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# Thermal hygrometric requirements for the rearing and release of *Tamarixia radiata* (Waterston) (Hymenoptera, Eulophidae)

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ABSTRACT. Thermal hygrometric requirements for the rearing and release of *Tamarixia radiata* (Waterston) (Hymenoptera, Eulophidae). *Tamarixia radiata* is the main agent for the biological control of *Diaphorina citri* in Brazil with a parasitism rate ranging from 20 to 80%. This study investigated the influence of temperature on the development, fecundity and longevity of adults of *T. radiata* and the effect of relative humidity (RH) on their parasitism capacity and survival rate in the pre-imaginal period. The effect of temperature was assessed in the range between 15 and 35 ± 1ºC, 70 ± 10% RH, and a 14-h photophase. The RH effect was evaluated in the range from 30 to 90 ± 10%, temperature at 25 ± 1ºC, and photophase of 14-h. At 25°C, circa 166.7 nymphs were parasitized, the highest parasitism capacity observed compared to other treatments. The longest longevity of females was observed at 25°C, although the rate did not differ in the 20–30°C temperature range. The threshold temperature (TT) was 7.2°C, and 188.7 degrees-day were required for the development (egg-to-adult period). The parasitism rate and longevity were higher at 50 and 70% of RH. This shows that temperature and RH may affect the parasitism capacity of *T. radiata* on nymphs of *D. citri*, which can explain the great parasitism variation for *D. citri* observed in citrus groves in São Paulo State, Brazil.

KEYWORDS. Biological control; capacity; fecundity; longevity; psyllid.

*Diaphorina citri* Kuwayama, 1907 (Hemiptera, Liviidae) is one of the most important pests for citrus producing areas in Brazil. It is the insect vector of the bacteria "*Candidatus* Liberibacter americanus" and "*Candidatus* Liberibacter asiaticus", that are associated to the main citrus disease in the world, the Huanglongbing (HLB) also known as Citrus Greening Disease (Martinez & Wallace 1967; Teixeira *et al*. 2005). Although *D. citri* is considered an exotic insect and it was first detected in Brazil at the end of the 1930's (Costa Lima 1942; Parra *et al*. 2010), only after the occurrence of HLB that its control started to be systematically carried out with chemical pesticides (Belasque Júnior *et al*. 2010). Chemical pesticides, therefore, became the main control management procedure for *D. citri* in citrus groves. The frequent use of pesticides has caused several problems. They kill natural enemies and other important insects for citriculture, compromising the Integrated Pest Management (IPM) in groves where the biological control was already implemented (Yamamoto & Parra 2005). Because *D. citri* is a widespread pest in Brazil and given its potential as a vector insect for HLB, it is feared that the Brazilian citrus industry be compromised, similarly to what has occurred in other countries (Fundecitrus 2004; Parra *et al*. 2010).

The search for alternative control methods of *D. citri* employs biocontrol agents such as the cenobiont ectoparasitoid *Tamarixia radiata* (Waterston, 1922) Hymenoptera, Eulophidae). This parasitoid has been widely used in several countries due to its high parasitism capacity, good establishment and field adaptation, constituting an important biological control agent of *D. citri* (Chien & Chu 1996; Hoy & Nguyen 2000; Étienne *et al*. 2001).

In Brazil, the occurrence of *T. radiata* was first registered in 2004 in the municipalities of Piracicaba and Jaboticabal, São Paulo State, immediately after the detection of HLB. In the period from March to July 2005, this parasitoid was registered in all citrus groves in São Paulo State, Brazil, showing natural parasitism rates between 27.5 to 80.0% (Gómez-Torres *et al*. 2006). This parasitism capacity has been greatly reduced due to the systematic use of chemical products for the control of *D. citri* (J.R.P. Parra, unpubl. data).

