LOWER THRESHOLD OF DEVELOPMENT AND THERMAL REQUIRMENTS OF COTTON LEAFWORM, *SPODOPTERA LITTORALIS* (LEPIDOPTERA: NOCTUIDAE) FROM EMAMECTIN BENZOATE- RESISTANT AND -SUSCEPTIPLE COLONIES

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Abstract

Lower threshold of insect development and thermal requirements were estimated in the laboratory at five constant temperature regimes (15-35ºC). The two biological parameters were established for different developmental stages of cotton leafworm, *Spodoptera littoralis* from emamectin benzoate-susceptible and resistant strains. From this study, the lower threshold of development was only species dependent (T_0 ranged from 9.1ºC (for larval stage) and the greatest was 11.77 ºC (for adult stage)). In contrast, thermal requirements were mostly colony, stage, sex and temperature dependent. Larval stage, pupal stage, generation time and life cycle for individuals from the resistant colony required greater thermal units than those from the susceptible colony and the difference was highly significant. Statistically, the greatest thermal requirements were for the larval stage followed by the pupal stage and the least were for the development of egg stage. In general, life cycle required significantly greater heat constant than insect generation. Thermal requirements were highly significant greater for oviposition period than for the two other physiological periods of adult female longevity. Thermal units were greater for female pupae (ranged from 144.97 d-d at 15ºC to 166.51 d-d at 20ºC) than those for male pupae (129.49 d-d at 15ºC to 157.41 d-d at 20ºC), however this variation was significant at 15 ºC and was insignificant at 20ºC. For adult females, thermal units were insignificantly greater (ranged from 95.70 at 15ºC to 146.78 at 25ºC) than those for adult males (ranged from 88.38 at 15ºC to 141.3ºC at 25ºC). For each of the two tested colonies, thermal requirements for most of the developmental stages were significantly different between the five constant temperatures and the minimum requirements of heat were mostly at 15ºC, however the maximum fluctuated between 20, 25 and 30ºC based on the tested age or stage. It could be concluded that the developing resistance to emamectin benzoate in the field is possible when cotton leafworm larvae expose to any of avermectin insecticides that share the same mode of action. As a result, avermectin resistant colonies start their activity normally as susceptible colony; however the heat requirements to complete the life cycle will be greater due to the elongation of life cycle for resistant colony.

Introduction

Emamectin is prepared as a salt with benzoic acid "emamectin benzoate" (Waddy et al, 2007). It works, like other avermectins, as a chloride channel activator by binding gamma amino butyric acid (GABA) receptors and glutamate-gated chloride channels disrupting nerve signals within arthropods (Grant, 2002). The stronger binding of GABA increases the cells permeability to chloride ions and neurotransmission is thereby reduced by subsequent hyperpolarization and the elimination of signal transduction (Rodríguez et al, 2007 and Andersch et al, 2011).

Emamectin benzoate could be used in pesticide rotation to reduce the development of resistance and to minimize the impacts on environment. Also the residue profile is very favorable, leading to a very low maximum residue level and short pre-harvest interval in all edible crops (Ishtiaq and Saleem, 2011).

In Egypt, cotton leafworm, *Spodoptera littoralis,* is one of the key pests of cotton and other field and vegetable crops (soybean, corn, tomato, and sugar beet). One of the recent recommended insecticides in controlling this insect species is emamectin benzoate. Because of the quick degradation of this product, there is a chance for insects to be exposed to sub lethal concentrations which raises the possibility of developing resistance.

Because insects are exothermic (“cold-blooded”) and require a consistent amount of heat accumulation to reach certain life stages, degree day values are an important component of an Integrated Pest Management program, providing a cost effective tool to reduce insect feeding damage. Resistant insect species may need more heat accumulation (Murray 2008). The sum of effective temperatures, that is, day degrees above the lower developmental threshold control the development of ectotherms, are used in phenology models to predict time at which the development of individual stages of a species will be completed (Jarosík et al. (2011). Insecticides that are applied based on a calendar date often result in poor insect control and a waste of resources. Insect activity varies from year to year depending on weather. Estimation of thermal units is important to choose the suitable time to insecticide treatments based on accurate weather data can be obtained, using degree days for the

optimum time treatments is more reliable than a calendar date. So the objective of this study is to compare those two biological parameters (lower threshold of development and thermal units) between emamectin benzoate-susceptible and - resistant strains in relation to insect ages, stages, sex and temperature ranged from 15 to 35^{o}C.

Materials and methods

Insect Rearing

Experiments were run with susceptible and emamectin benzoate resistant colonies in Heraeus® (Model BK 500) incubators fitted with timing devices automatically adjusted to set up the required period of light (electric switch-timer) and equipped with fluorescent lamp (32 watt) providing the light. Five different temperature regimes were tested on different biological aspects of the two colonies. Temperature regimes processes from 15°C to 35°C in 5°C increments during the whole period of experiments under continuous complete darkness and 60-70% R.H. The fluctuation of each temperature was approximately ± 0.5°C.

Five egg masses of 0-12 hr. old from each of the two tested colonies were incubated at the previously mentioned temperature regimes, observed daily for hatching. Date of egg hatching for each egg mass was recorded, and then neonates in the first day of hatching were carefully transferred individually into plastic vials provided with a piece of fresh castor-oil plant leaves (*Ricinus communis*) as a food supply and then covered with a pore lid for air refreshment. Larvae were observed daily to change the food supply and to change the vial if necessary.

Date of pupation was recorded, then pupae transferred to clean vials and observed for adult emergence. Dates of emergence and sex of emerged adults were also recorded. Just after emergence five couples of male and female from the same age were placed in glass jar of half kilogram capacity, provided with a small branch of Tafla plant (*Nerium oleander*) and a piece of cotton soaked in 10% sugar solution. Female and male were observed daily to record the pre-oviposition, oviposition and post-oviposition periods.

