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Full Length Research Paper

Effect of temperature on the development of the mealybug, *Phenacoccus solenopsis* Tinsley (Hemiptera: Pseudococcidae)

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An invasive mealybug *Phenacoccus solenopsis* Tinsley (Hemiptera: Pseudococcidae) has attacked cotton (*Gossypium hirsutum L.)* seriously in Pakistan and India, and caused great loss in the production of cotton in recent years. This kind of pests can probably spread rapidly and would threaten cotton and other crops in many infested and non-infested countries. *P. solenopsis* has first been reported to severely damage Chinese hibiscus (*Hibiscus rosa-sinensis*) in Guangzhou, in December, 2008. In order to understand the effects of temperature on the development of *P.solenopsis*, the developmental threshold temperature (C_t) and effective accumulated temperature (*K*) were calculated for each developmental stage. A total of 30 *P. solenopsis* individuals were divided into 6 groups and reared at six constant temperatures (18, 21, 24, 27, 30, and 33℃). Total developmental duration ranged from 81.3 days at 18℃ to 24.06 days at 30℃ for female mealybug, and from 44.21 days at 18℃ to 12.95 days at 30℃ f or male mealybug. Degree-day values for each developmental stage for both male and female mealybugs were determined. Total degree-day values required for full development for female and males were 414.9 and 218 degree-days respectively. Estimated threshold temperatures for different life stages ranged from 12.8℃ (pre-oviposition) to11.7℃ (3^{rd} instar) for female mealybugs, and 16.5℃ (prepupa) to 11.5℃ (2^{nd} instar) for male mealybugs.

Key words: Mealybug, *Phenacoccus solenopsis*, development, temperature.

INTRODUCTION

Mealybugs (Hemiptera: Pseudococcidae) are major plant pests worldwide (McKenzie, 1967; Williams, 1985; Williams and Granara de Willink, 1992). *Phenacoccus solenopsis* Tinsley first appeared on cotton (*Gossypium hirsutum* L.) in 2005 and caused serious damage in Sindh and Punjab districts of Pakistan and north-western India (Abbas et al., 2010). The mealybugs have also been found on a wide variety of host plants including economic species (Arif et al., 2009). *P. solenopsis* has also been reported as a pest of *Hibiscus rosa-sinensis* L. in Guangzhou city, which is the first report of this kind of pests in mainland China (Wu and Zhang, 2009).

The life cycles of male and female *P. solenopsis* are different. Sex is not distinguishable until they reach the end of second nymphal instar. Similar with the mealybug, *P. madeirensis*, the male second-instar nymphs can secrete waxy filamentous tests just before molting and then finish an additional nymphal stage (prepupal and pupal) inside the tests (Chong et al., 2003). Adult males emerge from the tests as non-feeding, winged individuals when compared with wingless adult females such as *P. madeirensis* Green (Chong et al., 2003). The third-instar females reveal similar external morphology as adult females and do not secrete tests. Little effect of temperature on the development of *P. solenopsis* is known. The objective of this study is to determine the

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development of *P. solenopsis* on Chinese hibiscus (*H. rosa-sinensis L.*) at various constant temperatures providing the optimal timing of insecticide applications for achieving effective control of *P. solenopsis*.

MATERIALS AND METHODS

Experimental conditions

All experiments were conducted in environment-controlled chambers (model RXZ, Ningbo Jiangnan Instrument Factory) maintained at six constant temperatures (18, 21, 24, 27, 30, and 33℃), and a light-dark period of 12:12 h (L:D). The re lative humidity of chambers was 70%.

Preparation of host plants

Chinese hibiscus (*H. rosa-sinensis L.*) were used as the host plants in this study and grown under natural light in greenhouses in the SCAU campus, Guangzhou, Guangdong province, China.

Mealybug development

Colonies of *P. solenopsis* were collected from a green belt in Guangzhou and maintained on Chinese hibiscus foliage (*H. rosa-sinensis L.*). The adults' *P. solenopsis* were fed Chinese hibiscus leaves and reared at 28℃. After 5 generat ions, the 1st instar nymphs were collected for the future research.

Determination of nymph

After the females started oviposition, the 1st instars nymphs were collected from the ovisac of a female and transferred individually into Petri dish. All mealybugs in the Petri dish were fed with fresh and young Chinese hibiscus leaves and placed in environment –controlled chambers with the constant temperatures of 18, 21, 24, 27, 30 and 33℃ respectively. At each temperature con dition, more than 30 nymphs were used. Chinese hibiscus leaves were changed within every 3 days.

