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Effect of temperature on the life cycle and pupal color of lime swallotail butterfly, *Papilio demoleus* (Lepidoptera: Papilionidae)

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Abstract
Papilio. demoleus (L) is considered among the major insect pest of citrus cultivated areas in Bangladesh. In this study, we investigated the effect of winter months temperature (18.55^{0}C to 25.37^{0}C) and warmer months temperature (27.67^0 to 30.53^0 C) on development cycle and pupal coloration of *P. demoleus*. Results showed that development of the *P. demoleus* was slow at lower temperature and at the high temperature, developmental rate was fast. Towards the end of the November when temperature was 24.12^{0}C, *P. demoleus* pupae entered into diapause and spent following months; December, January and middle of February in dormancy/diapause. Adults emerged at the end of February when temperature range was 25.5^{0}C to 27^{0}C with average 26.44^{0}C, for this reason winter months pupal period was about six times longer than pupal period of warm months. We found that temperature had a significant effect on *P. demoleus* pupal coloration. Larvae maintained in winter environment had more brown pupae (76.19%) and less green pupae (9.53%), whereas more green pupae (65.38%) and less brown pupae (11.54%) were found when larvae maintained in warmer environment.

Keywords: temperature regimes, *Papilio demoleus*, developmental periods, pupal colorations

1. Introduction

The lime swallowtail butterfly (*P. demoleus*) is one of the most wide spread members of the family Papilionidae and it is found throughout the tropical and subtropical areas disseminated from Southern and Southeast Asia (Corbet and Pendlebury, 1992)[8]. Caterpillars/larvae of *P. demoleus* are a serious insect pest feeding on citrus leaves and blossoms and it is a potential threat to citrus nursery stocks and other young citrus plants in Asia and the Middle East (CABI, 2013 ; Lewis, 2012) [6, 16]. In Bangladesh *P. demoleus* is identified as major pest of citrus plant e.g. lime, lemon, shaddock, mandarin, shatkora, adajamir and rough lemon and it has the potentiality of rapid population growth under warmer environment. A positive relationship between temperature and insect development is well established: insects develop faster in warmer temperatures. However, this positive relationship is unlikely to be linear. Ectotherms have temperature optima above and below which grow this negatively affected (Gilbert *et al.* 1976) [9]. Therefore, insect development can vary dramatically in calendar days if temperatures vary. But Grassberger and Reiter (2001)[10] reported that the succession of arthropods development is mostly affected and influenced by temperature and humidity. In warmer temperature and high humidity, insects have also been grow faster. The opposite conditions have also been noted more retard insect growth significantly. Globally, to develop improved strategies of managing *P. demoleus*, numerous studies have been conducted in various localities to provide valuable information on its biology (Ramarethinam and Loganathan, 2001; Singh and Gangwar, 1989) [20, 23] and ecology (Badawi 1981; Nandni *et al.* 2012) [2, 18] from subtropical to tropical regions. Clarke and Sheppard (1972) [7] reported that the pupae of many species of Lepidoptera, especially butterflies are variable or polymorphic in color triggered by temperature, humidity or photoperiod during late larval or pre-pupal stage, The pupae of swallowtail butterflies are often dimorphic, being either green or brown in the North American species, *Battus philenor* (Clarke and Sheppard, 1972) [7], *Papilio xuthus*, *Papilio protenor demetrius* (Ohnishi and Hidaka, 1956) [19].

The aim of this study was (i) to provide data on the development of *P. demoleus* at different temperature regimes of warm and winter months which might help to proper management of this pest at the appropriate stage and (ii) whether temperature affecting the pupal coloration of *P. demoleus*

2. Materials and Methods:

2.1 Maintenance of the butterflies in the laboratory: The study was carried out in the laboratory in Radiation Entomology and Acarology Division at Institute of Food and Radiation Biology in Atomic Energy Research Establishment (AERE); Dhaka, Bangladesh. Adult male and female butterflies were collected from AERE campus. After collection they were kept in rearing cage (44cm×38cm×38cm) covered with mosquito net. Pure culture of *P. demoleus* was obtained by separating eggs or larvae of one female and cultured them for further experiment. The butterflies were fed with 10% sugar solution daily to ensure reproductive success. Fresh young leaves with branches of citrus plant were used as oviposition. Larvae culture was provided with fresh citrus leaves as a food till the pre-pupae stage. Experiments were carried out on citrus leaves at 27.67^0 to 30.53^0 C for warmer months (March to October) and at 18.55^{0}C to 25.37^{0}C for winter months (November to February) in laboratory. Daily temperature of the individual day of single month was recorded from the Digital LCD hygrometer.