T_0 Estimation

Records for duration of different stages, total life cycle and generation time were used to estimate the lower threshold of insect development (T_0) as follows: Duration of each developmental stage (y) in days was plotted against temperature (T) in degree centigrade and the relationship is hyperbolic as commonly observed in many insect species (Peairs, 1914; Bean, 1961 and Miyashita, 1971). Multiplying the reciprocal for time (1/y) in days by 100 is plotted against temperature (T) in degree centigrade. The values on the ordinate (100/y) represent the average percentage of development made by the stage per day at the given temperature. Therefore, the distribution of the points indicates the course of temperature-velocity curve (Davidson, 1944). The values of the average percentage of development in one day which are presented within normal zone of development are fitted to straight line by method of least square (regression line). Theoretically, the point where the velocity line crosses the temperature axis is the threshold of development in degree centigrade (°C) (X- intercept method).

D-D_S Calculation

The degree days required to complete the development of each age or stage were determined according to the equation of thermal summation (Blunk, 1914) as follows: $K= (T-t_0)\ y$ where y= Developmental duration in days; T= Temperature in degree centigrade; t_0= lower threshold temperature of development, in degree centigrade and K= thermal units (degree-days).

Statistical analysis

For each colony, data were compared among ages and stages using analysis of variance followed by Duncan Multiple Comparison Range test ($LSR_{0.05}$). At each developmental stage, data were compared between resistant and susceptible colony using unpaired t-test. For each colony at each developmental stage, the effect of temperature on thermal requirements was compared based on ANOVA followed by the least significant difference ($LSD_{0.05}$).

Results and Discussion

Lower Developmental Threshold (T_0)

In this study, the upper threshold of insect development was not calculated because the range of temperature from 15 to 35^{o}C is within the optimum zone of insect activity and development. This means that the lower developmental threshold is below 15^{o}C and the upper is over 35^{o}C. The lower developmental thresholds (baseline) for both susceptible and resistant colonies of the cotton leafworm seemed to be a genetic trait. For each of the two tested colonies, the lower thresholds of zero development were insignificantly different (Table

1) between all tested stages (based on Duncan Multiple Comparison test followed by the $LSR_{0.05}$). The least value of zero development was (9.1ºC for larval stage) and the greatest was (11.77ºC for adult stage). However, this range of variation was not significant. Moreover, the variations in the lower threshold of developments for each tested stage were insignificantly different between the two colonies (based on the unpaired *t*- test, $P_{0.05}$).

In the current study, lower developmental thresholds seemed to be species dependent since different colonies and different stages from this insect species start their development at approximately the same temperature regime. In the present study the upper threshold of development was not estimated. Previous study by Murray (2008) confirmed our finding, she mentioned that the lower and upper thresholds vary among insect species and some insect have no upper developmental. Calculating the lower threshold of development gave idea about the suitable temperature for starting activity for all stages. Approximately, similar results were obtained in previous study with the same insect species by Younis (1992) who found that the lower temperature thresholds for egg, larval, pre-pupal, pupal and adult stages were 12.1, 12.7, 10.9, 11.0 and 13.0 ºC, respectively. Statistically, the previous author did not statistically analyze the obtained data; however his data numerically confirmed that the range of variations between different stages was very limited. In another study with the same insect species, El-Malki (2000) confirmed that eggs of cotton leafworm did not hatch at 10 and 37.5 ºC. In the present study, the development of cotton leafworm was evaluated at a range of temperature from 15 to 35 and this range was suitable for egg hatching. El-Malki (2000) confirmed what recently established in the present study, he estimated the lower threshold temperatures of embryonic, larval, pupal, preoviposition, oviposition and generation to be 11.81, 12.50, 11.33, 10.66, 10.80 and 12.60 ºC, respectively. Previous study by Doerr *et al.* (2002) with another insect species, *Lacanobia subjuncta*, from the same family (Noctuidae), revealed that the lower developmental thresholds for eggs, larvae, and pupae were very much lower than those for cotton leafworm; they were 6.6, 6.7, and 4.9 ºC, respectively. This confirms that insect species varied in the lower temperature at which insect start the activity. The significant variations among insect species in the lower and upper threshold of development were also confirmed by Murray (2008). More recent study (Kandil, 2013) with another insect species (*Earias insulana*) from Noctuidae reveals that the thresholds of development (T_o) were very much greater than what established in the recent study with cotton leafworm. The estimations in previous study (Kandil, 2013) were 15.95, 14.41, 13.32, 17.63 and 12.85 ºC for eggs, larvae, pupae, pre- oviposition and generation period, respectively.

Thermal requirements in relation to tested age or stage at different temperature regimes

Each insect species requires a consistent amount of heat accumulation to reach certain life stage which called thermal requirements or thermal units or degree-days. For each developmental stage, thermal units are based on the rearing temperature, the lower threshold for starting the activity and the time was taken to complete the development. The calculation of thermal units for each age or stage is based on the lower threshold of development, the rearing temperature and the time is taken to complete the development. As previously established (Table 1), the lower threshold of development is species dependent and it seems to be a genetic trait. As a result the quantity of constant heat for completing any developmental stage is essential depend on the duration of this stage and the dominant temperature. Generally, as duration increases, thermal requirements increases and as a result, thermal requirements are age or stage dependent. At each temperature regime, thermal requirements were compared among different developmental stages based on the least significant range at 5% level of probability ($LSR_{0.05}$). Different developmental stages from each of the two colonies revealed approximately similar pattern with different values.

