Biology of *Diaphorina citri* (Hem., Psyllidae) on different hosts and at different temperatures

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Ms. received: January 16, 2007; accepted: July 17, 2007

Abstract: The objective of this work was to study the effect of different hosts and temperatures on *Diaphorina citri* Kuwayama (Hem., Psyllidae) biology. *Citrus limonia* (Rangpur lime), *Murraya paniculata* (orange jessamine) and *Citrus sunki* (Sunki mandarin) were used as hosts. Measurements included duration and viability of the egg and nymphal stages, sex ratio, fecundity and longevity. In order to verify the effect of temperature on *D. citri* biology, the duration and viability of its developmental stages and biological cycle were compared at seven different temperature conditions. Durations of the embryonic and nymphal stages were similar for Rangpur lime, orange jessamine and mandarin. However, the nymphal viability obtained on mandarin was lower than the values obtained on the other hosts. Fecundity was higher on orange jessamine and, in all instances, females had greater longevity than males. A fixed number of instars (five) was obtained on the three hosts. Rangpur lime and orange jessamine provided better *D. citri* development when compared with mandarin. Duration of the egg and nymphal stages varied from 2.6 to 7.7 and from 9.4 to 35.8 days, respectively, at temperatures from 18 to 32°C. Egg viability was higher than 81.6% at the temperature range studied (18–32°C); nymphal viability was higher than 70% at the 18–30°C range, differing from viability at 32°C, which was dramatically reduced. The *D. citri* lower temperature development threshold (TT) and thermal constant (*K*) values for the egg, nymphal and biological cycle (egg–adult) stages were 12.0°C and 52.6 Degree-Day (DD); 13.9°C and 156.9 DD; and 13.5°C and 210.9 DD respectively.

Key words: *Murraya paniculata*, citrus, citrus pest, huanglongbing, temperature requirements

1 Introduction

Citriculture is an important source of hard currency for Brazil, because citric products are among the country's top agricultural exports, in the form of both juice and fresh fruits, representing an income of more than US$1.5 billion per year. The state of São Paulo is responsible for 80% of this total (FNP CONSULTORIA & COMÉRCIO 2004).

Even though Brazil uses cutting-edge citrus production technology, some aspects still deserve attention, particularly with regard to phytosanitary problems that reduce yield and make it harder to export to more demanding consumer markets, which establish non-tariff barriers. Exotic citrus pests and diseases, introduced to Brazil during the last decades, resulting from the globalization of economic markets, have caused high yield losses and raised concerns among professionals (researchers, agriculturists and technical persons) dealing with citrus production measures.

In July 2004, citrus huanglongbing was observed for the first time in Brazil. Huanglongbing is reported to cause total yield loss (Bové 2006). This disease is caused by the bacteria *Liberobacter asiaticum*, *Liberobacter africanum*, and the newly discovered *Liberobacter americanum* (Teixeira et al. 2005; Bové 2006). The last two bacteria have been identified in citrus orchards in the state of São Paulo. These bacteria are transmitted by the psyllid *Diaphorina citri* Kuwayama (Hem., Psyllidae) (Bové 2006).

For many years *D. citri* was considered an insect causing little damage and occurring only endemically in citrus orchards in several states of Brazil (Silva et al. 1968), including São Paulo (Gallo and Montenegro 1960). Chemical control was therefore performed only sporadically (Nakano et al. 1999). However, there is now increasing concern that the introduction of citrus huanglongbing may dramatically decrease citrus yields in Brazil, and may turn the state of São Paulo into a risk area. This would render it unsuitable for citriculture, as reported from other regions of the world, specifically China, South Africa, Thailand and the Philippines (Martinez and Wallace 1967; Fraser 1978; Bové 1986). Therefore, the objective of this study was to analyse the biology of *D. citri* on different hosts and at different temperatures, in order to establish continuous rearing for biological control purposes.

