Biological Parameters of the Bark Beetle, *Xylosandrus crassiusculus* (Motschulsky) Under Controlled Laboratory Conditions

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Abstract

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Biological parameters of the bark beetle, *Xylosandrus crassiusculus* (Motschulsky) (Coleoptera: Curculionidae: Scolytinae) was investigated under different temperature regimes (27, 29, 31 and 35ºC). The females reared at 29°C, lived 30.93±2.36 days for oviposition. The female laid a maximum of 6.71±1.89 eggs per day and a maximum of 6.99±1.33 batches per female. Each batch had an average of 13.78±2.01 eggs. The mean total fecundity recorded was 67.8±4.25 eggs, with a shortest egg incubation period. At 29°C, the duration of the larval stage was 20.17±2.89 days, the pupal stage 5.03±1.11 days, and the life span of the adult insect was 29.44±3.19 days. Significant differences (P<0.05) were noted when the duration of the different life stages under 29 and 35ºC were compared, with longer duration at 29°C. Insects reared at 35°C had narrower bodies and smaller sizes. Furthermore, the developmental stages period, including the ovipositional period (days) were negatively correlated with temperature.

Keywords: *Xylosandrus crassiusculus*, bark beetle, biological parameters, temperature regimes.

Introduction

Mango (*Mangifera indica* L., Anacardiaceae) is a major fruit crop in tropical and subtropical areas of the world (Masood *et al.*, 2010). Mango production has been decreased due to different biotic and abiotic factors (Jiskani *et al.*, 2007). Mango sudden death syndrome (MSDS) is one of the major threats of the mango industry (Khuhro *et al*., 2005). In Pakistan, the yield of mango trees has been reduced by primary insect pests such as mealybug (*Drosicha mangiferae*) (Green), hopper *(Idioscopus clypealis)*, midge *(Erosomya indica)*, scale insect *(Aulacaspis tubercularis*) and the fruit fly *(Bactrocera dorsalis*) (Brown *et al*., 2006).

Bark beetles (Coleoptera: Scolytidae) are the most important forest insect pests, as more than 6000 species have been recorded in tropical and sub-tropical regions of the world (Kazmi *et al*., 2005). The mango bark beetle, *Xylosandrus crassiusculus* (Motschulsky) (Coleoptera: Curculionidae: Scolytinae) has frequently been found on diseased mango trees (Saeed *et al*., 2011). The biology of *X. crassiusculus* is very important, especially investigations of it's role as a pest and as a possible carrier of MSDS. Effect of abiotic factors such as temperature on biological parameters of the bark beetle *i.e.* developmental time, life time, egg production, sex ratio, life span and morphological characteristics of the beetle were investigated (Hanula *el al*., 2008). Cool or warm environment can have adverse effects on life history traits of the beetle (Steininger *et al*., 2015) and optimum temperature is required for the proper development of the beetle (Ranger *et al.*, 2010). Temperatures may be too low for metabolic reactions in beetles (Werle *et al.*, 2016). Successful ambrosia beetle dispersal depends on suitable temperature, light intensity, wind direction and speed (Gandhi *et al*., 2010). All of these factors can affect the ability of the females to detect and orient towards stress volatiles from a new host. This is known as directed dispersal and it provides a greatest opportunity for successful colonization. Temperature is an important factor, if it is too cold, metabolic reactions necessary for flight may be compromised, and the ectothermic beetles may be unable to adequately operate flight muscles (Ranger *et al.*, 2013). Adults emergence depends mostly on air temperature in April-May every year (Mizell and Riddle, 2004). There is almost no systematic information on the biological parameters of *X. crassiusculus* in Sindh, except few reports related to its population in mango orchards (Khuhro *et al.*, 2005). In the present study, the life cycle and some biological parameters of *X. crassiusculus* at different temperature combinations under controlled laboratory conditions were investigated.