For explanation, at 15ºC (Table 2), developmental stages were significantly different in their day degrees requirements ($LSR_{0.05}$ for susceptible and resistant colony = 11.67 and 12.13, respectively). Statistically they arranged in ascending order based on their requirements of heat as follows: preoviposition period (19.09-20.63 d-d) = Postoviposition period (23.05-29.66) < egg stage (47.23-55.50) = oviposition period (48.02-49.04) < adult longevity (77.66-92.02) < pupal stage (137.39-165.14) < larval stage (218.11-249.51) < generation time (422.53-476.50) < life cycle (492.48-539.58 d-d). Regardless the tested colony and rearing temperature, life cycle requires significantly greater heat constant than insect generation. At this temperature regime, individuals from resistant colony mostly require greater thermal units than those from susceptible colony and the difference was highly significant during larval stage, pupal stage, generation time and life cycle. The significant variation between susceptible and resistant colony in heat requirements is only duration dependent.

Similarly to the finding at 15ºC, thermal requirements at 20ºC were age or stage dependent for each of susceptible and resistant colony ($LSR_{0.05}$ = 16.97 and 17.54, respectively, Table 2). However the pattern of arrangement was very little different as follows: preoviposition period (33.52-33.94 d-d) = postoviposition period (31.88-38.30) < oviposition period (55.78-59.28) ≤ egg stage (61.27-66.50) < adult longevity (116.86-

123.16) < pupal stage (162.03-186.18) < larval stage (265.96-288.52) < generation time (518.43-564.83) < life cycle (606.84-650.51d-d).

At 25ºC (Table 3), thermal requirements for different developmental stages were significantly different ($LSR_{0.05}$ = 13.82 and 14.32 for susceptible and resistant colony, respectively). The statistical arrangement of those developmental stages based on their requirements of accumulative heat are in descending order as follows: postoviposition period (29.46-31.06 d-d) ≤ preoviposition period (35.93-68.02) ≤ egg stage (51.79-57.75) ≤ oviposition period (73.06-82.78) < adult longevity (144.03-162.36) = pupal stage (149.26-164.69) < larval stage (211.41-245.97) < generation time (446.68-534.55) < life cycle (554.89-630.41 d-d).
At 30ºC (Table 3), the same pattern with little variation was achieved ($LSR_{0.05}$ = 10.34 and 14.32 for susceptible and resistant colony, respectively). Developmental stages (susceptible–resistant colonies) were arranged in descending order, based on thermal units as follows: preoviposition period (30.11-32.54) = postoviposition period (29.42-30.59) < egg stage (60.32-67.50) = oviposition period (68.75-69.12) < adult stage (124.15-125.19) < pupal stage (143.07-162.91) < larval stage (216.32-234.71) < generation time (447.96-493.64) < life cycle (542.93-587.33 d-d for susceptible and resistant colony, respectively).

At 35 ºC (Table 4), developmental stages from both colonies were significantly different in their thermal requirements ($LSR_{0.05}$ = 15.46 and 16.21 for susceptible and resistant colony, respectively). They arranged in descending order as follows: preoviposition period (28.58-32.31 d-d) = postoviposition period (28.07-33.77) < egg stage (53.54-59.38) = oviposition period (55.48-55.73) < adult stage (108.24-114.02) < pupal stage (151.45-175.36) < larval stage (237.91-270.39) < generation time (468.29-532.19) < life cycle (553.41-608.85 d-d). It is obvious that, among all immature stages, larval stage required the greatest accumulative heat to complete the development to pupal stage followed by pupal stage and the least requirements of heat was at egg stage.

Thermal requirements in relation to incubated temperatures

For each age or stage, from each of the two tested colonies, thermal units were calculated and statistically compared ($LSD_{0.05}$, Table 4) between the five temperature regimes used in this study. Regardless the tested colony, with the exception of egg stage, pre-oviposition period, and post oviposition period; thermal units for the rest of developmental stages were significantly different between different temperature regimes. It seems that the minimum degrees-day was at the lowest tested temperature (15ºC) and the maximum thermal units fluctuated between 20, 25 and 30ºC depending on the tested stage and colony. This means that there was no systematic change in thermal requirements in relation to temperature regimes and the pattern is differed according tested stage and colony (Table 4). Non-systematic variations among different temperature regimes could be explained based on the negative correlation between dominant temperature and insect duration and both are used in degrees day calculation. For each age or stage from each colony, thermal requirements were compared in relation to different temperature regimes.

For egg stage from each of the two colonies, thermal requirements did not significantly differ at the five constant temperature regimes. They ranged from 47.23d-d at 15ºC to 61.27d-d at 20ºC) for susceptible colony compared to 55.5 d-d at 15ºC to 67.5d-d at 30ºC for resistant colony. It seems that thermal requirements are slightly greater for resistant colony than those of susceptible colony; however the difference was insignificant in relation to tested temperatures (ANOVA, Table 4).

For larval stage, the differences in thermal requirements at the five constant temperature regimes were significant ($LSD_{0.05}$ = 12.51 for susceptible colony and 10.76 for resistant colony). The greatest thermal requirements were at 20ºC (265.96 and 288.52 d-d for susceptible and resistant colony, respectively). The two colonies are different regarding the lowest thermal requirements. For susceptible colony, the lowest thermal units were numerically at 25ºC and were statistically at 15, 25 and 30ºC (211.41-218.11d-d). Heat requirements for larval stage from resistant colony were statistically the lowest at 30ºC (234.71 d-d). Generally, heat requirements were greater for larval stage from resistant colony than those from susceptible one at all temperature regimes.