Table 1. *Mean (± SD) duration and survival of values for the egg and nymphal stages of* Diaphorina citri *and sex ratio of insects reared on different hosts*

Host	Duration (days)		Viability (%)		
	Egg^{ns}	Nymph^{ns}	Egg^{ns}	Nymph	Sex ratio^{ns}
'Rangpur' lime	3.61 ± 0.62	14.00 ± 0.56	87.50 ± 2.49	82.35 ± 6.27 a	0.50 ± 0.05
Orange jessamine	3.63 ± 0.49	14.11 ± 0.28	88.36 ± 1.93	88.82 ± 5.21 a	0.50 ± 0.06
'Sunki' mandarin	3.57 ± 0.64	13.46 ± 0.51	89.78 ± 2.35	44.61 ± 9.10 b	0.47 ± 0.07

Mean values followed by the same letter in the column are not different by Tukey test (P ≤ 0.05). ns, non-significant.
Temperature: 24 ± 2°C, RH: 70 ± 10%, and 14 : 10 h (light : dark) photoperiod.

2 Materials and Methods

2.1 Obtaining insects

Insects adults and nymphs were collected in citrus groves located at Escola Superior de Agricultura "Luiz de Queiroz", Universidade de São Paulo (ESALQ/USP). To accomplish this, citrus branches containing *D. citri* nymphs were cut and transported to the laboratory, and then placed in containers with cotton soaked in water, until adults emerged. Hand-held aspirators were used to collect adults. The insects were reared in the laboratory on *Citrus sinensis* plantlets for one generation before the studies were carried out. The rearing room was air-conditioned at a temperature of 25 ± 1°C, with a relative humidity (RH) of 70 ± 20%, and a 14 : 10 h (light : dark) photoperiod.

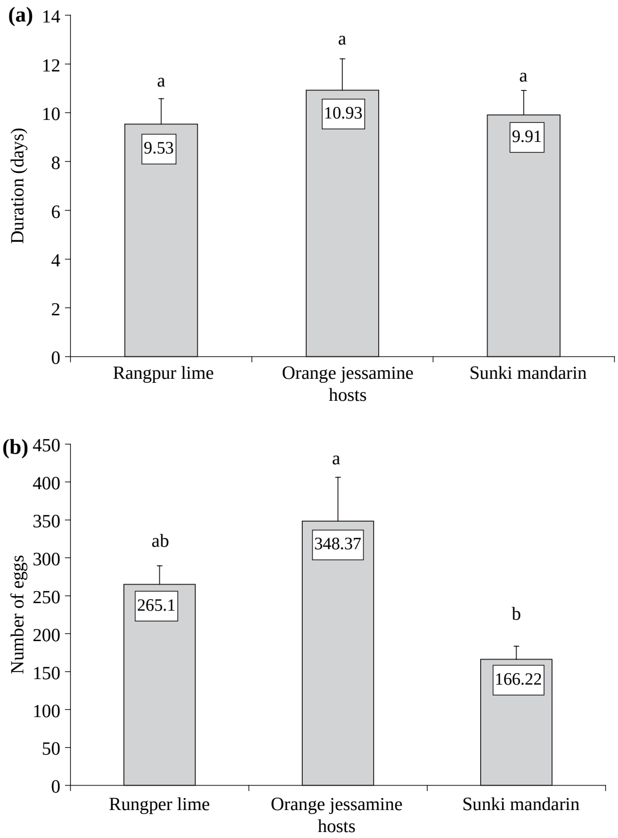

Fig. 1. *Pre-oviposition period (a) and mean number of eggs (b) of* Diaphorina citri *reared on different hosts. Temperature: 24 ± 2°C, RH: 70 ± 10% and 14 : 10 h (light : dark) photoperiod. Mean values followed by a common letter do not differ by Tukey test (P ≤ 0.05)*

2.2 Citrus and orange jessamine seedling cultivation

Seedlings of two citrus species (*Citrus limonia* and *Citrus sunki*) and the most common host of *D. citri* – orange jessamine (*Murraya paniculata*) (Fauvergue and Quilici 1991; Skelley and Hoy 2004) – were cultivated, individually in plastic seedling tubes (12 × 3.5 cm), containing a mixture of dung, compost and vermiculite (1 + 1 + 1) as substrate for citrus, and a mixture of dung, sand and compost (2 + 1 + 1) for orange jessamine. The plantlets were kept in a greenhouse until they reached approximately 25 cm, and were then pruned to obtain the shoots used in the experiments.