Materials and Methods

Biological parameters of *X. crassiusculus* was investigated at IPM Laboratory, Plant Protection Department, Sindh Agriculture University Tandojam-Pakistan during April, 2018. The laboratory stock culture of *X. crassiusculus* was maintained by placing infected logs (40-50 cm long and 20 cm in diameter), brought from the mango orchards of malir farm of Sindh Agriculture University, Tandojam and were

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kept in insect rearing chamber in the laboratory. A transparent collecting jar was fitted at the bottom end with an artificial light source. At 11 days intervals, 4-5 fresh logs were introduced to infected logs for the maintenance of stock culture. The life history traits of bark beetle were studied, using the disc method (Masood *et al*., 2009; Kendra *et al.* 2012; Gurpreet *et al*., 2013). The size of discs used was 4 cm in diameter and 2 cm thick, were cut from uninfected and healthy-looking mango tree stems by using a sharp knife. The newly hatched beetles were collected from the tree logs and reared on modified mango discs, at the rate of one pair (1:1) per disc. Each disc was then placed singly in a pressed thermopore glass having a filter paper at the base to absorb excessive moisture, to prevent the development of fungal growth. The discs were then placed in the incubating chambers at 27°C, 29°C, 31°C and 35°C at 70% RH. A total of 100 discs were used at each treatment. For daily observation, prepared discs were separated by the help of a sharp knife to investigate the different life stages of the beetle for each treatment. Two experiments were conducted; i) biological parameters (days) and fecundity of the bark beetle under different temperature regimes and ii) body size from egg to adult stage at the 2 temperature regimes 29 and 35°C only. Morphology of all the stages were investigated with the help of a magnified glass and under the stereomicroscope. The 1st and 5th larval instars were distinguished from each other based on molting and head capsule width by using a microscope with ocular micrometer (Fernandez *et al.*, 1999).

Statistical analysis

Biological parameters under different temperature regimes were compared through one-way analysis of variance (ANOVA) using least significant differences (LSD). However, the correlation between developmental period (days), ovipositional period (days) and fecundity of all treatments were estimated by using XLSTAT version 2018. The body size as affected by the two treatments (29 and 35°C) was analyzed by using a t-test. All statistical analyses were done through SPSS version 21 (IBM SPSS).

Results

Developmental period of *X. crassiusculus*

Temperature had a significant effect on the life history traits of the *X. crassiusculus*. The shortest egg incubation period was 04.01±1.99 days at 29°C, followed by 7.22±1.02 days at 27°C, 5.12±1.09 days at 31°C and 6.22±1.10 days at 35°C. The shortest larval stage duration was 20.17±2.89 days at 29°C, 25.17±2.06 days at 27°C, followed by 21.19±2.06 days at 31°C and 22.17±3.25 days at 35°C. However, the shortest duration of the pupal stage was 5.03±1.11 days at 29°C followed by 9.11±1.89 days at 27°C, 7.08±1.99 days at 31°C and 6.01±1.81 days at 35°C (Table 1). The longest adult stage was 29.44±3.19 days at 29°C and 26.74±2.56 days at 31°C, followed by 26.03±1.58 days at 35°C and 25.54±2.41 days at 27°C.

Table 1. Mean value (±SE) of biological parameters of *X. crassiusculus* reared at different temperature regimes under controlled laboratory conditions.

Parameters	**27°C**	**29°C**	**31°C**	**35°C**	**P**
Egg incubation period (days)	7.22±1.02 a (6-9)	04.01±1.99 c (2-5)	5.12±1.09 bc (4-6)	6.22±1.10 b (5-7)	<0.0001
Larval duration (days)	25.17±2.06 a (22-27)	20.17±2.89 c (17-23)	21.19±2.06 cd (18-22)	22.17±3.25 b (5-7)	<0.001
Pupal duration (days)	9.11±1.89 a (7-11)	05.03±1.11 c (4-7)	7.08±1.99 b (5-9)	6.01±1.81 ab (4-7)	<0.0017
Adult longevity (days)	25.54±2.41 bc (21-28)	29.44±3.19 a (26-31)	26.74±2.56 b (23-29)	26.03±1.58 ab (23-29)	<0.0023
Oviposition period (days)	26.03±1.29 b (24-27)	30.93±2.36 a (28-33)	24.09±1.99 bc (21-26)	25.13±1.89 c (23-27)	<0.001
No. of batches/female	4.15±1.01 bc (3-6)	07.02±1.33 a (5-10)	5.05±1.25 b (4-7)	4.20±1.66 ab (3-7)	<0.0014
No. of eggs/batch	10.01±2.14 c (8-12)	13.78±2.01 a (9-15)	12.01±2.74 ab (10-14)	11.88±2.33 bc (8-12)	<0.0038
Per day egg laying	4.01±0.77 bc (2-6)	06.71±1.89 a (4-9)	5.02±0.89 b (3-7)	5.01±0.99 b (3-6)	<0.051
Total fecundity (eggs)	59.8±3.25 bc (34-65)	67.8±4.25 a (55-80)	60.11±4.25 b (36-62)	55.8±4.25 c (41-60)	<0.0001

Values between brackets represent the range.
Values followed by the same letters in the same row are not significantly different at P=0.05.