Regarding susceptible colony, thermal requirements for each of female pupae, male pupae and both sex pupae significantly differed at different temperature regimes ($LSD_{0.05}$ = 6.59, 4.82 and 3.89, respectively). The maximum requirements of accumulative heat were at 20ºC (166.51, 157.41 and 162.03d-d, respectively) and the minimum requirements were at 15ºC (144.97, 129.49 and 137.39d-d, respectively). Similarly to the findings with susceptible colony, however with great values, thermal requirements for the development of female pupae, male pupae and both sexes from resistant colony are also temperature dependent ($LSD_{0.05}$ = 5.12, 5.38 and 4.80, respectively). For female pupae from the resistant colony, the maximum thermal requirements were at 20

(192.14d-d), followed by that at 35ºC (182.16d-d) and the minimum was statistically at 25ºC (168.99d-d) and 30ºC (165.66d-d). For male pupae, the maximum was at 20ºC (182.82d-d) and the minimum was statistically at 15ºC (161.62d-d), 25ºC (161.71d-d) and 30ºC (161.08d-d). For both sex pupae, the maximum was at 20ºC (186.18d-d) and the minimum was at 15 ºC (165.14d-d), 25 ºC (164.69d-d) and 30 ºC (162.91d-d). At all temperature tested and for both of the two tested colony, thermal units was greater for female pupae than those for male pupae; however, the variation between male and female pupae was significant at 15ºC and was insignificant at 20ºC ($LSR_{0.05}$ = 11.67 and 16.97 at 15ºC and 20ºC, respectively).

For adult stage (female, male and both sexes) from susceptible colony, thermal requirements were significantly different in relation to rearing temperatures ($LSD_{0.05}$ = 13.88, 11.16 and 11.65, respectively). The greatest thermal units were at 25ºC (146.8, 141.3 and 144.03d-d, respectively) and the lowest were at 15ºC (95.70, 88.38 and 92.02 d-d, respectively). Similarly with great values, thermal requirements for the development of adult female, adult male and both sexes from resistant colony are temperature dependent ($LSD_{0.05}$ = 10.67, 9.56 and 9.71, respectively). The maximum thermal units for adult female, adult male and both sexes were estimated at 25ºC (168.73, 156.11 and 162.36d-d, respectively) followed by those at 30ºC (128.04, 120.32 and 124.15 d-d, respectively). The least thermal units were estimated at 15ºC (85.03, 70.40 and 77.66d-d, respectively). For adult female from susceptible colony, thermal units were insignificantly greater (ranged from 95.70 at 15ºC to 146.78 at 25ºC) than those for adult male (ranged from 88.38 at 15ºC to 141.3ºC at 25ºC).

Female longevity was divided to three physiological periods (preoviposition, oviposition and postoviposition periods). For female from susceptible colony, thermal requirements for preoviposition period and postoviposition period did not significantly differ at the five constant temperatures (Table 4). Thermal constant for preoviposition period ranged from 20.63d-d at 15ºC to 35.93 d-d at 25ºC compared to somewhat greater estimation for postoviposition period (ranged from 29.66 d-d at 15ºC to 38.30 d-d at 20ºC). The greatest estimation of thermal requirements was for oviposition period and the differences at the five temperature regimes were significant ($LSD_{0.05}$ = 10.89). Statistically, the greatest thermal requirements for oviposition period of females from susceptible colony were estimated at 25ºC (82.78 d-d) and the lowest were statistically estimated at 15ºC, 20ºC and 35ºC (49.04, 55.78 and 55.73 d-d). Dissimilarly to what established with susceptible colony, the differences in thermal requirements were significant ($LSD_{0.05}$ = 6.07) for preoviposition period of females from resistant colony; the greatest thermal units were at 25ºC (68.02 d-d) and the lowest were at 15ºC (19.09d-d). Dissimilarly to the finding with susceptible colony, thermal requirements for oviposition period of females from the resistant colony were statistically dissimilar ($LSD_{0.05}$ = 11.35) at different temperature regimes, the maximum was statistically at 25 and 30ºC (73.06 and 68.75 d-d, respectively) and the minimum was at 15, 20 and 35ºC (48.02, 59.28 and 55.48d-d, respectively). Regardless the tested colony and temperatures, thermal units for pre and post oviposition period were insignificantly different, however was significantly greater for oviposition period. It seems that thermal requirement was very much greater for oviposition period than that for pre-oviposition period, particularly at 15ºC, probably because it is the longest physiological period compare to pre and post oviposition period.

Thermal requirements for the life cycle of susceptible colony were significantly different among the five constant temperatures ($LSD_{0.05}$ = 23.48). Statistically, the greatest estimation was at 20ºC (606.84 d-d) and the lowest was at 15ºC (492.48 d-d). However, they did not significantly differ between 25, 30 and 35ºC (554.89, 542.93 and 553.41 d-d, respectively). The pattern for generation time of the same colony was very little different, thermal requirements were statistically different among the five temperature regimes ($LSD_{0.05}$ = 18.21). However, the greatest thermal units were at 20ºC (518.43d-d) followed by that at 35ºC (468.29 d-d). The lowest thermal requirements were at 15ºC (422.53 d-d). For thermal units at 25 and 30ºC, the difference was insignificant (446.68 and 447.96 d-d, respectively). Similarly to what estimated with susceptible colony, life cycle for resistant colony required more accumulative heat than generation time. Also, there is a significant variation in mean thermal units among temperatures ($LSD_{0.05}$ = 19.19 and 16.09 for life cycle and generation time respectively). Compatible with the finding with susceptible strain, however with great values, the maximum thermal units was at 20ºC (650.51 and 564.83 d-d, respectively) and the minimum was at 15ºC (539.58 and 476.50 d-d, respectively). It seems that there is no systematic change in relation to temperature regimes. For generation time and life cycle, respectively, the statistical decrease in thermal units was as follows: (564.83 and 650.51 d-d) at 20ºC > (534.55 and 630.41 d-d) at 25ºC > (532.19 and 608.85 d-d) at 35ºC > (493.64 and 587.33 d-d) at 30ºC > (476.50 and 539.58 d-d) at 15ºC. Finally, at the range of 15ºC to 35ºC, thermal units were significantly greater for insect life cycle (492.48-606.84) than those for insect generation (422.53-518.43d-d). These data could be summarized as follows: in general, regardless the tested age or stage and the tested colony, thermal requirements were greater at 20ºC compared to those at 15 ºC. Approximately similar pattern was achieved when the two colonies were compared. Thermal requirements numerically

decreased at 25ºC than those at 20ºC for egg stage, larval stage, pupal stage, postoviposition period, life cycle period and generation time. In contrast, thermal requirements increased at 25ºC for adult stage, preoviposition and oviposition period. Thermal requirement were mostly decreased at 30ºC than those at 25ºC. There were few exceptions such as egg stage, larval stage from susceptible colony, and generation time of susceptible colony. Thermal requirements increased at 35ºC compared to those at 30 for larval stage, pupal stage, life cycle and generation time. The contrast was evident for the rest of development stages.