2.3 Rearing system

The *D. citri* rearing system consisted of a transparent acrylic cage for oviposition and another for nymphal development. Approximately 80 plantlets were placed in the egg-laying cage (34 × 34 × 40 cm), in seedling tubes arranged on a metal rack. One hundred insects were used for oviposition at a ratio of 1♀ : 1♂. After 2 days, the adults were removed with a hand-held aspirator and the seedlings were transferred into the nymphal development cage (70 × 50 × 50 cm). The seedlings of *C. sinensis* were irrigated daily and remained in the cage until adult emergence. Then, the seedlings were pruned to obtain shoots and were used again in the stock rearing.

2.4 Biology on different hosts

Citrus limonia, *C. sunki* and *M. paniculata* seedlings with a height of approximately 25 cm, containing shoots, were used in the study. Seedlings in the seedling tubes were placed

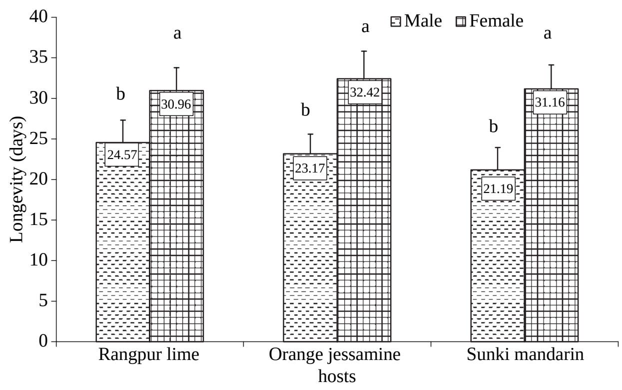

Fig. 2. *Mean longevity of* Diaphorina citri *males and females reared on different hosts. Temperature: 24 ± 2°C, RH: 70 ± 10% and 14 : 10 h (light : dark) photoperiod. Mean values followed by a common letter do not differ by Tukey test (P ≤ 0.05)*

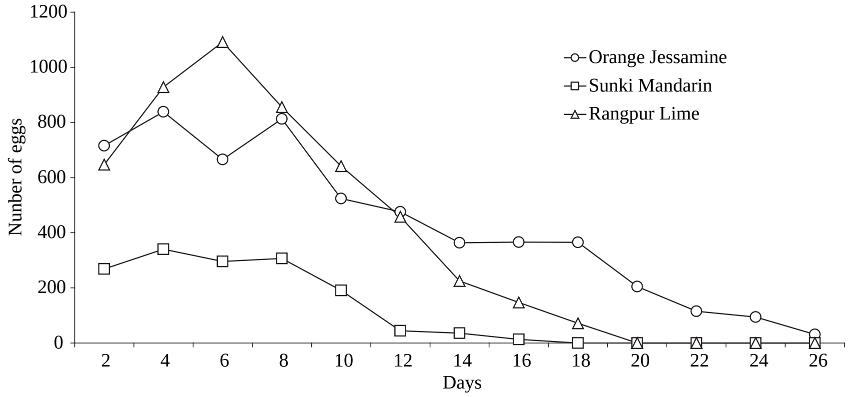

Fig. 3. *Diaphorina citri egg-laying rhythm when reared on different hosts. Temperature: 24 ± 2°C, RH: 70 ± 10%, and 14 : 10 h (light : dark) photoperiod*

Table 2. *Mean generation duration (T), net reproductive rate (R_0), reproductive capacity (r_m), and finite rate of increase (λ) of Diaphorina citri reared on three hosts.*

Hosts	T	R_o	r_m	λ
'Rangpur' lime	36.16	86.03	0.1232	1.130
Orange jessamine	44.42	92.15	0.1018	1.107
'Sunki' mandarin	37.55	13.70	0.0697	1.072

in plastic cups (12 × 3.5 cm) containing moistened vermiculite to maintain humidity and to support the seedling tube. Then, each seedling tube and plastic cup was placed inside a cage constructed from a 2-l soda bottle.