Developmental duration for each instar was recorded by checking its exuvia every 12 h. The developmental durations of third- and fourth instar males were determined by the removal of the tests and checking for exuvias. Average developmental duration of individual nymphs was calculated for each instar. The life span of females was simply calculated as the sum of the durations of each instar and the pre-oviposition. Individual male life span was measured from the beginning to adult emergence.

Estimation of degree-day requirements

Developmental rates for each immature life stage were calculated using the reciprocal of the average number of days (that is, 1 per day) required to complete a particular life stage. The relationship between developmental rate and temperature was described by a linear model (Arnold, 1959) through fitting the linear section of the data points. Temperature data above and below the linear portion of the developmental rate curve were not used to estimate degree-days or the lower threshold temperature. The lower temperature threshold for development was determined as the x-intercept (Arnold, 1959).

Statistical analysis

Significant differences among developmental durations for female and male at different stages were detected by using Fisher protected significant difference (least significant difference (LSD) test with the significance level at P = 0.05). A general linear model procedure (PROCGLM) was performed to determine the effect of temperature on developmental durations of *P. solenopsis* (SAS Institute 1985).

RESULTS

Developmental duration at different temperatures

The constant-temperature chambers temperatures provided the estimation of the lower threshold for the development of *P. solenopsis*. From 18 to 30℃, higher temperature shortened the developmental duration of *P. solenopsis* at stages by approximately 70.4% for female and 70.7% for male (Table 1). The fastest developmental rate for 1st instar and 2nd instar nymphs occurred at 30℃ and decreased at 33℃. The fastest developmental ra te for female 3rd instar nymph occurred at 27℃ and then decreased as temperature increases. The developmental rate of pre-oviposition stage, prepupa and pupa revealed an increase at the increase of temperature (Table 1). The developmental duration in every nymphal instar at 18℃ was three times as long as that at 30℃. Males requ ired a longer duration (1 to 8 days) for nymphal development than females from 27 to 18℃. (18℃, F = 153.08, df = 1, 29, $P<0.0001$; 21℃, F=91.53, df = 1, 28, $P<0.0001$; 24℃, F = 67.72, df = 1, 29, $P<0.0001$; 27℃, F = 23.45, df=1, 29, $P<0.0001$). The relationships between temperature and developmental rate at different stages were shown in Figures 1 and 2.

Effective accumulated temperature and threshold temperature

The developmental threshold temperature (*Ct*) and effective accumulated temperature (*K*) were determined. Degree-day values estimating female 1st instar, 2nd instar, 3rd instar, pre-oviposition, and all stage development in constant temperature experiments were 76.2, 52.8, 65.8, 209 and 414.9 respectively. Similarly, degree-day values estimating 1st instar, 2nd instar, 3rd instar, pre-oviposition, and all stage development were 80.1, 73.5, 20.7, 64.8 and 218 respectively (Table 2).

DISCUSSION

Temperature has a significant effect on the developmental rate of *P. solenopsis.* Female *P. solenopsis* can complete their development at 30℃ within 24.06 days, when

Table 1. Developmental durations for laboratory-reared female and male *P. solenopsis* mealybugs at six temperatures, based on materials from a colony maintained at South China Agricultural University, Guangzhou, GD.

Temperature (℃)	Female development [a] (d)					Male development [a] (d)				
	Nymph			Pre-oviposition	Hatch to oviposition	Nymph		Prepupa	Pupa	Hatch to adult
	1st instar	2nd instar	3rd instar			1st instar	2nd instar			
18	14.68±0.27a	9.0±0.17a	12.38±0.21a	45.24±0.59a	81.30±0.74a	14.23±0.10a	10.3±0.19a	6.47±0.10a	13.21±0.28a	44.21±0.40a
21	9.67±0.19b	5.95±0.21b	7.12±0.20b	23.70±0.40b	46.44±0.50b	9.58±0.18b	8.73±0.30b	5.21±0.30b	6.22±0.22b	29.74±0.61b
24	5.16±0.23c	4.45±0.25c	4.97±0.19d	16.75±0.16c	31.33±0.41c	5.90±0.22c	5.53±0.28c	3.87±0.23c	5.03±0.17c	20.33±0.58c
27	5.00±0.40c	4.0±0.26c	3.80±0.25e	15.40±0.16d	28.20±0.51d	5.38±0.20d	4.95±0.19d	2.04±0.19d	3.63±0.18d	16.00±0.39d
30	3.63±0.16d	2.83±0.08d	4.04±0.19e	13.56±0.16e	24.06±0.35e	4.01±0.11e	3.88±0.10e	1.56±0.10de	3.50±0.14d	12.95±0.20e
33	4.10±0.14d	4.10±0.31c	5.98±0.24c	13.44±0.17e	27.62±0.42d	4.06±0.10e	4.63±0.11d	1.20±0.08e	3.12±0.11d	13.01±0.20e