Thermal requirements at each age or stage in relation to tested colony

At each temperature regime, thermal requirements for each age or stage were compared between susceptible and resistant colony using unpaired t-test. At 15ºC, the difference between the two colonies was insignificant for egg stage, adult female, adult male, adult both sexes, preoviposition period, oviposition period and postoviposition period. In contrast, thermal requirements for larval stage, female pupae, male pupae, both sex pupae, life cycle and generation time from resistant colony were highly significant greater than those from susceptible strain.

The pattern was very little different at 20ºC, thermal requirements at each of egg stage, female adult, adult both sexes, and the three physiological period of adult female did not significantly differ between susceptible and resistant colony. However, the rest of insect developmental periods (larval stage, female pupae, male pupae, both sex pupae, male adult, life cycle and generation time from resistant colony required greater degree days than those from susceptible colony and the differences were highly significant.

When the thermal requirements of two colonies at each developmental stage were compared at 25ºC, the difference was insignificant for egg stage and postoviposition period; however was highly significant for the rests of developmental stages. Thermal requirements at larval stage, pupal stage, adult stage, preoviposition period, life cycle period and generation time was highly significant greater for resistant colony than those for susceptible colony. Dissimilarly, thermal requirements for oviposition period were highly significant greater for susceptible colony compared to that from resistant strain.

At 30ºC, resistant strain required greater thermal units at larval stage, female pupae, male pupae, both sex pupae, life cycle and generation time when compared with those from susceptible strain and the difference was highly significant. Contrary for adult female, adult male, adult both sexes, preoviposition period, oviposition period, the difference was insignificant.

At 35ºC, larval stage, female pupae, male pupae, both sex pupae, life cycle and generation time from resistant colony required very much greater thermal units than those from susceptible colony, moreover, the difference was highly significant. For the rest of developmental stages, their day degrees requirements did not significantly differ between the two colonies.

In summary, thermal requirements for egg stage was insignificant between the two tested colonies at any of temperature regime. In contrast, thermal requirements for male and female adults were significantly different between the two colonies at 15 and 25ºC. At the three other temperatures, the difference was insignificant. For the three physiological periods of adult females from resistant and susceptible colony, the difference was only significant with oviposition period at 25ºC. for larval stage, pupal stage, life cycle and generation time, the different in thermal requirements between susceptible and resistant colony was highly significant at all temperature regimes.

To our knowledge and based on literature searching, there is no available literature regarding the comparison of the lower threshold of development and thermal requirements between susceptible and resistant colonies of any insect species toward any insecticide. The only available literatures were to compare these parameters in relation to constant and fluctuating temperatures and different host plants. In the present study, when the three immature stages were compared, the greatest accumulative heat was for larval stage followed by pupal stage and the least was for egg stage. Mean accumulative heat for larval stage from susceptible colony was 211.41d-d at 25ºC compared to 214.06d-d in the previous study by Younis (1992). He estimated the thermal requirements at 25ºC for egg, larval, pupal and adult stages from the same insect species to be 44.17, 214.06, 143.19 and 180.23 day-degrees, respectively. In previous study conducted by Doerr *et al.* (2002) with another insect species (*Lacanobia subjuncta*), however from the same family, thermal requirements for this insect species were very much greater than those for cotton leafworm, *Spodoptera littoralis*. They estimated the required degree-days to complete egg, larval and pupal stages to be 75, 476, and 312, respectively. In another study with another species from Pyralidae family, *Diaphania indica* (Saunders), Kinjo and Arakaki (2002) confirmed that the T_o of this species was very much higher than that for cotton leafworm. The authors estimated the development

thresholds to be 22.43, 18.37 and 16.63 ºC for eggs, larvae and pupae, respectively. The corresponding thermal constants were 36.17, 178.0 and 147.55 degree-day (DD), respectively and a total of 351.72 DD was required for the development from egg to adult in the laboratory. It seems from the previous study that there is a negative correlation between T_0 and D-Ds; for the study by Kinjo and Arakaki (2002), the threshold of development was greater for *Diaphania indica* than what established in our study with *Spodoptera littoralis* in the current study and as a result thermal requirements for *Diaphania indica* was very much lower than that for cotton leafworm. In another study by Bartekova and Praslicka (2006) with *Helicoverpa armigera*, the lower thermal threshold for the development of eggs, larvae and pupae was 14.8, 11.3 and 8.2ºC, respectively and the corresponding thermal constant was 64.1, 344.8 and 222.2 d-d, respectively. The lower thermal threshold for total development of *H. armigera* was 11.5 ºC and the thermal constant was 625.0 day-degrees. It seems that the thermal requirements for *H. armigera* were more close to those of *S. littoralis*. In more recent study conducted by Kandil (2013) with *Earias insulana* from field strain, the thresholds of development (T_o) were 15.95, 14.41, 13.32, 17.63 and 12.85ºC for eggs, larvae, pupae, pre- oviposition and generation period, respectively and the corresponding thermal units were 23.15, 131.33, 90.79, 20.83 and 346.19D-Ds, respectively. With another insect species, *Athetis lepigone* (Moschler) (Lepidoptera: Noctuidae), Li-Tao *et al.* (2013) determined the effect of temperature (18, 21, 24, 27 and 30ºC) on the growth, development and fecundity. Approximately 95% of mature larvae stopped pupating at 18 ºC, and about 70% of mature larvae stopped pupating at 21ºC. When the growth chamber temperature was above 24 ºC, no growth arrest was observed.

