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Studies on some aspects of the biology and ecology of Citrus butterfly *Papilio demoleus* (Papilionidae: Lepidoptera) on citrus in Vietnam

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Abstract

The citrus butterfly *Papilio demoleus* L. (Papilionidae: Lepidoptera) is considered among the most important insect pests of citrus trees in Vietnam. Observations on the mean development times (in days) of various life stages of *P. demoleus* on *Citrus sinensis* L. were carried out under laboratory conditions. Our results show that the egg-hatching time and development period of *P. demoleus* immature stages decreased as temperature increased from 25^0 to 30^{0}C. The citrus butterfly had a life cycle of 35.50 days at 25^{0}C and 22.88 days at 30^{0}C. Eggs at 25^0, 26.5^0, 28.5^0 and 30^{0}C hatched in 3.55, 3.48, 3.08 and 2.06 days, respectively. This pest typically has five instars and duration of larval stage ranged from 11.50 days at 30^{0}C to 17.93 days at 25^{0}C. The average time of pupal stage was reduced from 12.46 days at 25^{0}C to 7.33 days at 30^0. Adult longevity of the pest on honey diets was about 2-5 days. The average reproduction rate per female on honey diet was low at 21.67 eggs. Leaf-feeding capacity of *P. demoleus* larvae on citrus leaves had a positive correlation with nymphal ages. The mean leaf quantity consumed by the fifth instar was highest (2.68±0.15g/day). Results of study on biological traits of *P. demoleus* would be valuable scientific evidence on building citrus butterfly management programs.

Keywords: caterpillar, fecundity, herbivory, life history, temperature

Introduction

Papilio demoleus L. (Papilionidae: Lepidoptera), commonly known as citrus butterfly (Figure 1), is found throughout tropical and subtropical areas of Southern and Southeast Asia (Guerrero *et al.* 2004). This butterfly ranges widely and is an extremely successful invader. Moreover, *P. demoleus* whose caterpillars are a serious insect pest feeding on citrus leaves and blossoms, are a potential threat to citrus nursery stocks and other young citrus trees in Asia and the Middle East (Lewis 2012; CABI 2013). In Vietnam, to date, this species is distributed widely in many citrus production regions (NIPP, 1999). Globally, to develop improved strategies of managing *P. demoleus,* numerous studies have been conducted in various localities to provide valuable information on its biology (Singh & Gangwar 1989; Ramarethinam & Loganathan 2001) and ecology (Badawi 1981; Nandni *et al.* 2012) from subtropical to tropical regions. More importantly, precise understanding of life-history parameters of insect pests is essential for monitoring and studies of population dynamics. In Vietnam, however, knowledge of the biology of this species is rarely known at both local and national scales. This research carried out studies on the biology, food consumption of larvae and longevity of this insect under laboratory conditions.

Methods

Experimental setup: *P. demoleus* pupae (Figure 1) were collected originally from citrus orchards in Vietnam's northern (20°42'08.4"N 105°20'14.0"E). Pupae were placed into plastic vials (15cm in diameter and 25cm in height) until emergence. Each vial has a mesh lid for ventilation. One-day-old adults were collected and mated after sexual identification. A young citrus seedling with young shoot-tip leaves was put in net cages (50x50x50cm) with mated adults for 2-day reproduction periods in order to obtain a cohort of eggs for the experiments. At least 10 plants were used at each treatment condition. The eggs on leaves were marked and observed daily using magnifiers to determine incubation time, development time of nymphal stages and various other life cycle parameters of *P. demoleus* under different temperatures (n>10 at each treatment).

Fecundity: To determine fecundity of *P. demoleus* female adults, fifteen couples were transferred individually into plastic vials covered with net lids (30cm in diameter and 35cm in height). An artificial diet (honey 100%) was used as a food source for adults. The plastic vials were checked daily to count mean numbers of eggs produced from the 1st day to the 5th day by using magnifiers. Similarly, to investigate longevity of adults, five artificial diets (Distilled water, honey 20%, 50%, pure honey and solution of sugar 50% and water) were designed. One *P. demoleus* couple was transferred into a transparent plastic vial to measure mean longevity of *P. demoleus* adults. Ten couples were designated on each diet.