Fecundity of *X. crassiusculus*

Temperature had a significant effect on the oviposition period of *X. crassiusculus*. The oviposition period was longest (30.93±2.36 days) when females were reared at 29°C, whereas the shortest period (26.03±1.29 days) was at 27°C, followed by 25.13±1.89 days at 35°C and 24.09±1.99 days at 31°C. Each female laid a maximum of 6.71±1.89 eggs per day when reared at 29°C, whereas the lowest per day egg production was 4.01±0.77 eggs at 27°C, followed by 5.01±0.99 eggs per day at 35°C and 5.02±0.89 eggs per day at 31°C. Females reared at 29°C laid a maximum of 07.02±1.33 batches per female and a minimum of 5.05±1.25 batches/female at 31°C, followed by 4.20±1.66 batches at 35°C and 4.15±1.01 batches/female at 27°C. The mean number of eggs laid by a single female was 13.78±2.01 eggs per batch when reared at 29°C, followed by 12.01±2.74 eggs/batch at 31°C, 11.1.88±2.33 eggs/batch at 35°C and 10.01±2.14 eggs/batch at 27°C. The mean maximum fecundity was 67.8±4.25 eggs at 29°C. Whereas, the lowest egg production recorded was 55.8±4.25 eggs at 35°C, followed by 59.8±3.25 eggs and 60.11±4.25 eggs at 27 and 31°C, respectively. The sex ratio was 1:19 (male: female) at 29°C and 1:10 at 27°C, followed by 1:6 and 1:04 at 31°C and 35°C, respectively. Males of ambrosia beetle could not fly and were used solely for mating purposes only.

Correlation of life-history traits and different temperature regimes

Figure 1 represents the correlation between different temperatures and average developmental period (days) from egg to adult of the bark beetle *X. crassiusculus*. The regression equation was y=-0.5856x+79.712 and the correlation coefficient was R^2=0.3134. Developmental period (days) was negatively correlated with temperature. Furthermore, developmental period (days) fluctuated with the temperature; as temperature decreased, the developmental period increased. The same trend was observed in relation to the ovipositional period (days) and fecundity/female as they were also negatively correlated with temperature. The regression equation for the ovipositional period was: y=-0.3534x+37.325 and correlation coefficient was R^2=0.1588, when temperature increased the ovipositional period decreased (Figure 2).

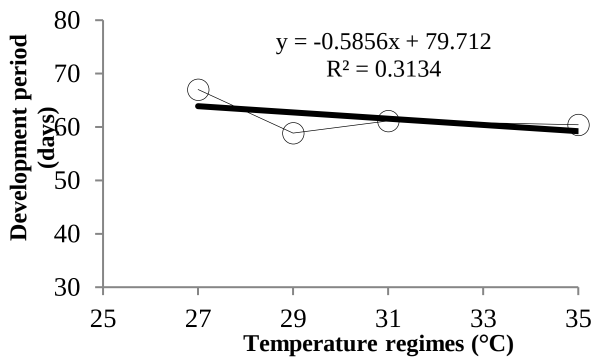

Figure 1. Correlation between developmental period (days) and different temperature regimes used.

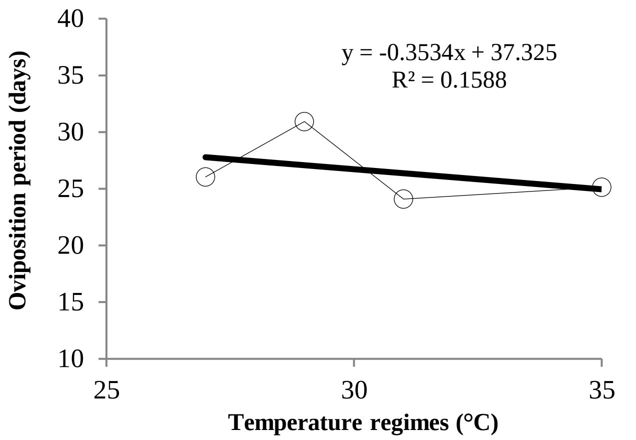

Figure 2. Correlation between the ovipositional period (days) and different temperature regimes used.