2.2 Monitoring developmental periods and pupal color of *P. demoleus*: From laboratory culture, freshly laid eggs with citrus leaves transferred in to plastic container constantly observed the days required for egg hatching, larvae development, pre pupal period, pupal period and adult emergence. The larval instars were determined by the presence of exuviae**.** The developmental periods of different life stages was carried out by taking 10 replication of each stage viz., egg, 1st instar, 2nd instar, 3rd instar, 4th instar and 5th instar larvae as well as pre-pupa, pupae and adult emergence for linear measurements and also monitoring and counting the pupal color of *P. demoleus* in both temperatures conditions at 27.67^0 to 30.53^{0}C (warm months) and 18.55^{0}C to 25.37^{0}C (winter months). Preliminary observations during rearing of papilionidae butterflies in outdoor conditions revealed green and brown pupal morphs. Given the variation in temperature across the warmer and winter months, we tested the role of temperature in determining the pupal color. For this observation, 26 larvae were reared at higher temperature (warm months) and 23 larvae were reared at lower temperature (winter months).

3. Results and Discussion

As expected, temperature had a significant effect on *P. demoleus* development time from egg to adult (Table 1). At lower temperature (18.55^{0}C to 25.37^{0}C), the life cycle (time elapsed between oviposition and adult emergence) of the *P. demoleus* was completed in 96.123 days whereas, it was 29.331 days at treated in higher temperature (27.67^0 and 30.53^{0}C). Indicating that higher the temperature (warm months), insects grow faster or lower the duration of each stage and opposite condition was more retard or slower insect growth significantly. Incubation period, time elapsed between oviposition and egg hatch which prominently affected by temperature. At 18.55^{0}C to 25.37^{0}C (November to February) temperature, the incubation period was 4.812±0.403 days (represents 5.006% of the complete development cycle time). When temperature range between 27.67^0 and 30.53^0 C for warmer months, its incubation periods was 3.032±0.221 days (represents 10.337% of the complete development cycle time). The present observation is more or less similar with that of Hoang *et. al.* (2015) [11] findings. They found that the incubation period of *P. demoleus* was 2.60±0.10 days, 3.08±0.14 days, 3.48±0.15 days and 3.55±0.20 days at 30^{0}C, 28.5^{0}C, 26.5^{0}C, and 25^{0}C temperature respectively. Our findings were also consistent with Hoang *et al* (2015) [11] studies because of incubation periods increased with the decreased of temperature and it becomes shorten with enhanced temperature. The larvae/caterpillar of *P. demoleus* typically had five instars and total larval development time (time elapsed between egg hatch and 5th instar/final instar) significantly varied at different temperatures (P<0.05). At lower temperature (winter months) increase to the completion of different larval period of *P. demoleus* in 1st instar 3.187±0.834 days, 2nd instar 3.250±0.447 days, 3rd instar 3.250±0.577days, 4th instar 4.062±0.680 days and 5th instar 7.250±0.125 days than those reared at higher temperature (warmer months) in 1st instar 2.562±0.403 days, 2nd instar 2.680±0.403 days, 3rd instar 2.812±0.403 days, 4th instar 2.625±0.387 days and 5th instar 4.468±0.427 days. At lower temperature (18.55^{0}C to 25.37^{0}C) total larval period was on an average of 20.999±3.66 days (represents 21. 845% of the complete development cycle time) whereas, 15.147±2.21 days spent to complete their total larval period (represents 51.641% of the complete development cycle time) at higher temperature (27.67^{0}C to 30.53^{0}C). The present findings are differed with those of 3.2±0.7 days in 1st instar, 2.3±0.7 days in 2nd instar, 2.7±0.7 days in 3rd instar, 3.3±1.2 days in 4th instar, 2.7±0.7 days in 5th instar and total larval period was 14.2 days when its reared from June to September at 30±4^{0}C (Sharif and Zarea, 1970) [21]. Karim *et. al.* (2007) [14] found 5.5 ± 0.2 days for 1st instar, 4.6 ± 0.2 days for 2nd instar, 2.6 ± 0.1 days for 3rd instar, 2.4 ± 0.1 days for 4th instar, 4.0 days for 5th instar and total larval period was 19.1 days when *P. demoleus* larvae reared from September to December, 2006. At low temperature, the metabolic rate may be markedly reduced and this could result in slower the larval development**.** Increases in temperature causes an increase in metabolic activity and reduce the development time (Anderson, 2000) [1]**.** The variation in the duration of different larval instar and total larval period may be due to different temperature and host plants but the present findings are in confirmation with those findings that the higher temperature accelerates the larval growth whereas lower temperature extend the period of larval development of *P. demoleus*. Similar result has shown in *Lucilia cuprina* (Kotze *et al*., 2015) [15]. It suggested that larvae of *P. demoleus* living in environments with a temperature 25.37^{0}C or lower have shown stunted growth rates compared to larvae in warmer temperature (27.67^0 C to 30.53^{0}C).