Leaf-feeding capacity: To measure leaf-feeding capacity of age-specific larvae of *P. demoleus,* ten larvae at different ages were placed individually into plastic vials with fresh young citrus leaves. Leaf weight was measured (g) before and after feeding of larvae. Leaves were renewed daily. Leaf-feeding capacity (g/day) of age-specific larvae was measured daily the difference in the leaf weight.

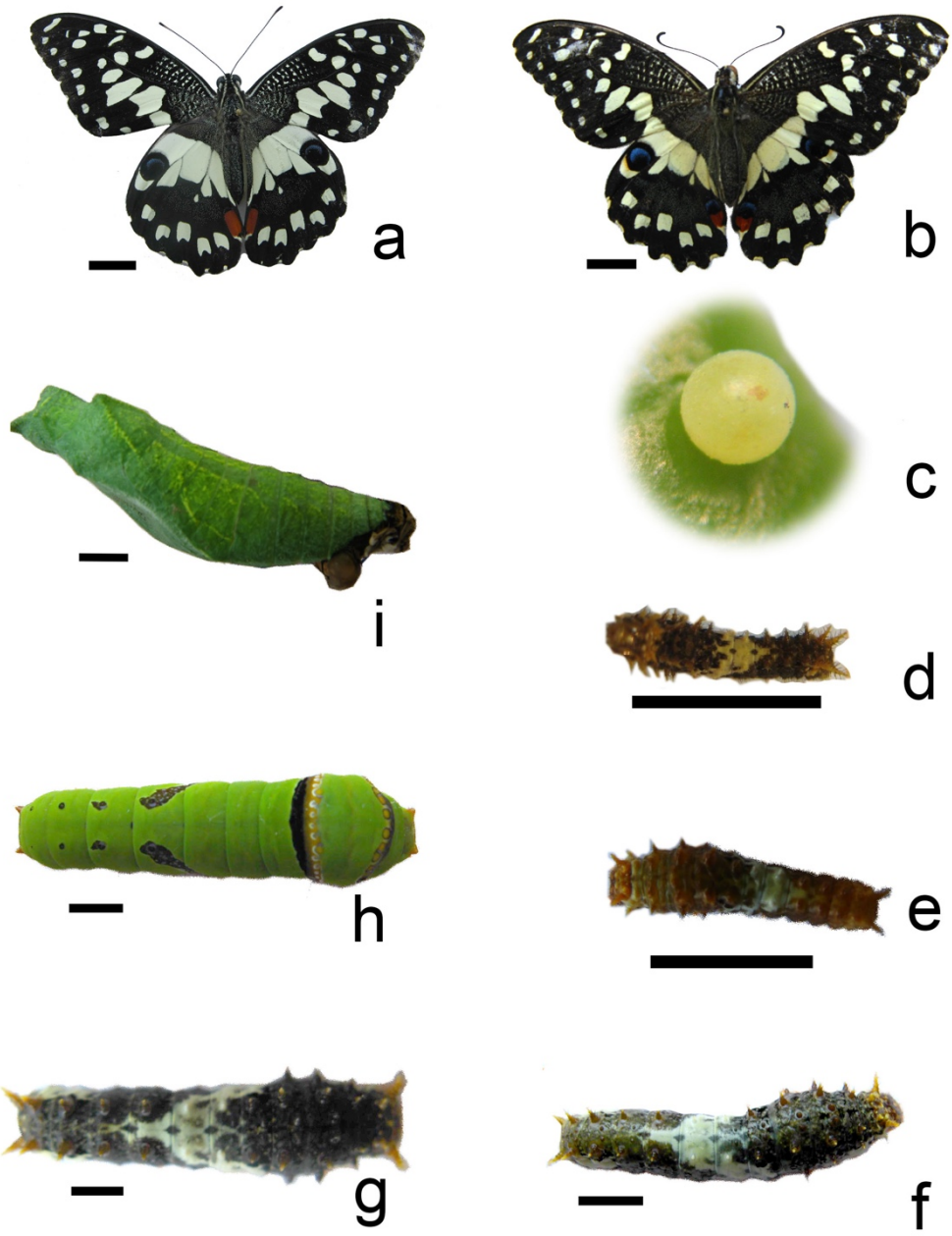

Figure 1. Lifecycle of The citrus butterfly *Papilio demoleus*: **a**. Female butterfly; **b**. Male butterfly; **c**. Egg; **d**. 1st instar larvae; **e**. 2nd instar larvae; **f**. 3rd instar larvae; **g**. 4th instar larvae; **h**. 5th instar larvae; **i**. Pupa. Scale bars: **a** & **b**, 10 mm; **c**–**i**, 5 mm.