Complex hygrometric variables can affect the development, emergence and fecundity of this parasitoid, and temperature and RH are the most relevant (King *et al*. 1985). Thus, this study investigated the thermal hygrometric requirements of *T. radiata* for its production, mass rearing and establishment after releases in different sites as part of the IPM used for the psyllid control, vector insect for HLB.

## MATERIAL AND METHODS

Insect rearing. We used *D. citri* and *T. radiata* from the rearing of Laboratory of Insect Biology from the Department of Entomology and Acarology from *Escola Superior de Agricultura "Luiz de Queiroz"– Universidade de São Paulo* (ESALQ-USP). The rearing of *D. citri* was adapted from the methods described in Skelley & Hoy (2004) and Nava *et al*. (2007), using seedlings of the orange jasmine (*Murraya paniculata* (L.) Jack), 25–30 cm high, cultivated in a substrate of vermiculite and vegetal compound at 1:1 ratio, and maintained in incubators (30 ± 1°C, 60 ± 10% RH, and a 14-h photophase). The plants with new sprouts were transferred to acrylic cages (34 x 34 x 40 cm) containing approximately 50 couples of *D. citri* to obtain eggs in a 24-h period. After oviposition, the plants were transferred to cages for nymph development (70 x 50 x 50 cm) and maintained in an incubators at 25 ± 1ºC, 70 ± 10% RH, and a 14-h photophase.

For the rearing of *T. radiata*, we used seedlings of the orange jasmine infested with nymphs in the 4th and 5th instars of *D. citri* (Chu & Chien 1991; Skelley & Hoy 2004; Nava *et al.* 2007). The seedlings of the orange jasmine containing nymphs in the 4th instar were placed for parasitism in cages (45 x 35 x 37 cm) for a 24-h period. Afterwards, the plantlets containing nymphs supposedly parasitized were placed in cages (60 x 50 x 52 cm) in an acclimatized room for *T. radiata* development. At emergence, 80% of the parasitoids were used for the experiments and the rest was used to maintain the rearing.

Effect of temperature on the parasitism capacity and survival rate of *T. radiata*. Twenty females of *T. radiata*, 24-h of age, mated, were placed individually in cylindrical plastic cages (15.5 x 5.5 cm), containing five holes at the top that were covered with *voile* fabric to allow aeration. The females were fed with a mixture of pure honey and pollen (1:1) (Chien *et al.* 1994), offered as fillets fixed to the inner sides of the cages. The nymphs of *D. citri* were exposed to *T. radiata* females for 24 h in incubators at 15, 20, 25, 30, and 35 ± 1°C, 70 ± 10% RH, and a 14-h photophase.

For each female of *T. radiata*, we offered daily an orange jasmine plants with 30 nymphs of *D. citri* in the 5th instar, according to Chu & Chien (1991). After parasitism, the females were removed from the cages using a glass tube (12 x 75 mm). The nymphs of *D. citri* and the orange jasmine plantlet were placed in an acclimatized room kept under the same environmental conditions (temperatures, RH and photophase) during the parasitism period. We evaluated the number of nymphs parasitized daily, the accumulated parasitism rate, the total number of nymphs parasitized per female and longevity of females of *T. radiata*.

The experiment design used was a completely randomized with five treatments (temperatures) and four repetitions, containing four orange jasmine plantlets with 30 nymphs each.

Effect of temperature on the egg-to-adult period and thermal requirements. The orange jasmine plantlets with nymphs of *D. citri* in the 5th instar were offered to the females of *T. radiata* for a period of 24 h at 25 ± 1ºC, 70 ± 10% RH, and a 14-h photophase, in acrylic cages (15.5 x 5.5 cm) with holes on top covered with *voile* fabric to allow aeration. Afterwards, the females of *T. radiata* were removed from the cages and the orange jasmine plantlets with nymphs were placed in acclimatized rooms at 18, 20, 22, 25, 28, 30 and 32ºC, 70 ± 10% RH, and a 14-h photophase. Three hundred nymphs were observed at each temperature (treatments), they were divided into 10 repetitions (plantlets) with 30 nymphs per plant. Based on data from the egg-to-adult period, we calculated the threshold temperatures (TT), and the thermal constant (K) (Haddad *et al.* 1999).

