.9a
	BtK	86.3a	1: 3.2a
EBS	Control	96.6a	1: 2.7a
	EB	95.3a	1: 2.8a
*BtK*S	Control	93.3a	1: 3.3a
	BtK	89a	1: 3.2a

Generally, the number of *D. semiclausum* male emerged from the hosts was larger than the females, but the head capsule width of the *D. semiclausum* females was greater than the male. Our results were similar to those reported by Yang *et al.* 1993 and Gols *et al.* 2009, who investigated that the *D. semiclausum* sex ratio was found to be biased and head capsule width of the male was smaller than the female emerged from the same *P. xylostella* host.

When parasitized larvae of the three strains were fed on free-toxin cabbage plants, the only *D. semiclausum* parameters measured that was affected by the treatments was the developmental time of the males, which was significantly greater with the *BtK*S strain than the WS and EBS strains. There was no significant difference in survivorship, head capsule width of adults, sex ratio or female developmental time between the WS control, EBS and *BtK*S strains In contrast, the endoparasitoid *Venturia canescens* which developed in *Bt* tolerant flour moths, *Ephestia kuehniella* in the absence of toxin had significantly longer developmental time and larger head capsule widths compared to the *Bt* susceptible *Ephestia kuehniella* host, although there were no difference in the emergence rate of the adult parasitoids from the two host types (Rahman *et al.* 2004).

Although the results reported by Schuler *et al.* (2004) were inconclusive, they found in one experiment that male *Cotesia plutellae* parasitoid was significantly smaller on the *Bt* plants compared to non-*Bt* plants on resistant *P. xylostella* hosts, but the female progeny did not differ between *Bt* or non-*Bt* plants. In addition, Schuler *et al.* (2004) indicated that the C. *plutellae* parasitoids which developed to maturity in *Bt*-resistant *P. xylostella* hosts fed on *Bt* or non-*Bt* plants exhibited no effects on time of emergence from the host or percentage survivorship. Although the *P. xylostella* used in our study exhibited a completely different tolerance mechanism to that of the *P. xylostella* in the Schuler *et al.* (2004) study, it is of interest to observe a similar developmental disadvantage of both parasitoid species in these two studies.

In conclusion, our results indicated that the insecticides resistance host status and its associated fitness effect can influence *D. semiclausum* developmental performance, but these effects are clearly depended upon the type of the host tolerance, as evidenced by the observed differences in response to the *BtK*S versus EB host tolerance status. However, these experiments were all conducted under optimal laboratory conditions. Therefore, it would be useful to

complement these studies with i) further, experiments conducted under sub-optimal to extreme temperature condition, as environmental stressors may influence the parasitoid's response to host tolerance and toxin exposure, and ii) multiple doses to determine dose-dependency of these response. Further, this study precluded several potential parasitoid effects of host tolerance and toxin exposure, namely female fecundity and ovipositional choice/preference; these potential effects require further investigation.

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تأثير صفة المقاومة للمبيدات لحشرة العثة ذات الظهر الماسي في تطور وحياتية متطفل اليرقات *Diadegma semiclausum*

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الملخص

يعد المتطفل *Daidegma semiclasusum* (Hymenoptera: Ichneumonidae) من أهم المتطفلات على يرقة العثة ذات الظهر الماسي *Plutella xylostella* (Lepidoptera : Plutellidae)، التي تعد من الآفات الاقتصادية على محصول اللهانة. الهدف الأساس من هذا البحث هو دراسة تاثيرالمقاومة التي تكتسبها الآفة نتيجة الاستخدام المتكرر للمبيدات الكيميائية والإحيائية على هذا المتطفل. استخدمت في هذه الدراسة ثلاث سلالات للحشرة ذات الظهر الماسي وهي السلالة الحساسة و السلالة المقاومة للمبيد الكيميائي Emamectin benzoate والمبيد الحيوي *Bacillus thuringiensis* (*BtK*) حيث إن حالة المقاومة هي حالة مرتبطة ارتباطا وثيقا مع ميكانيكية المقاومة التي تتولد وتتطور في بعض الآفات الحشرية نتيجة الاستخدام غير الرشيد للمبيدات. وبما إن هذه الصفة مرتبطة بسايكولوجية الحشرة والتي قد تحدث تغيرات في فسيولوجية هذه الآفة وكذلك فان المتطفل يقضي أو يكمل دورة حياته داخل الطور اليرقي لهذه الحشرة، لذلك فقد تم دراسة بعض الخصائص المورفولجية للعائل الذي يمتلك صفة المقاومة لتراكيز معينة من المبيدين الكيميائي والحيوي، وكذلك دراسة بعض الخواص المورفولوجية للمتطفل مثل فترة التطور والنسبة الجنسية وكذلك حجم المتطفل بالإضافة إلى دراسة التأثير على حياتية المتطفل الذي يكمل دورة حياته داخل العائل الذي يمتلك صفة المقاومة. أظهرت النتائج بان حالة المقاومة للعائل بالمبيد الكيميائي ليس لها تأثير على حياتية وصفات المتطفل، بينما أظهرت النتائج بان حالة المقاومة للعائل مع المبيد الحيوي فقط أثرت على تطور ذكور المتطفل بالمقارنة مع السلالة الحساسة فقط دون التأثير على حياتية المتطفل. وبالتالي فأن هذه الدراسة تبين تأثير حالة المقاومة بالمبيد الحيوي *Bt* بالمقارنة مع المبيد الكيميائي على حياتية وتطور متطفل *D. semiclausum*.

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