Values are Means±SE, n=3; Means in columns followed by the same letter are not significantly different (P>0.05; Fisher's protected LSD); [a] Nymph developmental durations were measured from the day of hatching; prepupa, pupa, and pre-oviposition durations were determined when 2nd instar nymph stopped moving, pupating, and adult emergence. Experiments began with more than 30 larvae (0 d old).

Table 2. Linear regressions of development rate on rearing temperature used to estimate the lower developmental threshold and degree-days (DD) for development of *P. solenopsis.*

Sex	Life stage	No.	Model	F	P	r^2	Lower development threshold, ℃[a]	Estimated stage duration in DD[b]
Female	1st instar	5	Y = 0.01335x - 0.1595	27.3	0.0064	0.872	11.9	76.2
	2nd instar	5	Y = 0.01888x - 0.2317	82.6	0.0028	0.965	12.3	52.8
	3rd instar	4	Y = 0.01521x - 0.1783	27.1	0.0138	0.900	11.7	65.9
	pre-oviposition	6	Y = 0.00486x - 0.0623	35.6	0.0270	0.947	12.8	209.0
	Hatch to adult oviposition	5	Y = 0.0024x - 0.0294	93.8	0.0023	0.969	12.3	414.9
Male	1st instar	6	Y = 0.01268x - 0.1524	78.4	0.0009	0.951	12.0	80.1
	2nd instar	5	Y = 0.01363x - 0.1566	96.2	0.0023	0.970	11.5	73.5
	prepupa	6	Y=0.047361x-0.779469	72.4	0.0011	0.948	16.5	20.7
	pupa	6	Y = 0.01596x - 0.1874	73.9	0.0010	0.949	11.7	64.8
	Hatch to adult emergence	6	Y = 0.0046x - 0.0614	1278.7	0.0001	0.998	13.3	218.0

No. Number of temperature data points along linear portion of development curve used in equation; a; Lower temperature for development calculated as x-intercept of regression.

compared with 81.3 days at 18℃. Immature development in males is slightly slower than that in females, which probably ensures insemination of females when adult females have developed completely to mate after their emergence. Adult males reverse their body out of the tests after emergence, and then search for adult females in a purposeful way through mid-veins. This phenomenon can result in the co-evolution in both