We found that towards end of the fifth instar there was a distinct short period during which the larva did not feed or move. This period reported by other investigators is considered a pre-pupal stage (time elapsed between end of the final instar larvae or post feeding stage and pupal stage). In this stage body gradually shorten in length by spilled excreta with water from the body and body bending upwards mid-dorsally. Before going to pupal stage, there is a pre-pupal stage that is recognized by their conspicuous segmentations with curved form (Karim *et al.* 2007) [14]. As usual as the pre-pupal period persist 1 day but when the temperatures become low then this period become extended. The duration of this stage was found 1.0-3.0 days with a mean of 2.062±0.442 days (represents 2.145% of the complete development cycle time) at lower temperature (18.55^{0}C to 25.37^{0}C) and 1.0-1.5 days with a mean of 1.062±0.171 days (represents 3.620% of the complete development cycle time) at higher temperature (27.67^0 to 30.53^0 C). Jhanavi *et al*. (2018) **[12]** found 1.02-1.06 days (1.062±0.171 days) and Sharifi and Zarea (1970) **[21]** reported 1-2 days with a mean of 1.1 ±0.3 days at 30±4^{0}C.

The duration of pupal stage (time elapsed between pupal stage and adult emergence) and pupal color of *P. demoleus* was very sensitive to temperature. In this study the pupal stage of *P. demoleus* remained for 50.0-83.0 days (68.250±10.389 days) i.e. represents 71.002% of the complete development cycle time at lower temperature (18.55^{0}C to 25.37^{0}C) while at higher temperature (27.67^{0}C to 30.53^{0}C) it was 9-11 days (10.090±0.841days) i.e. represents 34.400% of the complete development cycle time. Result showed the pupal duration was about 6.8 times longer at lower temperature (winter months) than higher temperature (warmer months). It is indicated that *P demoleus* pupae undergo diapauses at lower temperature and we observed pupae of *P. demoleus* hibernated from middle of the November (25.37^{0}C) continued middle of February (24.27^{0}C) and diapauses termination or adult emerged in the end of February when temperature range was 25.5^{0}C to 27.0^{0}C with average 26.44^{0}C. Sharif and Zarea (1970)**[21]** reported that the pupal stage of *P. demoleus* is very sensitive to temperature and they found 10.8±1.4 days of pupal duration when larvae reared in June to September at 30±4^{0}C. They also found that it hibernated in winter, and when rise in temperature above 25.0^{0}C caused adult to emerge. On the other hand, Karim *et al*. (2007) **[14]** reported that the pupal period lasted for 10.0 - 11.1 days with a mean of 10.7 ± 0.2 days when *P. demoleus* larvae reared in September to December. These results indicated that temperature has a significant effect on pupal duration and it mentioned that pupal diapause in *P. demoleus* are regulated by temperature. In our study, larvae reared from the egg stage under winter months (November to February) at lower temperature entered into diapauses pupae and opposite condition have been recorded to non-diapause pupae. Several Neotropical papilionids, *Battus polydamus* (Muyshondt, 1974) **[17]**, *Papilio polyxenes stabilis* (Blau, 1980) **[4]**, *Parides bunichus*, *P. proneus*, and *P. agavus* (Brown *et al*. 1980-1981) **[5]** have extended pupal stages suggestive of diapause. Sims and Shapiro (1983) **[22]** reported that the induction of pupal diapause in *B. philenor* under laboratory conditions is primarily controlled by temperature and they observed high temperatures promote continuous development, whereas lower temperatures induce diapause. The longer periods were reflective of cool-weather for egg hatching, larval development and pupal development. Like other ectotherms, development of insect species is influenced by climatic conditions, especially temperature (Bale *et al.* 2002) **[3]**. Temperature is one of the most critical environmental factors influencing rate of insect growth and development (Taylor 1981) **[27]**. Thermal requirements for intrusive insect pests' development has important implications for control programs, as temperature determines the population growth and size of intrusive pests and their variation under different conditions (Kang *et al.* 2009) **[13]**.