Statistical Analysis: Statistical analysis was performed using SigmaPlot version 12.2. Means were compared using Student-Newnan-Keuls method in one-way ANOVA to assess impact of temperature on incubation period and development time of the immature stage of *P. demoleus* and impact of artificial diets on adult longevity. Differences in probability level ($P \leq 0.05$) were considered significant.

Results and Discussion

Development time of eggs and immature stages of *P. demoleus*

The development periods of incubation and immature stage of *P. demoleus* on citrus trees at various temperatures are summarized in Table 1. Our results indicated that the egg-hatching time and development period of *P. demoleus* immature stages decreased as temperature increased. All of the developmental stages of the species were affected by change in temperature ranging from 25^0 to 30^0C ($P<0.001$). However, there were no significant differences in incubation period at 25^0C and 26.5^0C ($P=0.436$). The larvae typically had five stadia and total development time of nymphal stages varied at different temperatures ($P<0.001$). The longest development duration of larvae was observed at 25^0C valuing 17.93 days, while the shortest was 11.5 days at 30^0C. The development period of larval stage was about 4-5 times longer than that of incubation time at all temperatures tested. Pupal period at 25^0C and 30^0C was 12.46 days and 7.33 days, respectively.

Table 1. Mean (days±SE) development period of incubation and immature stage of *Papilio demoleus* L. on citrus trees at various temperatures

Development stages	Mean (days±SE) development duration of egg and immature stages			
	30^0C, 70%r.h.	**28.5^0C, 77.2%r.h**	**26.5^0C, 86.5%r.h.**	**25^0C, 70%r.h.**
Incubation period	2.60 ± 0.10^{c} (n=25)	3.08 ± 0.14^{b} (n=35)	3.48 ± 0.15^{a} (n=33)	3.55 ± 0.20^{a} (n=20)
1st larvae	1.70 ± 0.12^{c} (n=25)	2.31 ± 0.08^{b} (n=30)	2.61 ± 0.13^{a} (n=29)	2.78 ± 0.17^{a} (n=18)
2nd larvae	1.96 ± 0.13^{c} (n=24)	2.19 ± 0.08^{c} (n=27)	2.56 ± 0.12^{b} (n=27)	2.94 ± 0.13^{a} (n=17)
3rd larvae	1.61 ± 0.12^{c} (n=23)	2.29 ± 0.09^{b} (n=25)	2.64 ± 0.12^{a} (n=24)	3.00 ± 0.16^{a} (n=16)
4thlarvae	2.60 ± 0.13^{b} (n=22)	2.70 ± 0.11^{b} (n=25)	3.42 ± 0.10^{a} (n=24)	3.57 ± 0.14^{a} (n=14)
5th larvae	3.60 ± 0.13^{d} (n=20)	4.08 ± 0.11^{c} (n=25)	4.91 ± 0.14^{b} (n=23)	5.43 ± 0.17^{a} (n=14)
Total larval period	11.50 ± 0.27^{d} (n=20)	13.60 ± 0.25^{c} (n=25)	16.17 ± 0.35^{b} (n=23)	17.93 ± 0.32^{a} (n=14)
Pupal period	7.33 ± 0.37^{c} (n=18)	7.39 ± 0.28^{c} (n=23)	10.68 ± 0.39^{b} (n=22)	12.46 ± 0.45^{a} (n=13)

Means followed by the different letter within a row are significantly different (Student-Newnan-Keuls test one-way ANOVA; $P\leq0.05$)

Like other ectotherms, development of insect species is influenced by climatic conditions, especially temperature (Bale *et al.* 2002). In this study, it could be suggested that duration of *P. demoleus* development stages was significantly dependent on changes in temperature under laboratory condition. Badawi (1981) reported that the longest and shortest periods recorded were 6.1 and 3.1 days for the egg stage, 22.7 and 12.9 days for the larval stage and 22.4 and 8.0 days for the pupal stages respectively, depending on temperature regimes tested. The infestation of *P. demoleus* increased significantly with the increase in maximum temperature (Chatterjee *et al.* 2000). The egg, larval, pupal and adult stages lasted 3.4 days, 13.1 days, 9.9 days and 1.5 days, respectively (Dang 2005). The total larval period of *P. demoleus* was observed to be 17.98 ± 2.08 days. The pupal period was 11.57 ± 0.92 days (Nandni *et al.* 2012).