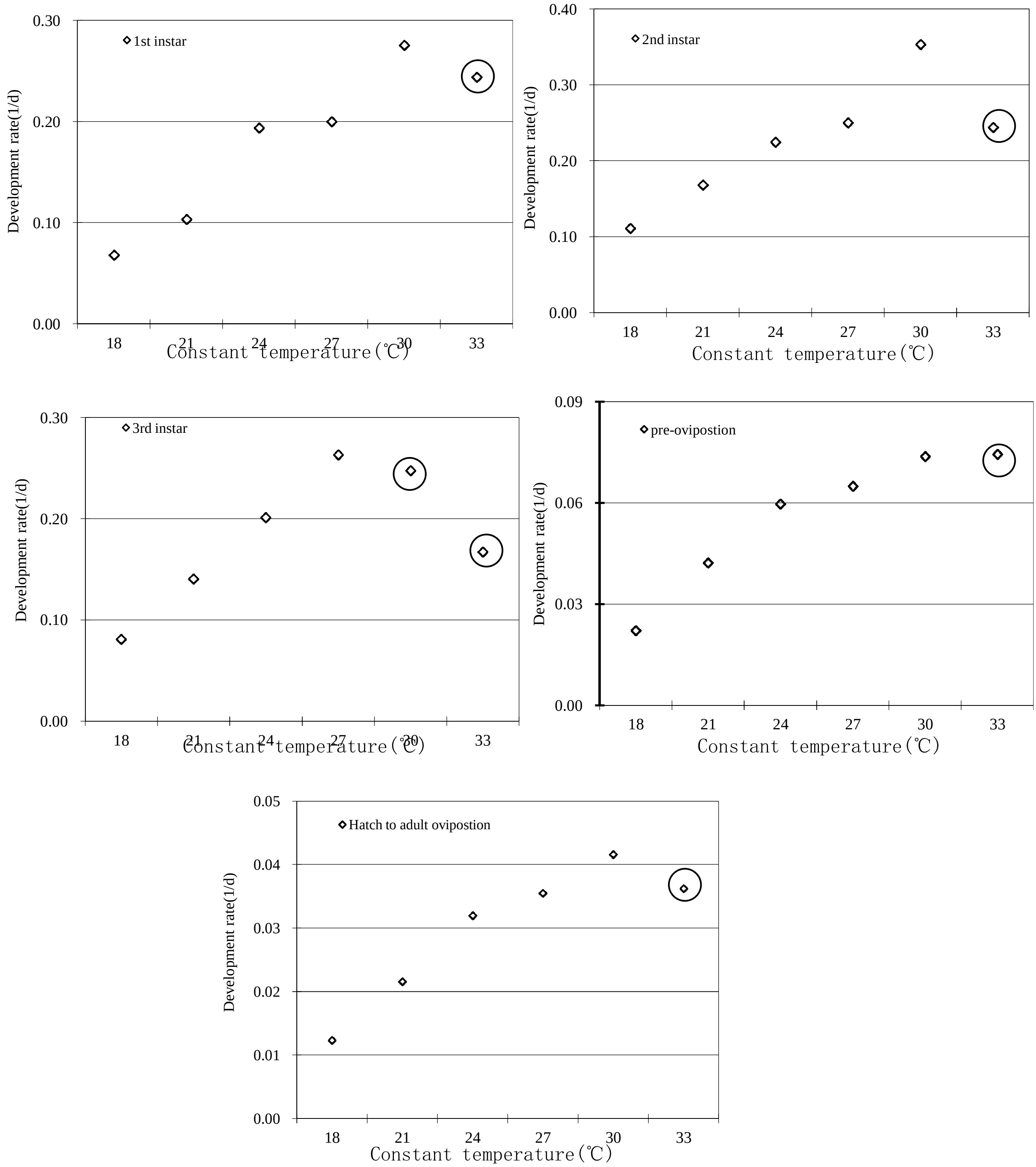

Figure 1. Development rates for nymph and adult pre-oviposition of female *P. solenopsis* reared under constant temperatures in the laboratory. Circled data points were not included in linear regression.

sexes, because the adult females prefer to feed in juicy mid-veins.

The developmental threshold and effective accumulated temperatures of *P. solenopsis* between laboratorial and