Table 1. Lower threshold of development (t_0 expressed as ºC) calculated for different developmental ages and stages from susceptible (SS) and emamectin benzoate resistant (RS) strain of cotton leafworm, *Spodoptera littoralis* (Boisd.).

Cotton leafworm ages and stages	Lower threshold of development (Mean ± SD)		Unpaired t-test
	SS	RS	$P_{0.05}$, $P_{0.01}$
Egg stage	11.72 ± 0.19	11.25 ± 0.24	0.22, 0.45
Larval stage	9.14 ± 0.18	9.10 ± 0.22	0.35, 0.70
Female pupae	10.25 ± 0.16	10.40 ± 0.25	0.46, 0.92
Male pupae	10.74 ± 0.11	10.67 ± 0.19	0.42, 0.84
Both sex pupae	10.49 ± 0.35	10.59 ± 0.27	0.39, 0.79
Adult female	10.61 ± 0.23	11.17 ± 0.76	0.11, 0.21
Adult male	10.87 ± 0.37	11.77 ± 0.43	0.19, 0.39
Both sex adults	10.74 ± 0.16	11.47 ± 0.26	0.24, 0.48
Preoviposition period	11.18 ± 0.33	11.92 ± 0.21	0.34, 0.68
Oviposition period	9.67 ± 0.23	9.78 ± 0.29	0.45, 0.89
Postoviposition period	10.88 ± 0.65	11.61 ± 0.54	0.44, 0.88
Life cycle	10.25 ± 0.48	10.37 ± 0.69	0.36, 0.71
Generation time	10.17 ± 0.42	10.27 ± 0.67	0.35, 0.69
$LSR_{0.05}$	NS	NS	---

Table 2. Thermal units (day degrees) required for the successive stages of susceptible and resistant cotton leafworm, *Spodoptera littorals* reared for one generation at 15 and 20°C.

Age or stage	15°C		Unpaired t-test	20°C		Unpaired t-test
	SS	SR	P 0.05, P 0.01	SS	SR	P 0.05, P 0.01
Egg stage	47.23 ± 2.93G	55.5 ± 3.14B	0.15, 0.29	61.27 ± 4.54F	66.5 ± 4.79F	0.057, 0.11
Larval stage	218.11 ± 0.92C	249.51 ± 1.23C	1.32 E-14, 2.63 E-14	265.96 ± 3.24C	288.52 ± 4.65C	4.36 E-09, 8.73 E-09
female pupae	144.97 ± 1.35D	172.81 ± 1.19D	7.03 E-21, 1.41 E-20	166.51 ± 4.90D	192.14 ± 7.58D	2.29 E-08, 4.57 E-08
male pupae	129.49 ± 0.90E	161.62 ± 0.95E	1.84 E-24, 3.69 E-24	157.41 ± 4.74D	182.82 ± 9.16D	1.77 E-07, 3.53 E-07
Both sex pupae	137.39 ± 1.01DE	165.14 ± 0.71DE	1.05 E-24, 2.11 E-24	162.03 ± 1.74D	186.18 ± 2.71D	7.08 E-16, 1.42 E-15
Female adult	95.70 ± 3.67F	85.03 ± 4.37F	0.00033, 0.00066	125.83 ± 0.71E	121.85 ± 7.39E	0.26, 0.51
Male adult	88.38 ± 2.26F	70.40 ± 2.70G	7.25 E-07, 1.45 E-06	120.52 ± 11.9E	111.93 ± 7.36E	0.10; 0.21
Both sex adult	92.02 ± 2.98F	77.66 ± 3.35FG	3.00 E-10, 5.99 E-10	123.16 ± 0.74E	116.86 ± 7.02E	0.069, 0.14
Preoviposition	20.63 ± 2.09H	19.09 ± 2.58I	0.17, 0.33	33.52 ± 7.38G	33.94 ± 6.76G	0.46, 0.92
Oviposition period	49.04 ± 4.45G	48.02 ± 4.37H	0.36, 0.72	55.78 ± 5.66F	59.28 ± 11.19G	0.28, 0.55
Postoviposition	29.66 ± 1.84H	23.05 ± 2.84I	0.34, 0.68	38.30 ± 7.63G	31.88 ± 3.75G	0.24, 0.48
Life cycle	492.48 ± 7.20A	539.58 ± 5.27A	1.1 E-06, 2.2 E-06	606.84 ± 8.16A	650.51 ± 8.61A	1.54E-05, 3.08E-05
Generation time	422.53 ± 5.51B	476.50 ± 3.96B	4.62E-08, 9.24E-08	518.43 ± 8.79B	564.83 ± 8.14B	7.97E-06, 1.59E-05
LSR 0.05	11.67	12.13	---	16.97	17.54	

For each stage, at each temperature degree, p values expressed the statistical variation between susceptible and resistant colony.
For each column, data followed by the same letter are not significantly different (based on the value of $LSR_{0.05}$).

Table 3. Thermal units (day degrees) required for the successive stages of susceptible and resistant cotton leafworm, *Spodoptera littorals* reared for one generation at 25 and 30ºC.