We believe that differences in total development period of *P. demoleus* could be attributed to differences in environmental factors, rearing methods or host plant quality.

Table 2. Mean (days±SE) pre-reproduction and generation time of *P. demoleus* L. on citrus trees at various temperatures and relative humidity levels

Temperature and humidity	Pre-reproduction time		Generation time	
	n	Mean (days±SE)	n	Mean (days±SE)
30^0C, 70% r.h.	16	1.13±0.08^c	13	22.88±0.48^d
28.5^0C, 77.2% r.h	22	1.40±0.11^c	20	25.40±0.44^c
26.5^0C, 86.5% r.h.	20	1.47±0.11^b	17	31.50±0.59^b
25^0C, 70% r.h.	11	2.27±0.14^a	11	35.50±0.73^a

Means followed by the different letter within a column are significantly different (Student-Newnan-Keuls test one-way ANOVA; P≤0.05)

Life cycle of *P. demoleus* on citrus trees at different temperatures

The results of the experiments revealed that pre-oviposition and generation time of *P. demoleus* were affected by temperature, which decreased following increase in temperature (Table 2). The duration of pre-reproduction ranged from 1.13 days to 2.27 days at different temperatures tested; however, no significant difference was found at 28.5^0 and 30^0C. The pre-reproduction period in temperature 30^0C was about half that of in temperature 25^0C. Similarly, mean generation time of *P. demoleus* was significantly longer at 25^0C with a value of 35.5 days than at any other temperature regimes tested, ranging from 22.88 days at 30^0C, 25.4 days at 28.5^0C and 31.5 days at 26.5^0C.

Our findings were consistent with previous studies. For example, Rafi *et al.* (1996) showed that pre-oviposition period was 1-2 days. Lewis (2012) reported that the average length of a *P. demoleus* generation varied from 26 to 59 days, and this pest is capable of producing multiple generations in a year depending on environmental variables, including temperature. Dang (2005) also found that life cycle of *P. demoleus* was approximately 25–36 days, the average being 29.5 days at 28.6^0C and 77.5% r.h. Similar results reported by Nandni *et al.* (2012) suggest that average generation time of *P. demoleus* reared on citrus leaves was approximately 29.55 ± 3.06 days.

Fecundity and longevity of *P. demoleus*

In this study, the fecundity of *P. demoleus* reared on honey 100% was low, with production an average of 21.67eggs/female (Table 3). The average egg production of a female was highest (13.06 eggs/female) on the 2nd date after adult emergence. *P. demoleus* fecundity decreased steadily on the 5th day with only 0.27 eggs/female. Our results were similar to an earlier study by Dang (2005), who reported that reproduction capacity of *P. demoleus* was observed to be very low, with only 22.9 eggs/female at temperature 28.4^0C and 75.2%r.h. Similarly, Rafi *et al.* (1996) showed that the number of eggs produced by a single female of *P. demoleus* ranged from 10 to 45 eggs, depending significantly on environmental variables and

other biotic factors. However, reproduction values of *P. demoleus* on citrus leaves obtained by Mishra and Pandey (1965) were higher than those on honey, ranging from 10 to 183 eggs/female. Also, on citrus plants in Southern Iran, Sharifi and Zarea (1970) found that a female may lay up to 110 eggs. Our results find that, compared to many of insect pests belonging to Lepidoptera family, the fecundity of *P. demoleus* was still low in despite of availability of food sources. This is a reason why *P. demoleus* rarely become an epidemic species on citrus in Vietnam.