Effect of RH on the parasitism capacity and development of the larval stages of *T. radiata*. The experiment was carried out in acclimatized rooms with regulated to 30, 50, 70 and 90 ± 10% HR, 25 ± 1ºC and, a 14-h photophase. Females of *T. radiata*, 24-h of age and mated, were placed individually in acrylic cages (35 x 35 x 45 cm) where they were fed with a droplet of pure honey and pollen (1:1) placed on the inner side of the cage.

An orange jasmine plantlet with 30 nymphs of *D. citri* in the5th instar was offered to each female of *T. radiata*. After 24 h, the females were removed from the cages using a glass tube (12 x 75 mm) and the nymphs were kept in acclimatized rooms under the environmental conditions described above.

We carried out daily observations to determine the rates parasitism and emergence of *T. radiata*. The parasitism rate was evaluated on the 5th day after offering the plantlets to the nymphs, when the parasitized nymphs had the mummy formation.

The experiment comprised a completely randomized design with five treatments (RH) and four repetitions, containing four plantlets with 30 nymphs each.

Data analysis. To determine the effect of temperature on the number of parasitized nymphs, the data were subjected to the analysis of variance (ANOVA) and the means were compared by the Tukey test ($p \leqslant 0.05$). Data on longevity were analyzed in the Kaplan-Meier estimator and later compared in the long-rank test using the R program (R Development Core Team 2011).

The temperature requirements were calculated in the hyperbole method (Haddad *et al.* 1999). To determine the effect of RH on rates of parasitism and emergence, the data were analyzed in the polynomial regression model. The analyses were preformed in the SAS program (Statistical Analysis System), version 9.2, 2002–2008 (SAS Institute 2002).

## RESULTS

The total parasitism capacity of *T. radiata* greatly differed among the temperatures tested ($F = 3.310$; $gl = 4$; $P = 0.039$), and the highest parasitism capacity was observed at 25°C followed by 30°C. At 20, 35 and 15°C, we observed a smaller number of nymphs parasitized, indicating that these temperatures are less adequate for the reproduction of *T. radiata* (Fig. 1). Regarding the daily parasitism, we observed that at 25°C, the females parasitized a larger number of nymphs, followed by 30°C (Fig. 2A).

In the other temperature conditions, the daily parasitism was lower, and at 15 and 35°C, parasitism occurred on the 1st day of oviposition and did not surpass 10 parasitized nymphs (Fig. 2A). Furthermore, we observed that because these extreme temperatures (15 and 35ºC) negatively affected the life span of *T. radiata*, there was a shorter period of parasitism and 100% of parasitism was attained at these temperatures on the 6th day, while 100% of parasitism was attained between the 11th and 13th day at 20, 25 and 30ºC (Fig. 2B).

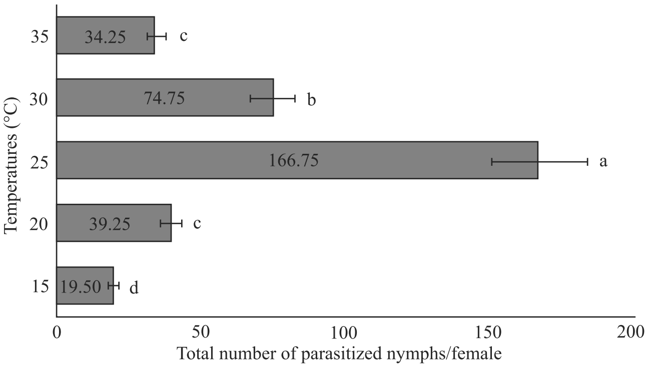

Fig. 1. Parasitism capacity of *Tamarixia radiata* on nymphs in the 5th instar of *Diaphorina citri* at different temperatures. 70 ± 10% RH, and a 14 h photophase. Means followed by the same letter, in the bars, are not significantly different, as assessed in the Tukey test ($P \leqslant 0.05$).