For nymphal development females in the oviposition period were placed in cages, having several seedlings of the mentioned hosts at their disposal. After 24 h, the females were removed and only 20 plantlets of each host tested containing eggs of the psyllid were used. The eggs were counted and the plantlets individualized in the cages. Evaluations included viability of the egg, duration of the egg and nymphal stages, and sex ratio.

In addition, the number of instars was determined for the nymphal stage, based on daily head capsule width measurements of 14 nymphs. Measurements were obtained using a ocular micrometer attached to a stereoscopic microscope. The number of instars was determined based on Dyar's (1890) method.

For tests of the adult stage, 20 adult pairs < 24 h old were placed in the cages previously described. Separation by sex was based on Aubert (1987). Determinations included the pre-oviposition period, fecundity and longevity of males and females.

2.5 Biology at different temperatures

Citrus limonia seedlings, grown in seedling tubes as described previously, were used as hosts. The egg and nymphal stages were monitored at temperatures of 18, 20, 22, 25, 28, 30 and 32°C, all with 70 ± 10% RH and 14 : 10 h (light : dark) photoperiod.

For the embryonic period, *C. limonia* shoots containing 24-h-old eggs were collected. The shoots were distributed among acrylic plates (6.0 × 1.5 cm), containing moistened filter paper at the bottom. One hundred and fifty eggs were used at each temperature, with a total of six replicates. The dishes were sealed and kept in incubators to determine the duration and viability of the egg stage.

For the nymphal stage, 20 insects up to 24 h of age were transferred to Rangpur lime seedlings with six replicates, totalling 120 insects per temperature value. Each seedling tube containing a plantlet was placed into a cup containing vermiculite, in order to fix the plant and maintain moisture. Daily observations were made to evaluate mean nymphal duration and viability. The experimental design was completely randomized with seven treatments (temperatures) and six replicates (acrylic dishes and plants).

2.6 Data analysis

The data were submitted to analysis of variance and the means were compared by Tukey tests ($P \leq 0.05$) with the program SAS (SAS Institute 2000). Percentage data were transformed to arcsine $\sqrt{P/100}$. A fertility life table was used to determine the best host for *D. citri* development. Calculations were made for net reproductive rate (R_0), mean time for each generation (T), intrinsic rate of population increase (r_m), and finite rate of increase (Λ). Based on the duration values for the egg and nymphal stages and for the egg–adult period, the lower temperature development threshold (T_b) and thermal constant (K) were determined, using the hyperbole method (Arnold 1960).

3 Results and Discussion

3.1 Biology on different hosts

The hosts evaluated did not affect the duration and viability of the *D. citri* egg stage, which were around 3.6 days and 88% respectively (table 1). Duration of the nymphal stage was also similar in the three hosts, between 13.5 and 14 days. Viability was significantly lower for insects reared on Sunki mandarin (table 1), indicating that the best hosts to rear the psyllid are Rangpur lime and orange jessamine. Catling (1970) reported a duration of 3 days for the embryonic period and 11–15 days for the nymphal stage, at temperatures of 25–26°C. Even though this psyllid has a relatively large number of hosts in the Rutaceae family (*Murraya paniculata*, *Citrus aurantifolia*, *C. lemonon*, *C. sinensis*, *C. medica*, *C. nobilis*, *C. reticulate*, *C. deliciosa*, *C. paradise*, *C. hystrix*, *C. grandis*, *Microcitrus australisiaca*, *Trifhasia trifoliate*, *Fortunella* sp., *Poncirus trifoliate*, *M. koenigii*, *Toddalia asiatica*, *Vepris lanceolata*, *Coriea* sp., *Atalantia* sp., *Clausena lansium*), they cannot develop completely in all of them (Aubert 1985, 1987). *M. paniculata* and *C. aurantifolia* are the preferred species for feeding, egg-laying and nymphal development (Aubert 1985). Our egg-stage results are very

Table 3. *Mean values (± SD) (values between parentheses indicate the variation interval) for* Diaphorina citri *head capsule width, Dyar's constant and coefficient of correlation, when reared on different hosts, with respective equations*