Fecundity regression equation was: y=-0.8527x+86.885 and correlation coefficient R^2=0.3373, when temperature was decreased to 27°C, the fecundity of females was lowest, and the fecundity was highest by increasing of temperature to 29°C. However, when temperature was increased to 31 or 35°C, the fecundity decreased (Figure 3).

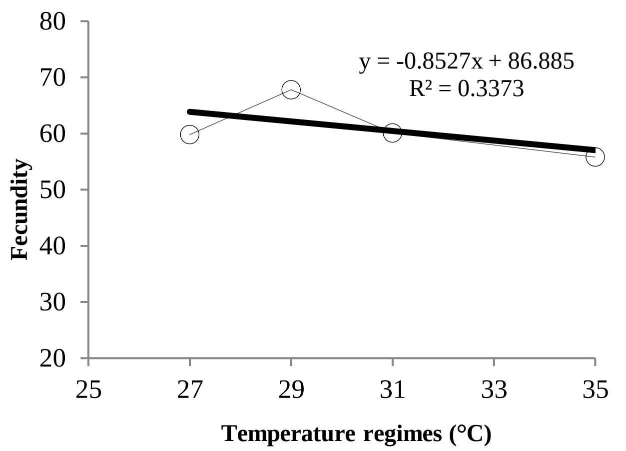

Figure 3. Correlation between female fecundity and different temperature regimes used.

Morphological characteristics

Significant difference in egg size was observed when the insect was reared at different temperatures. Egg size was largest (0.41±0.030 mm wide, 0.61 ±0.041 mm long) when the female was reared at 29°C as compared to females reared at 35°C. Whereas, smallest size (0.25 ±0.025 mm wide, 0.53±0.033 mm long) was observed when the insect was reared at 35°C, with significant differences among treatments (t=4.13, F=0.33; df=119, P<0.007). In shape, eggs were rod-shaped, shining and soft white in color, with both ends largely curved (Table 2).

Table 2. Mean life stage body size (±SE) of the insect *X. crassiusculus* when reared at different temperatures.

	Body measurement (mm) (n=20)			
	Width		Length	
Life stage	**29°C**	**35°C**	**29°C**	**35°C**
Egg	0.41 ±0.030	0.25 ±0.025	0.61 ±0.041	0.53±0.033
1st larval Instar	0.39±0.051	0.21±0.039	0.77±0.010	0.63±0.004
5th Instar/ Full grown	0.71 ±0.043	0.65±0.025	3.01±0.099	2.06±0.070
Pupa	0.89 ±0.064	0.78 ±0.044	2.82±0.089	2.01±0.069
Adult	0.92±0.002	0.69±0.007	1.99±0.098	1.58±0.077

The larvae were white in color, legless and slightly curved. The size of 1st instar larvae was 0.39±0.051 mm wide and 0.77±0.010 mm long when reared at 29°C, and was smaller (0.21±0.039 mm wide and 0.63±0.004 mm long) when reared at 35°C, with significant difference among treatments (t=5.09, F=0.54; df=115, P<0.001). The mean size of 5th instar (full grown larvae) was 0.71 ±0.043 mm wide and 3.01±0.099 mm long when reared at 29°C, and 0.65±0.025 mm wide and 2.06±0.070 mm long when reared at 35°C. The t-test showed a significant difference between the temperature treatments (t=6.01, F=0.56; df=75, P<0.021). The new white pupae turned to light brown just before adult emergence and with a mean size of 0.89 ±0.064 mm wide and 2.82±0.089 mm long when reared at at 29°C and 0.78 ±0.044 mm wide and 2.01±0.069 mm long when reared at 35°C, with significant difference in pupa's width (t=3.03, F=0.45; df=59, P<0.037) but without significant difference in pupa's length (t=0.25, F=0.16; df=19, P<0.747). The adults were cylindrical with an average body size of 0.92±0.002 mm wide and 1.99±0.098 mm long when reared at 29°C and 0.69±0.007 mm wide and 1.58±0.077 mm long when reared at 35°C, with significant differences between the two treatments (t=4.20, F=0.53; df=198, P<0.001) (Table 2).