Table 3. Fecundity of *P. demoleus* L. female adults reared on a 100% honey diet

Observation dates	**Number of eggs/female/day**		
(after adult emergence)	Minimum	Maximum	Mean (eggs±SE)
1st day	3	12	5.86±0.86 (n=15)
2nd day	6	21	13.06±1.35 (n=15)
3rd day	0	5	1.80±0.44 (n=15)
4th day	0	2	1.00±0.23 (n=11)
5th day	0	1	0.27±0.14 (n=11)
Mean eggs/female			21.67±1.07

Table 4. Longevity of *P. demoleus* L. adults reared on different artificial diets (at 30^0C and 70%r.h.)

Artificial diets	**n**	**Mean longevity of *Papilio demoleus* L. adults**		
		Minimum	Maximum	Mean (±SE)
Disttiled water	10	1	2	1.10±0.18[c]
Honey 20%	10	2	4	3.23±0.26[a]
Honey 50%	10	2	5	3.50±0.42[a]
Pure honey	10	3	5	4.17±0.25[a]
Sugar 50 % + water solution	10	1	4	2.52±0.40[b]

Means followed by the different letter within a column are significantly different (Student-Newnan-Keuls test one-way ANOVA; P≤0.05)

In nature, adult female *P. demoleus* require a source of nutrition, provided by sources such as pollen, nectar and honeydew or by host feeding, to promote longevity and facilitate egg production. Longevity of the female adult is an important life table parameter to conserve generation development and increase species population growth. In this study, longevity of *P. demoleus* was examined on different diets of honey, sugar and water considered as natural food source of the species (table 4). Results of statistical analysis revealed that differences in concentration of honey in diets did not affect adult lifespan of *P. demoleus* (P>0.05). The mean

adult longevity of the species was lowest (1.1 days) reared on distilled water, while this value was 2.52 days on a diet of sugar solution. Maximum longevity of *P. demoleus* adults reared on honey diets was about 5 days. A similar study by Dang (2005) showed that the longevity of female adult was about 2-5 days on pure honey diet at 28.4^{0}C and 75.2%r.h. Additionally, seasonal climatic condition may influence adult longevity of the pest. For example, Badawi (1981) reported that adult longevity on citrus ranged from 4 to 6 days with an average of 5.1 days during spring. Similar report on mandarin plants carried out by Singh and Gangwar (1989), mean lifespan of male and female adult was 5.1 days and 5.8 days, respectively.

Leaf-feeding capacity of *P. demoleus* larvae at different ages

Larvae of the citrus butterfly showed a different capacity of food consumption during the larval instars. The mean leaf quantity consumption in gram was presented in Table 5. Leaf-feeding capacity of *P. demoleus* larvae on citrus leaves had a positive correlation with nymphal ages. Leave feeding activities of 4th and 5th larvae were rapid in comparison to other larval stages. Our results are consistent with the results of Nandni *et al.* (2012) and Dang (2005).

Table 5. Mean leaf consumption of age-specific larvae

Age-specific larvae	Leaf consumption (g/day)		
	Minimum	Maximum	Mean (±SE)
1st larvae	0.008	0.34	0.13 ± 0.02^e
2nd larvae	0.08	0.71	0.30 ± 0.02^d
3rd larvae	0.16	1.22	0.63 ± 0.05^c
4th larvae	0.26	3.43	1.19 ± 0.13^b
5th larvae	0.66	4.56	2.68 ± 0.15^a

Means followed by the different letter within a column are significantly different (Student-Newnan-Keuls test one-way ANOVA; P≤0.05)

Conclusion

Our results show that temperature is a key factor influencing the development of *P. demoleus.* The egg-hatching time and development period of *P. demoleus* immature stages decreased as temperature increased from 25^0 to 30^{0}C. Also, temperature plays an important role in the decrease of populations in winter moths and in the relative abundance of *P. demoleus* in warm months where mean generation time is shorter. Fourth and fifth larvae of *P. demoleus* showed a high capacity of citrus leave consumption, compared to other instars. Moreover, adult longevity of the species was short ranging from 2 to 5 days when rearing on honey diets. In term of fecundity, the mean number of eggs laid by a *P. demoleus* female were low (21.67 eggs) in despite of availability of food source and rearing condition. Due to low reproduction rate, *P. demoleus* is less likely to damage severely on citrus plats in Vietnam. Results of study on biological traits of *P. demoleus* would be valuable scientific evidence on building citrus butterfly management programs.

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