Age or stage	25ºC		Unpaired t-test	30ºC		Unpaired t-test
	SS	SR	$P_{0.05}$, $P_{0.01}$	SS	SR	$P_{0.05}$, $P_{0.01}$
Egg stage	51.79 ± 7.27F	57.75 ± 11.50F	0.18, 0.36	60.32 ± 15.29H	67.5 ± 12.22F	0.18, 0.36
Larval stage	211.41 ± 2.82C	245.97 ± 3.49C	4.22 E-14, 8.44 E-14	216.32 ± 3.34C	234.71 ± 6.89C	1.51 E-11, 3.02 E-11
female pupae	153.37 ± 3.83D	168.99 ± 1.86D	4.35 E-10, 8.7 E-10	148.52 ± 5.60D	165.66 ± 5.81D	4.21 E-09, 8.43 E-09
male pupae	145.83 ± 4.93D	161.71 ± 2.23D	2.53 E-08, 5.06 E-08	137.93 ± 2.88EF	161.08 ± 4.02D	5.5 E-10, 1.1 E-09
Both sex pupae	149.26 ± 3.63D	164.69 ± 1.81D	7.63 E-11, 1.53 E-10	143.07 ± 2.89DE	162.91 ± 4.53D	3.46 E-12, 6.92 E-12
Female adult	146.78 ± 2.04D	168.73 ± 11.57D	0.0013, 0.0026	127.97 ± 10.62FG	128.04 ± 15.75E	0.40, 0.80
Male adult	141.30 ± 9.99D	156.11 ± 11.83D	3.05E-13, 6.1E-13	122.43 ± 10.47G	120.32 ± 9.98E	0.38, 0.75
Both sex adult	144.03 ± 11.25D	162.36 ± 11.14D	3.12E-12, 6.24E-12	125.19 ± 8.12G	124.15 ± 12.51E	0.19, 0.38
Preoviposition period	35.93 ± 7.57G	68.02 ± 10.94EF	1.17E-08, 2.33E-08	30.11 ± 10.31I	32.54 ± 8.09G	0.31, 0.63
Oviposition period	82.78 ± 8.39E	73.06 ± 6.81E	0.0022, 0.0045	69.12 ± 11.14H	68.75 ± 11.07F	0.49, 0.97
Postoviposition	31.06 ± 11.81G	29.46 ± 11.20G	0.065, 0.13	30.59 ± 10.67I	29.42 ± 10.07G	0.30, 0.61
Life cycle	554.89 ± 16.81A	630.41 ± 12.24A	7.91E-13, 1.58E-12	542.93 ± 17.66A	587.33 ± 16.42A	9.09E-21, 1.82E-20
Generation time	446.68 ± 14.83B	534.55 ± 8.07B	1.01E-06, 2.03E-06	447.96 ± 17.74B	493.64 ± 16.51B	1.82E-16, 3.64E-16
$LSR_{0.05}$	13.82	14.32		10.34	14.32	

For each column, data followed by the same letter are not significantly different (based on the value of $LSR_{0.05}$).

Table 4. Thermal units (day degrees) required for the successive stages of susceptible and resistant cotton leafworm, *Spodoptera littorals* reared for one generation at 35ºC and the LSD values for susceptible and resistant colonies at the five temperature regimes.

Age or stage	35ºC		Unpaired t-test	$LSD_{0.05}$ in relation to temperature regimes (15-20-25-30-35ºC)	
	SS	SR	$P_{0.05}$, $P_{0.01}$	SS	SR
Egg stage	53.54 ± 11.54F	59.38 ± 8.39F	0.22, 0.44	NS	NS
Larval stage	237.91 ± 3.52C	270.39 ± 6.04C	6.43 E-22, 1.29 E-21	12.51 (c-a-c-c-b)	10.76 (c-a-c-d-b)
female pupae	156.99 ± 4.61D	182.16 ± 6.45D	1.17 E-06, 2.34 E-06	6.59 (d-a-bc-cd-b)	5.12 (c-a-cd-d-b)
male pupae	145.92 ± 4.75D	171.77 ± 5.16D	8.1 E-12, 1.62 E-11	4.82 (d-a-b-c-b)	5.38 (c-a-c-c-b)
Pupae both genitors	151.45 ± 2.68D	175.36 ± 4.21D	2.14 E-10, 4.27 E-10	3.89 (d-a-b-c-b)	4.8 (c-a-c-c-b)
Adult female	117.07 ± 20.41E	114.38 ± 10.66E	0.49, 0.99	13.88 (c-b-a-b-b)	10.67 (d-bc-a-b-c)
Adult male	110.99 ± 13.22E	102.21 ± 12.72E	0.34, 0.69	11.16 (d-bc-a-b-c)	9.56 (d-bc-a-b-c)
Adult both genitors	114.02 ± 16.37E	108.24 ± 12.15E	0.41, 0.81	11.65 (d-bc-a-b-c)	9.71(d-bc-a-b-c)
Preoviposition	28.58 ± 10.65G	32.31 ± 12.64G	0.46, 0.68	NS	6.07 (c-b-a-b-b)
Oviposition period	55.73 ± 11.33F	55.48 ± 11.28F	0.24, 0.48	10.89 (c-c-a-b-c)	11.35 (c-bc-a-ab-c)
Postoviposition	33.77 ± 21.57G	28.07 ± 10.46G	0.24, 0.48	NS	NS
Life cycle	553.41 ± 20.71A	608.85 ± 61.82A	5.33E-12, 1.07E-11	23.48 (c-a-b-b-b)	19.19 (e-a-b-d-c)
Generation time	468.29 ± 14.59B	532.19 ± 28.19B	1.07E-20, 2.14E-20	18.21 (d-a-c-c-b)	16.09 (e-a-b-d-c)
$LSR_{0.05}$	15.46	16.21		….	….

For each stage, at each temperature degree, p values expressed the statistical variation between susceptible and resistant colony.
For the first and second columns, data followed by the same letter are not significantly different (based on the value of $LSR_{0.05}$).