We investigated the influence of one environmental factor, temperature, on *P. demoleus* pupal coloration. Temperature had a significant effect on pupal coloration and both (warmer and winter) temperature influenced the rate of pupal color (Table- 2 and Fig. 1). After pupation from both temperature conditions, pupae were classified four types with respect to body color i. e. green, pale green, brown and dark brown (Fig. 1). When larvae were maintained under a range of temperature at 18.55^{0}C to 25.37^{0}C (winter months), developed into green type (9.53%), pale green (4.76%), brown (76.19%) and dark brown type pupae (19.05%). In contrast, when larvae reared under a range of temperature 27.67^{0}C to 30.53^{0}C (warmer months), developed into green (65.38%), pale green (15.38%), brown (11.54%) and dark brown type pupae (7.69%). The results indicate that, pupae of *P. demoleus* were either brown or green or very rarely intermediate in both temperatures. Proportions of green and brown pupae were expected to vary across low and high temperature, with larvae in the cold (winter months) treatment having the highest proportion of brown pupae comparison to green pupae and larvae in the warmer months treatment having highest proportion of green pupae comparison to brown pupae. Because coloration affects absorption of radiant energy, color variation in response to temperature may be an ectothermic adaptation to suboptimal thermal conditions. Yamamoto *et al*. (2011) [28] reported that brown type pupae of *Byasa alcinous* are induced by lower temperature and humidity conditions, whereas yellow type are induced by higher temperature and humidity conditions. Interestingly, in other tropical species, *Danaus chrysippus* (Smith *et al.*, 1988) **[25]** and *Papilio polytes*, *Papilio demoleus* and *Papilio polyxenes* (Smith, 1978) **[24]** showed that high relative humidity (RH) induced formation of predominantly green pupae while low RH induced formation of predominantly brown pupae (*Papilio polytes*, *Papilio demoleus* and *Papilio polyxenes*) or pink pupae (*Danaus chrysippus*). Talbot (1939) [26] reported that greenish pupal color was due to pupal development in proximity to leaves or any other green object, and brown pupal color was due to proximity to brown objects. Yamanaka *et al.* (2004) **[30]** mentioned that the degree of development of orange coloration in pupae of *Papilio xuthus* varies with differences in pupation site. They observed SD-larvae developed into diapauses-green (12.4%), orange (59.9%) and brownish-orange type pupae (22.7%) in containers of smooth-surfaced transparent plastic under constant light at 25^{0}C while, a large proportion (95.3%) of larvae developed into orange type pupae in rough-surfaced paper containers under the same condition and less than 5.0% pupae developed into brownish-orange type. Yamanaka *et al.* (2009) **[29]** also reported that environmental cues affecting the pupal coloration of *Vanessa cardui* butterfly, over 80% of larvae reared at 16^{0}C developed into dark type pupae, whereas over 82% of larvae reared at 32^{0}C developed into white pupae irrespective of long/short photoperiod conditions. In this study, larvae reared from egg stage under low temperature, highest number of brown pupa were found. The opposite conditions have found maximum number of green pupae. In our observation, we obtained both color green and brown pupae on green leaves and branches of citrus plant and in the transparent white plastics box. It is not clear whether pupal color influences substrate choice or whether substrate influences pupal color. Therefore, we may conclude that pupal color does not depend not only relative humidity (RH) or on pupal development objects/substrates but also depends on ambient temperature conditions of our environment.

Our present outcomes indicated that the development periods of *P. demoleus* was momentously influenced by the temperature regimes of warm and winter months. Regarding the data obtained from this study, the development periods of *P. demoleus* decreased as temperature increased and also development period increased as temperature decreased. Those recorded data of the range of duration of different life stages of *P. demoleus* agree with above mentioned authors investigation because those literatures showed that the development periods increased as temperature decreased and decreased as temperature increased of different species of butterflies and other insects.