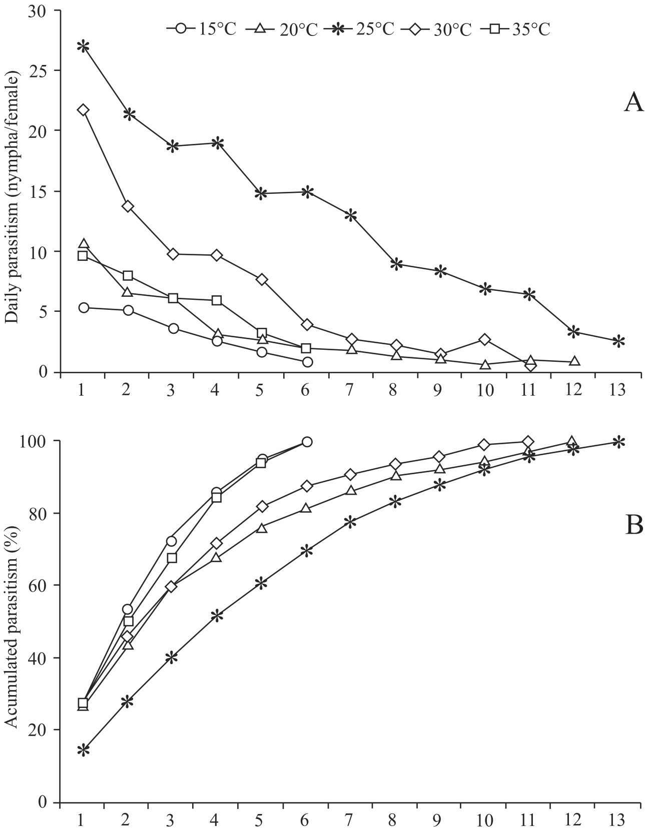

Fig. 2. Average parasitism rate daily (A) and accumulated (B) of *Tamarixia radiata* on *Diaphorina citri* at 15, 20, 25, 30 and 35 ± 1°C, 70 ± 10% RH, and a 14h photophase.

Longevity of females of *T. radiata* subjected to parasitism of *D. citri* was affected by the different temperatures ($\chi^2 = 12.3$; $df = 4$; $P = 0.001$). The longest life span was observed at 20, 25 and 30ºC, respectively, which were markedly different from values found at 15 and 35ºC (Fig. 3).

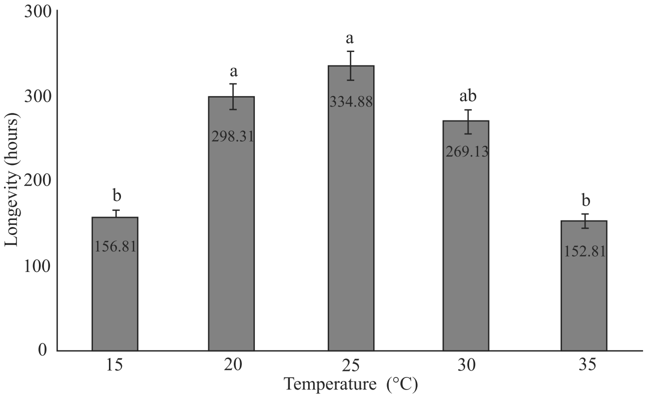

Fig. 3. Longevity of females of *Tamarixia radiata* at different temperatures, 70 ± 10% RH, and a 14h photophase. Averages of longevity were analyzed in the Kaplan-Meier estimator. Means followed by the same letter, in the bars, are not significantly different in the Tukey test ($P \leqslant 0.05$).