Summary

Lower threshold of insect development and thermal requirements were estimated in the laboratory at five constant temperature regimes (15-35ºC). The two biological parameters were established for different developmental stages of cotton leafworm, *Spodoptera littoralis* from emamectin benzoate-susceptible and -resistant strains. From this study, the lower threshold of development (T_0) was only species dependent. The least value of zero development was 9.1ºC (for larval stage) and the greatest was 11.77ºC (for adult stage); however, the range of variations from 9.10 to 11.77ºC was insignificant. In contrast, thermal requirements (day-degrees or degree days) were mostly colony, stage, sex and temperature dependent. When the two colonies were compared, larval stage, pupal stage, generation time and life cycle for individuals from resistant colony required greater thermal units than those from susceptible colony and the difference was highly significant. For all immature stages from each of the two tested colony, statistically, the greatest thermal requirements were for larval stage followed by pupal stage and the least were for the development of egg stage. In general life cycle required significantly greater heat constant than insect generation. Regardless the tested colony and temperature regimes, thermal requirements were highly significant greater for oviposition period than for the two other physiological periods of adult female longevity. Variations in thermal requirements were also compared between males and females at all temperature regimes tested and for both of the two tested colonies. Thermal units were greater for female pupae (ranged from 144.97 d-d at 15ºC to 166.51 d-d at 20ºC) than those for male pupae (129.49 d-d at 15ºC to 157.41 d-d at 20ºC), however this variation was significant at 15ºC and was insignificant at 20ºC. For adult females, thermal units were insignificantly greater (ranged from 95.70 at 15ºC to 146.78 at 25ºC) than those for adult males (ranged from 88.38 at 15ºC to 141.3ºC at 25ºC). For each of the two tested colony, thermal requirements for most of the developmental stages were significantly different between the five constant temperatures and the minimum requirements of heat were mostly at 15ºC, however the maximum fluctuated between 20, 25 and 30ºC based on the tested age or stage. It could be concluded that the developing resistance to emamectin benzoate in the field is possible when cotton leafworm larvae expose to any of avermectin insecticides that share the same mode of action. As a result, avermectin resistant colonies start their activity normally as susceptible colony, however the requirements of heat for complete the life cycle will be greater due to the elongation of life cycle for resistant colony.

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References

Andersch, W.; P. Evans and B. Springer 2011. Combinations of biological control agents and insecticides or fungicides. Published 2011-05-12, assigned to Bayer Crop science.

Bartekova, A. and J. Praslicka 2006. The effect of ambient temperature on the development of cotton bollworm (*Helicoverpa armigera* Hubner, 1808). Plant Protection Science; 42 (4): 135-138.

Bean, J. L. 1961. Predicting emergence of second instar spure bud-worm larvae from hibernation under field conditions in Minnesota. Ann. Ent.Soc. Am.; 54 (2): 175-177.

Blunk, H. 1914. Entwicklung des *Dytiscus marginalis* L. Z. Wiss. Zool.; 111: 76-151.

Davidson, J. 1944. On the relation between temperature and rate of development of insects at constant temperatures. J. Anim. Ecol.; 13: 26-38.

Doerr, M. D.; J. F. Brunner and V. P. Jones 2002. Temperature-dependent development of *Lacanobia subjuncta* (Lepidoptera: Noctuidae). Environmental Entomology; 31 (6): 995-999.

El-Malki, K. G. 2000. Thermal requirements and prediction models of cotton leafworm, *Spodoptera littoralis* (Boisd). 2000 Proceedings Beltwide Cotton Conferences, San Antonio, USA; 2: 1019-1021.

Grant, A. N. 2002. Medicines for sea lice. Pest Management Science; 58 (6): 521–527.

Ishtiaq M. and M. A. Saleem 2011. Generating susceptible strain and resistance status of field populations of *Spodoptera exigua* (Lepidoptera: Noctuidae) against some conventional and new chemistry insecticides in Pakistan. J. Econ. Entomol.; 104 (4): 1343-1348.

Jarosík, V. 1.; A. Honek; R. D. Magarey and J. Skuhrovec 2011. Developmental database for phenology models: related insect and mite species have similar thermal requirements. J. Econ. Entomol.; 104 (6): 1870-1876.

Kandil, M. A. A. 2013. Relationship between temperature and some biological aspects and biochemical of *Earias insulana* (Boisd.) (Lepidoptera: Noctuidae). Egyptian Academic Journal of Biological Sciences: Entomology; 6 (1): 11-20.

Kinjo, K. and N. Arakaki 2002. Effect of temperature on development and reproductive characteristics of *Diaphania indica* (Saunders) (Lepidoptera: Pyralidae). Applied Entomology and Zoology; 37 (1): 141-145.

Li-Tao, L.; W. Yu-Qiang; M. Ji-Fang; L. Lei; H. Yan-Tang; D. Chao; G. Yao-Jin; D. Zhi-Ping and W. Qin-Ying. 2013. The effects of temperature on the development of the moth, *Athetis lepigone* and a prediction of field occurrence. Journal of Insect Science (Madison); 13 (103): 1536-2442.

Miyashita, K. 1971. Effective of constant and alternating temperatures on the development of *Spodoptera litura* F. (Lepidoptera: Noctuidea). Appl. Ent. Zool., 6 (3): 105-111.

Murray, Marion S. 2008. Using Degree Days to Time Treatments for Insect Pests. Published by Utah State University Extension and Utah Plant Pest Diagnostic Laboratory.

Peairs, L. M. 1914. Some phases of the relation of temperature to the development of insects. West Virginia Agr., Exp. Sta. Bull., 208: 62pp.

Rodríguez, E. M.; D. A. Medesani and M. Fingerman 2007. Endocrine disruption in crustaceans due to pollutants: A review. Comparative Biochemistry and Physiology - Part A: Molecular and Integrative Physiology 146 (4): 661–671.

Waddy, S.; V. Merritt; M. Hamilton-Gibson; D. Aiken and L. Burridge 2007. Relationship between dose of emamectin benzoate and molting response of ovigerous American lobsters (*Homarus americanus*). Ecotoxicology and Environmental Safety; 67 (1): 95–99.

Younis, A. M. 1992. Some biological aspects of cotton leafworm, *Spodoptera littoralis* (Boisd.) under different constant temperature regimes. Bulletin of the Entomological Society of Egypt; 70: 171-180.