Host	Mean head capsule width (mm)					Dyar's Constant (k)	Correlation Coefficient (R)	Equation
	1st instar	2nd instar	3rd instar	4th instar	5th instar			
Rangpur lime	0.11 ± 0.008 (0.08–0.13)	0.18 ± 0.007 (0.15–0.19)	0.32 ± 0.01 (0.26–0.35)	0.43 ± 0.009 (0.40–0.45)	0.56 ± 0.011 (0.51–0.61)	1.45	0.999	$y = 0.3735x - 2.3824$
Orange jessamine	0.12 ± 0.006	0.18 ± 0.02	0.35 ± 0.03	0.44 ± 0.01	0.57 ± 0.04	1.44	0.999	$y = 0.3656x - 2.3204$
Sunki mandarin	0.12 ± 0.005 (0.09–0.14)	0.19 ± 0.006 (0.17–0.21)	0.32 ± 0.01 (0.30–0.34)	0.43 ± 0.01 (0.39–0.46)	0.56 ± 0.02 (0.50–0.61)	1.42	0.999	$y = 0.3517x - 2.2908$

*$P \le 0.05$.
Temperature: 24 ± 2°C, RH: 70 ± 10%, and 14 : 10 h (light : dark) photoperiod.

near to those reported by Tsai and Liu (2000) on four different hosts: *C. jambhiri*, *C. aurantium*, *C. paradisi* and *M. paniculata*. They also observed differences in the nymphal stage. The nymphal stage duration was 12.8 and 12.6 days when reared on *M. paniculata* and *C. paradisi*, respectively, which differed from that when reared on *C. jambhiri* and *C. aurantium*, with values of 13.5 and 13.1 days respectively. In general, these values are lower than those verified in the present study for Rangpur lime, orange jessamine and mandarin, i.e. 14.0, 14.1 and 13.5 days respectively (table 1). These duration value variations might probably be related to the use of different hosts and temperatures, as well as different rearing techniques and insect strains. There was no influence of nutrition on either sex, as the sex ratio was nearly one male to one female on all hosts studied (table 1).

Diaphorina citri fecundity was higher on orange jessamine plantlets (348.4 eggs) and Rangpur lime (265.1 eggs), differing significantly from that in Sunki mandarin (166.2 eggs) (fig. 1b). Higher values than those recorded in the present research were observed by Liu and Tsai (2000) on orange jessamine (626 eggs) at 25°C. Despite the mean number of 348.4 eggs per female on orange jessamine, there was a variation from 79 to 668 eggs; consequently, there were females with higher egg-laying capacity, with numbers equivalent to those recorded by the authors cited. The great variation observed for these values could be related to several factors, such as the host used and rearing techniques developed to maintain *D. citri*. In addition, the biology of this citrus psyllid may be influenced by insect adaptations under laboratory conditions. In this study we worked with the second laboratory generation, but this information is not provided in Liu and Tsai's (2000) research.

The pre-oviposition period was similar for the three treatments, i.e. nearly 10 days (fig. 1a), indicating that upon emerging *D. citri* does not have a mature reproductive system. Consequently, in order to facilitate laboratory rearing, the insects can remain in a cage until approximately 10 days, without host replacement for oviposition, reducing labour costs.

Male longevity on the three species was similar, which was similar to that of the female (fig. 2). However, there was a significant difference on male and female longevity for each host, with females having greater longevity than males. Female longevity was near 30 days, i.e. lower than the 39.7 days recorded by Liu and Tsai (2000) on orange jessamine.

With reference to egg-laying rhythm, it was observed that the peak *D. citri* oviposition, when fed on Rangpur lime occurred from day 4 to 8, decreasing later until day 20 (fig. 3). With regard to insects fed on Rangpur lime, the same tendency was observed, but with a larger egg-laying period, which was extended until day 26. For *D. citri* fed on Sunki mandarin, however, a smaller oviposition period was observed and, like for the two other hosts evaluated, the peak oviposition occurred in the first 8 days (fig. 3). As a result, Rangpur lime and orange

Table 4. Diaphorina citri *instar duration mean (± SD) when reared on different hosts*