Discussion

All developmental stages from eggs to adults were affected by temperature. Results obtained were in agreement with earlier reports (Iqbal *et al*., 2007; Ranger *et al.*, 2013) which indicated that temperature variations had an influence on the life cycle of ambrosia bark beetles. Usually, incubation period of bark beetle eggs depend on substrate (i.e., artificial diet vs. natural host) and temperature (Carrillo *et al*., 2014). However, Ranger *et al*. (2016) investigated the effect of different temperature levels on the life stages duration of the ambrosia beetle, reported that eggs hatched in 7-14 days and larvae pupated in 11-23 days at 29°C, but when reared at 22-24°C the eggs hatched in 14-35 days and the larvae pupated in 21-35 days. Likewise, earlier results on the biology of *X. crassiusculus* indicated better sex ratio, high fecundity rate, hatching ability and short life cycle when the beetle was reared at 29°C as compared to 27, 31 and 35°C. The maximum number of eggs laid was 67.8±4.25 at 29°C. Kazmi *et al*., (2005) reared *H. mangiferae, X. glabratus* and *X. crassiusculus* at three temperature levels and the results obtained were significantly different from each other. Furthermore, Carrillo *et al*., (2013) conducted laboratory experiments at two temperature levels and examined the life history traits of the bark beetle. The findings partially agree with those of Masood *et al*., (2009), who conducted an experiment under controlled laboratory conditions on the life cycle of mango bark beetle, *Hypocryphalus mangiferae*. He reported that at 25°C, larval duration was completed within 16.90±2.12 days, pupal period lasted 3.85±1.41 days and the adults emerged and survived for 22.9±3.53 days. The female was observed to lay more eggs up to 27 days when reared at 29°C, as compared to other temperature regimes. These results agree with those reported by Roeper *et al.,* (1980). Many other workers investigated the effect of environmental factors on the life stages of different bark beetle spp., especially temperature (Werle *et al.,* 2016; Ranger *et al.,* 2016; Beaver and Liu, 2010; Masood *et al*., 2009; Steininger *et al.,* 2015). The differences between these reported results (developmental times, fecundity and morphological characteristics) and those of the present study may be due to different species investigated, use of different artificial diets, natural hosts and different temperature regimes. However, all indicated the influence of temperature on the biological parameters of the mango bark beetle.

It can be concluded from the present study that the highest fecundity with a shortest egg incubation period (days), maximum hatching, faster larval and pupal development time, highest female ratio, and body size increase were recorded when the ***X.** crassiusculus* was reared at 29°C as compared to other temperatures. Accordingly, the rearing temperature of 29°C was found to be the most favorable (Optimum) temperature for ***X.** crassiusculus* beetle's development and reproduction.

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الملخص

قرشي، خالد حسين، عبد الوحيد سولانجي، عبد الغني لنجر، جان محمد مري، سجاد علي خوهرو وأسلام بوكيرو. 2021. العناصر الحياتية لخنفساء القلف (Motschulsky) *Xylosandrus crassiusculus* تحت ظروف المختبر المراقبة. مجلة وقاية النبات العربية، 39(2): 146–151.

تمت دراسة العناصر الحياتية لخنفساء القلف (Motschulsky) *Xylosandrus crassiusculus* (Coleoptera: Curculionidae: Scolytinae) عند مستويات حرارة مختلفة (27، 29، 31 و 35°س). وصل طول فترة وضع البيض للإناث 30.93 يوماً عند حرارة 29°س. وضعت الإناث 6.71 بيضة/اليوم في 6.99 لطخة لكل أنثى. احتوت كل لطخة على 13.78 بيضة. كان مجموع ما وضعته الحشرة الأنثى 67.8 بيضة. عند حرارة 29°س وصل طول فترة حياة اليرقة إلى 20.17 يوماً، فترة حياة العذراء 5.03 يوماً وفترة حياة الحشرة الكاملة 29.44 يوماً. كان هناك فرق معنوي لعناصر الحياة المختلفة للحشرة عند تربيتها عند حرارة 29°س مقارنة بتربيتها عند حرارة 35°س عند مستوى احتمال 5%، وكانت الفترات أطول عند مستوى حرارة 29°س. كان للحشرة عند تربيتها عند درجة حرارة 35°س أجسام أرفع وكانت أقل حجماً. كما كان ارتباط طول فترات الأطوار المختلفة، بما فيها فترة وضع البيض، سلبياً مع درجة الحرارة.

كلمات مفتاحية: *Xylosandrus crassiusculus*، عناصر حياتية، خنفساء القلف، درجة الحرارة.

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