4. Conclusions:

Proper management of any insect pest, it is important to know the reproductive cycle, ecology, seasonality dispersion and feeding behavior of the insect. Many insects have had their development cycles modeled in terms of degree-days. The degree days of an environment determines the optimal time for a specific insect outbreak. *P. demoleus* having great activities from the warm months and hibernated in winter months. So the present study revealed information on the effect of temperature regimes of warm and winter months on life cycle of lime swallotail butterfly *P. demoleus* might be helpful for proper determination of the relative abundance of this pest species and also helpful for pest management at appropriate time of the year. Based on our data and those of other studies, we mentioned that temperature affecting pupal coloration in *P. demoleus* butterfly. Further study should be conducted to clarify whether larval rearing substrate influences pupal color of *P. demoleus*.

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Table 1: Duration of different developmental stages of *P. demoleus* reared at two different temperature regimes, 27.67⁰C to 30.53⁰C (warmer months) and 18.55⁰C to 25.37⁰C (winter months)

Developmental Stages		Duration in days at 27.67⁰ to 30.53⁰ C				Duration in days at 18.55⁰C to 25.37⁰C			
		Min.	Max.	Mean±Sd	%	Min	Max.	Mean±Sd	%
Incubation period (O-E)		2.5	3.5	3.032±0.221[a]	10.337	4.0	5.0	4.812±0.403[b]	5.006
Larval period	1st instar	2.0	3.0	2.562±0.403[a]	16.914	2.0	5.0	3.187±0.834[b]	15.177
	2nd instar	2.0	3.0	2.680±0.403[a]	17.693	3.0	4.0	3.250±0.447[b]	15.477
	3rd instar	2.0	3.5	2.812±0.403[a]	18.565	3.0	5.0	3.250±0.447[b]	15.477
	4th instar	2.0	3.0	2.625±0.387[a]	17.330	4.0	5.0	4.062±0.680[b]	19.344
	5th instar	4.0	5.0	4.468±0.427[a]	29.498	5.0	9.0	7.250±0.125[b]	34.525
Total larval period (E— L_5)		12.0	17.5	15.147±2.21[a]	51.641	17.0	28.0	20.999±3.66[b]	21.845
Pre-pupal period (L_5-Pu)		1.0	1.5	1.062±0.171[a]	3.620	1.0	3.0	2.062±0.442[b]	2.145
Pupal period (Pu-Em)		9.0	11.0	10.090±0.841[a]	34.400	50.0	83.0	68.250±10.389[b]	71.002
Total life time (O-Em)		24.5	33.5	29.331±2.75 [a]	100.00	72.0	119.0	96.123±22.74[b]	100.00

O-E: time elapsed between oviposition and egg hatch; E-L_5: time elapsed between egg hatch and 5th/final instar larval stage; L_5-Pu: time elapsed between final instar larval stage and pupal stage; Pu-Em: time elapsed between pupal stage and adult emergence; O-Em: time elapsed between oviposition and adult emergence; %: percentage of development for a stage. Means followed by the different letter within a row are significantly different (Student test $P \leq 0.05$).

Table 2: Classification of pupal color types with percentage of *P. demoleus* at two different temperatures regimes

Larval rearing temperatures	No. of larvae reared	Pupal color type (%)			
		Green type	Pale green type	Brown type	Dark brown type
Warmer months (March to October) temperature (27.67⁰C to 30.53⁰C)	26	65.38	15.38	11.54	7.69
Winter months (November to February) temperature (18.55⁰C to 25.37⁰C)	23	9.53	4.76	76.19	19.05

Fig 1: Classification of pupal colors of *P. demoleus*, showing the color types of Green (right), Pale Green (2nd from right), Brown 3rd from right and Dark Brown pupa (left).

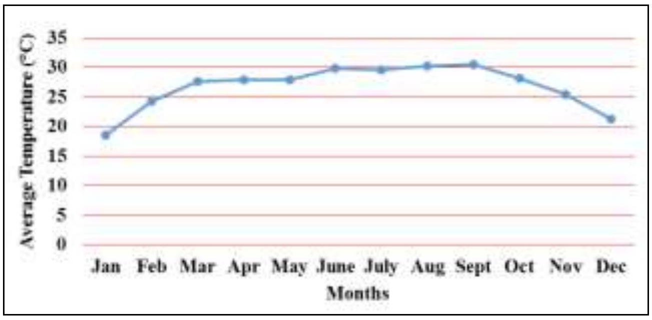

Fig 2: Monthly temperature data of the butterfly rearing laboratory during study period

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