Host	Duration (days) 1st instar	2nd instar	3rd instar	4th instar	5th instar	Total
'Rangpur' lime	2.57 ± 0.14 a (2–3)	2.21 ± 0.11 a (2–3)	1.79 ± 0.15 a (1–3)	2.43 ± 0.23 a (1–4)	5.57 ± 0.29 b (4–7)	14.57
Orange jessamine	2.43 ± 0.17 a (2–4)	2.12 ± 0.18 a (1–3)	2.71 ± 0.22 a (1–4)	3.07 ± 0.20 ab (2–4)	4.57 ± 0.20 b (4–6)	14.93
'Sunki' mandarin	2.38 ± 0.15 a (2–4)	2.08 ± 0.14 a (1–3)	2.00 ± 0.16 a (1–3)	2.69 ± 0.17 a (2–4)	5.00 ± 0.16 b (4–6)	14.15

Mean values followed by the same letter in the column are not different by Tukey test (P ≤ 0.05).
Temperature: 24 ± 2°C, RH: 70 ± 10%, and 14 : 10 h (light : dark) photoperiod.

jessamine are the best two hosts for rearing *D. citri* in the laboratory, as observed for nymphal development. Therefore, as it has been defined that the highest percentage of eggs are laid on the first 10 days, we can recommend insect elimination after this period, in order to optimize rearing. Under field conditions, *D. citri* has been frequently reported on orange jessamine plants (Silva et al. 1968; Cermeli and Godoy 2000; Tsai et al. 2000). The plant is therefore considered an ideal host for *D. citri* development (Aubert 1987).

The mean *D. citri* generation time (*T*) was 36.2, 44.4 and 37.6 days on Rangpur lime, orange jessamine and Sunki mandarin respectively (table 2). Net reproductive rate (R_0) was higher on orange jessamine (92.2) and Rangpur lime (86.0), and lower on Sunki mandarin (13.7) (table 2). The other fertility life-table parameters showed the same tendency as the biological results obtained, i.e. the insect prefers orange jessamine and Rangpur lime in relation to mandarin.

Based on the frequency distribution of head capsule width measurements of *D. citri* nymphs, five instars were determined for insects reared on orange jessamine, Rangpur lime and Sunki mandarin, with a coefficient of determination (R^2) near 1 (table 3). Dyar's constant (*K*) was near the mean value established by the author, that is, 1.4, and within the interval allowed by Dyar's rule, from 1.1 to 1.9 (table 3). However, we were not able to discriminate the best host based on number of instars, since it was constant and equal to 5.

The instars showed variable durations; the first four were similar, differing from the fifth instar, which had a value close to 5 days on the three hosts (table 4). These values are similar to those determined by Liu and Tsai (2000), who found durations of 2.00, 1.59, 1.68, 2.36 and 5.21 days for the first, second, third, fourth and fifth instars respectively.

3.2 Temperature effect and temperature requirements determination

The mean duration of the embryonic period varied from 7.7 days at 18°C, to 2.6 days at 32°C (table 5). The temperature range studied did not affect egg viability, which oscillated between 81.6% and 95.2% (table 6). These values are near those reported by Liu and Tsai (2000), who determined a duration of 4.1 days and a viability of 95.5% at 25°C on orange jessamine.

Table 5. *Mean duration (± SD) for the egg and nymphal stages and biological cycle (egg–adult) of* Diaphorina citri *reared on Rangpur lime and at different temperatures (ºC)*

Temperature (ºC)	Duration (days) Egg	Nymph	Biological cycle
18	7.7 ± 0.67 a	35.8 ± 0.71 a	43.5 ± 1.31 a
20	6.4 ± 0.17 b	24.5 ± 0.34 b	30.9 ± 0.52 b
22	5.9 ± 0.16 b	23.8 ± 0.36 b	29.6 ± 0.66 b
25	4.5 ± 0.11 c	12.6 ± 0.26 c	17.1 ± 0.29 c
28	3.2 ± 0.19 d	12.2 ± 0.25 c	15.4 ± 0.16 cd
30	2.9 ± 0.17 d	9.4 ± 0.41 c	12.4 ± 0.24 d
32	2.6 ± 0.23 d	9.4 ± 0.66 c	12.1 ± 0.37 d

Mean values followed by the same letter in the column are not different by Tukey test (P ≤ 0.05).
RH: 70 ± 10%, and 14 : 10 h (light : dark) photoperiod.