The duration of the egg-to-adult period of *T. radiata* was inversely proportional to the temperature rates studied, obtaining durations (± SEM) of 17.31 ± 0.13, 14.20 ± 0.12, 12.43 ± 0.13, 10.33 ± 0.13, 10.09 ± 0.11, 7.55 ± 0.19 and 7.59 ± 0.21 days for temperatures at 18, 20, 22, 25, 28, 30, 32ºC, respectively, significantly differing from one another ($F = 2.761$; $df = 6$; $P = 0.025$). Based on data on the egg-to-adult period duration of *T. radiata*, we determined the temperature threshold of 7.2°C and the thermal constant of 188.7 degrees-day ($R^2 = 0.9470$) (Fig. 4).

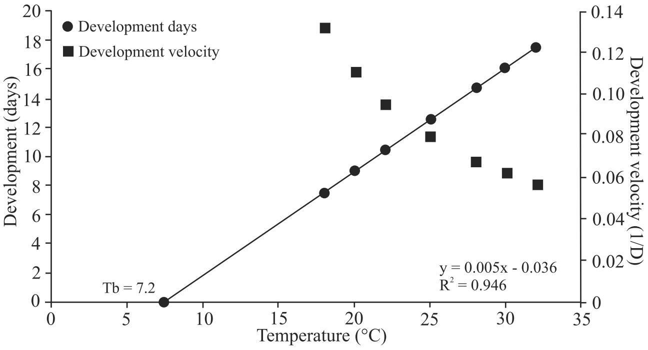

Fig. 4. Curve of development velocity (egg-to-adult period) of *Tamarixia radiata* at different temperatures. 70 ± 10% RH, and a 14 h photophase.

RH affected rates of parasitism and emergence ($F = 167.26$; $P = 0.0001$; and $F = 64.87$; $P = 0.0001$, respectively) (Figs. 5A and 5B). The highest parasitism capacity was observed at RH 70 and 50%, corresponding to 83.0 and 65.5%, respectively. At RH 30 and 90%, the parasitism rate was 29.8 and 37.3%, respectively. The optimal relative humidity estimated for the parasitism capacity of *T. radiata* was 61.95% where the parasitism rate was 79.53% (Fig. 5A). The best emergence rate occurred at RH 50 and 71%, corresponding to 57.4 and 82.3%, respectively, while at RH 30 and 90%, we observed a survival rate of 37.8 and 26.5%, respectively (Fig. 5B). The highest estimated emergence rate (74.58%) occurred at RH 59.1%.

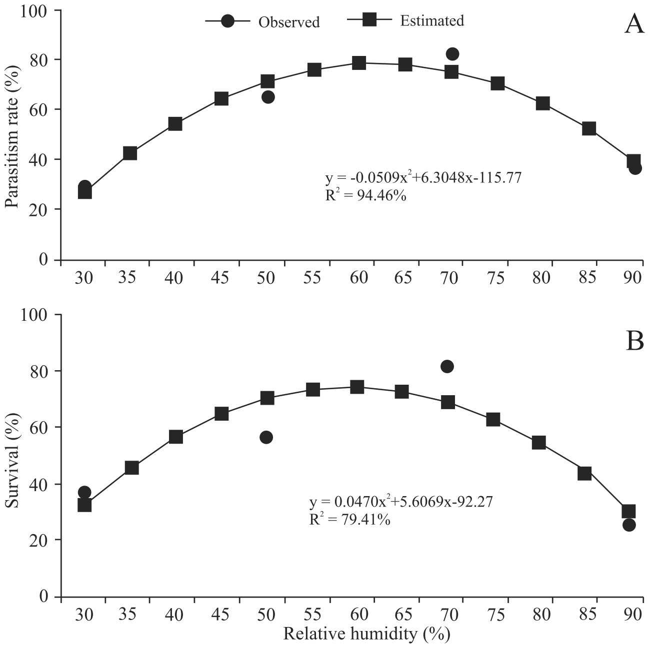

Fig. 5. Parasitism (A) and survival (B) rates of *Tamarixia radiata* on *Diaphorina citri*, at different RH. Temperature 25 ± 1°C, and a 14h photophase.