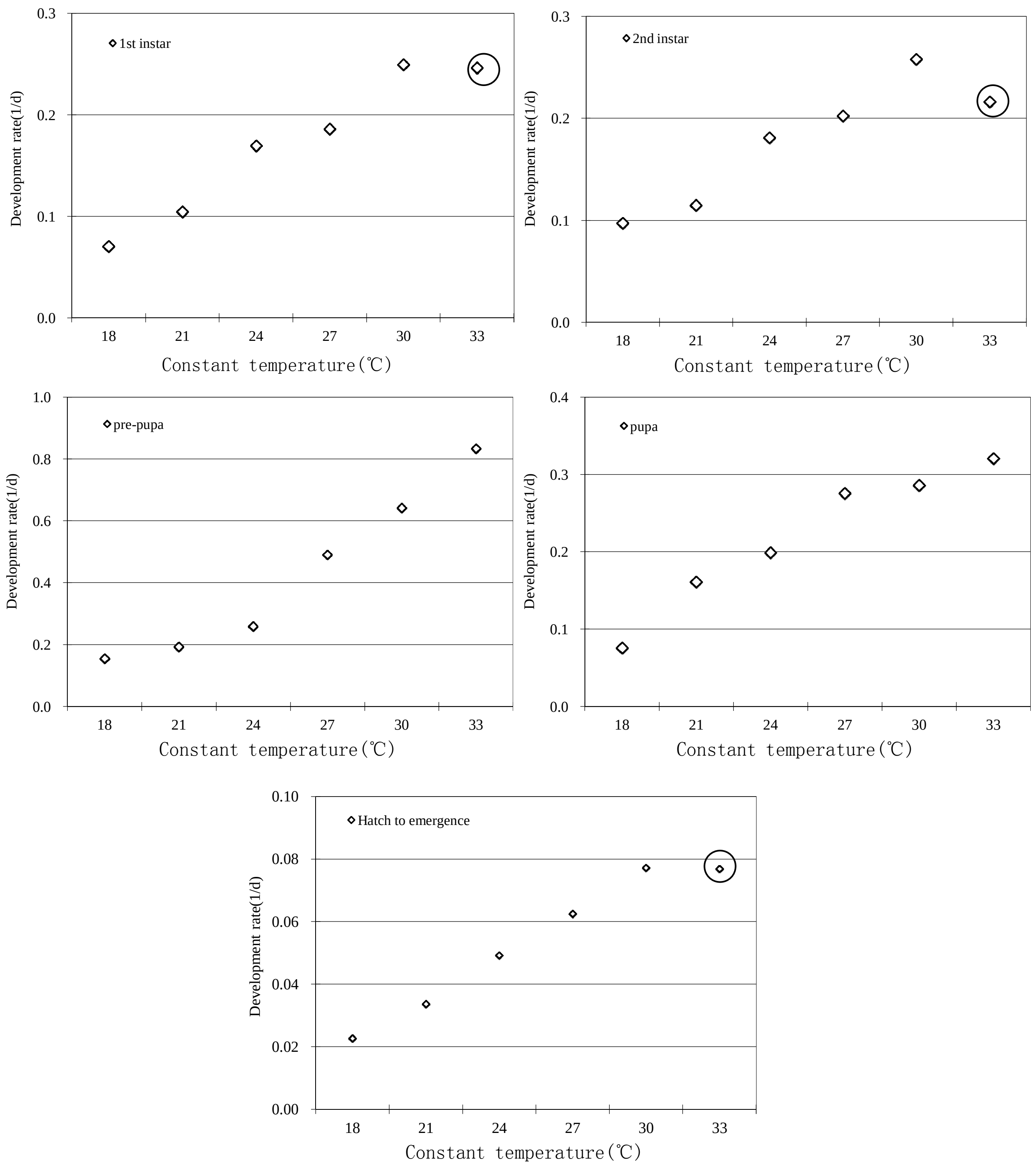

Figure 2. Development rates for nymph and pupa of male *P. solenopsis* reared under constant temperatures in the laboratory. Circled data points were not included in linear regression.

outdoor conditions should be similar somewhat, because the developmental duration may be influenced by factors such as variable temperature, humidity and food conditions. Therefore, laboratorial results should be validated in the field to ensure the reliable results that can be used to predict developmental rate of *P. solenopsis*.

The biological parameters especially for the developmental threshold and effective accumulated temperatures of mealybugs are necessary for the potential prediction of geographical distribution in this kind of invasive pests (Wang et al., 2010). The CLIMEX model has been used to analyze the potential distribution area in the world where *P. solenopsis* can frequently infest (Wang et al., 2010; Ma et al., 2011). Most of the tropical and sub-tropical regions worldwide are suitable for mealybugs, and all cotton-producing areas in China are potentially suitable for the pests. According to the developmental threshold and effective accumulated temperature of *P. solenopsis*, some parameters of this model can change, and the prediction of potential geographical distribution by this model will be more precise.

Understanding of the life history of *P. solenopsis* is crucial to the integrated management of this kind of pests. In a typical greenhouse situation with temperature ranging from 20 to 30℃, a female can complete its developm ent within one month and produces approximately 300 eggs in a week. Successful management of *P. solenopsis* depends on the early detection and control of mealybug population. Due to high survival rates, rapid development, enormous reproductive capacity, and lack of early management, the population of *P. solenopsis* can reach a high level and cause significant economic damage to many hosts such as greenhouse Chinese hibiscus and cotton. Because of the thick waxy secretion surrounding the body, adult mealybugs are difficult to control. Repeated applications of chemicals targeting immature stages of mealybugs are required for suppressing the population of mealybugs (Townsend et al., 2000). Control programs should take advantage of the fact that the development of *P. solenopsis* is temperature-dependent. At lower temperature, the immature mealybugs have longer developmental periods and thin waxy secretion, thus, leading to a wider window of opportunity for management. Because many insecticides are only effective against a specific developmental stage, the treatment schedule relies on developmental rate and the exposure to the duration of susceptible stages (Townsend et al., 2000). Most natural enemies employed in biological control programs also prefer specific developmental stages of the mealybugs. These data provided in this study will provide a better timing of insecticide applications for pests to release natural enemies' at the most susceptible immature stages.

Conclusions

Total developmental duration of female mealybug ranged from 81.3 days at 18℃ to 24.06 days at 30℃, and f rom 44.21 days at 18℃ to 12.95 days at 30℃ for male mealybug. Total degree-day values required for full development for female and males were 414.9 and 218 degree-days respectively. Developmental threshold for different life stages ranged from 11.7℃ □(3rd instar) to 12.8 ℃ (pre-oviposition) for female mealybugs, and 11.5℃ (2nd instar) to 16.5℃ (prepupa) for male mealybugs.

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