Table 6. *Mean viability (± SD) for the egg and nymphal stages and biological cycle (egg-adult) of* Diaphorina citri *reared on Rangpur lime and at different temperatures*

Temperature (ºC)	Viability (%) Egg^{ns}	Nymph	Biological cycle
18	95.0 ± 0.68	70.7 ± 3.04 a	67.2 ± 1.31 a
20	95.2 ± 0.92	70.0 ± 2.41 a	66.6 ± 1.94 a
22	88.4 ± 3.98	72.5 ± 2.72 a	64.1 ± 2.63 a
25	93.8 ± 3.40	74.0 ± 4.64 a	69.4 ± 2.98 a
28	89.8 ± 2.58	77.5 ± 3.98 a	69.5 ± 1.62 a
30	90.5 ± 5.03	73.8 ± 3.65 a	66.8 ± 0.83 a
32	81.6 ± 4.50	7.0 ± 2.77 b	5.7 ± 2.61 b

Means followed by the same letter in the column are not different by Tukey test (P ≤ 0.05).
ns, non-significant.
RH: 70 ± 10% and 14 : 10 h (light : dark) photoperiod.

Table 7. *Threshold temperature (TT) thermal constant (K), development equation (1/D), and coefficient of determination (R^2) of* Diaphorina citri *biological cycle stages when reared on Rangpur lime*

Stage/period	TT (ºC)	*K* (DD)	Equation (1/*D*)	R^2
Egg	12.07	52.61	$y = 0.019007x - 0.229488$	0.9773
Nymph	13.94	156.88	$y = 0.006374x - 0.088836$	0.9465
Egg–adult	13.53	210.91	$y = 0.004741x - 0.064134$	0.9670

The lower temperature development threshold (TT) for the egg stage was 12.0°C and the thermal constant (*K*) was 52.6 Growing Degree-Day (GDD) (table 7). Liu and Tsai (2000) determined a TT of 8.9°C and a thermal constant of 67.6 DD for *D. citri* reared on orange jessamine in the USA. Therefore, there was a 3.0°C difference. This difference could be due to the fact that different populations were studied.

For the nymphal stage, there was a variation in duration from 9.4 to 35.8 days in the temperature range from 32 to 18°C (table 5). In the temperature range from 25 to 30°C, duration did not differ among the temperatures studied. Viability was near 70%, and values for the different temperatures were statistically similar, except at 32°C, in which viability was 7% (table 6). Temperatures higher than 32°C are unfavourable for the insect. Therefore, better results are expected when the insect is reared within the 18 to 30°C range. Although further testing is needed, it can be inferred that the insect has a preference in the field, for regions with milder temperatures. For the nymphal stage, TT and *K* were 13.9°C and 156.88 DD (table 7), respectively, which were therefore higher than the 10.81°C and 185.19 DD values determined by Liu and Tsai (2000) on orange jessamine.

For the biological cycle (egg–adult), variation was from 43.5 to 12.1 days at temperatures from 18 to 32°C (table 5). A duration of 17.1 days was determined at 25°C. This value is near the 16.9 days reported by Liu and Tsai (2000). Viability for the egg–adult period in the temperature range from 18 to 30°C was statistically similar, ranging from 69.9% to 64.1%. It was different, however, from the viability recorded at 32°C (5.0%) (table 6). A similar result (75.4%) was recorded by Liu and Tsai (2000), when *D. citri* was reared on orange jessamine at 25°C in Florida, USA. TT and *K* for the egg–adult period were 13.5°C and 210.9 DD respectively (table 7). The studies are important for the rearing of the parasitoid to be released into citrus-growing areas, as *T. radiata* has been recently found in São Paulo, Brazil (Gomez-Torres et al. 2006).

Acknowledgments

To Fapesp for granting a post-doctoral fellowship (proceeding no. 04/14216-6), Fundecitrus for financial support, and Prof. Marinéia L. Haddad for her help with the statistical analyses.

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