## DISCUSSION

Temperature and RH influenced the reproductive activity of *T. radiata* and they can be determinant factors for the success of its use in biological control, for its mass rearing in laboratory, as well as its establishment in the citrus groves.

The parasitism capacity of *T. radiata* on *D. citri* is related to other factors, such as food and developmental stage (Chu & Chien 1991; Chien *et al*. 1994). In addition, temperature exerts great influence on the developmental and reproductive activities, as demonstrated by Gómez-Torres *et al*. (2012), where the highest parasitism rate (77.3%) occurred at 26.3ºC, similar to the rate found in our study at 25ºC, where each female of *T. radiata* parasitized on 166.8 nymphs (Fig. 1). At the other temperatures, higher or lower than 25ºC, the parasitism rate was reduced. According to Chu & Chien (1991), at 25ºC, fecundity of *T. radiata* may range from 98 and 156 eggs/females, similar to the findings in our study (166.75) under the same temperature condition.

It has also been observed a quadratic relation between fecundity of the parasitoid and temperature in Eulophidae, due to the significant interaction between this abiotic factor with the embryonary development and with life span of females (Patel & Schuster 1991). The authors also report that at low temperatures, the females laid fewer eggs when compared with females subjected to temperatures between 20 and 30°C. Hondo *et al*. (2006) evaluated the development, efficiency and reproduction of seven species of Eulophidae based on temperature tolerance and concluded that all species analyzed were adapted to high temperatures (25 and 30ºC).

The highest daily and accumulated rates of parasitism observed at 25ºC are probably related to the average life span of the females of *T. radiata*, given that at extreme temperatures (15 and 35ºC), the average life span was reduced by half, i.e., three days shorter than at 25ºC (Fig. 3).

The development of the egg-to-adult period of *T. radiata* at different temperatures was similar to that observed by Fauvergue & Quilice (1991), in which the authors determined for the constant temperatures of 20, 25, 27 and 30ºC, a duration of 16.8, 10.1, 9.1 and 8.5 days, respectively. For temperature requirements for the egg-to-adult development, our obtained data differ from threshold temperature of 11°C and the thermal constant 165GD, shown by Chien & Chu (1996) for a population of *T. radiata* in *Citrus sinensis* and *M. paniculata*. This difference of 3.8°C for the thresholds temperature and 23.7 degrees-day between the two studies is probably attributed to the geographic origin of the populations, once temperature requirements between species may vary according to the geographic sites (Honék & Kocourek 1990; Nava *et al*. 2010).

In our study, we observed that the parasitism rate of *T. radiata* on *D. citri* was higher at RH 70%; however, it is reduced at 30 and 90%. Likewise, Duale (2005) found similar results for another species of Eulophidae *Pediobius furvus* (Gahan, 1928), with high parasitism rates between RH 60 and 80%. Probably, extreme RH influences indirectly the parasitoids, once they are protected by the nymphs' exoskeleton. Unpublished data show that for *D. citri* RH below 30% is harmful to nymphs, therefore, the pre-imaginal development of *T. radiata* may have also been affected (J.R.P. Parra, unpubl. data).

The relation between a parasitoid and its host is highly complex, thus, it is essential to understand the responses of biological control agents to biotic and abiotic factors that may affect its reproduction, development and survival (Geden 1997; Almeida *et al*. 2002). Therefore, based on the data observed in this study, criteria must be used to obtain enough mass rearing of *T. radiata* to enable the use of this parasitoid for the control of *D. citri* in citrus groves. These criteria should be associated to temperature and hygrometric requirements determined in the current study, i.e., temperature between 25 to 30°C and RH near 70%. These parameters show great adequacy for the reproduction, development and survival of *T. radiata*. Moreover, the release and adaptation of *T. radiata* to field conditions will depend on thermal hygrometric requirements, which even allow occurrence delineation or identification of the number of generations for the regions in São Paulo State, Brazil's major citrus producer.

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