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EFFECT OF TEMPERATURE ON LIFE HISTORY AND POPULATION GROWTH PARAMETERS OF *PLANOCOCCUS CITRI* (HOMOPTERA, PSEUDOCOCCIDAE) ON COLEUS [SOLENOSTEMON SCUTELLARIOIDES (L.) CODD.]

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Abstract — The development, life history, reproduction, and population growth parameters of *Planococcus citri* Risso on coleus [*Solenostemon scutellarioides* (L.) Codd.] were studied at various temperatures ranging from 10 to 37°C, 70±10% RH, and photoperiod length of 16: 8 h (L: D). Females and males successfully developed into adults at from 15 to 32°C and 18 to 32°C, respectively. All first instars died at 10, 12, and 37°C. Lower temperatures (10, 12, and 15°C) caused higher egg mortality than did higher temperatures (32, 35, and 37°C). At all temperatures (except 15°C), the highest percentage of nymphal mortality was observed in the first instar. The sex ratio was female-biased between 15 and 30°C, but there was a slightly higher number of males at 32°C. The highest adult longevities of females and males were obtained at 18 and 25°C, respectively. The pre-oviposition, oviposition, and post-oviposition periods were significantly different at various temperatures. The highest fecundity was observed at 23°C. The shortest and longest oviposition periods occurred at 32 and 18°C, respectively. Maximum values of the intrinsic rate of natural increase (r_m), net reproduction rate (R_0), and finite rate of increase (λ) and the shortest mean generation time (T) and doubling time (DT) were obtained at 25°C. Our findings showed citrus mealybug performances to be highly affected by temperature.

Key words: Planococcus citri, temperature, development, reproduction, population parameters

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INTRODUCTION

The citrus mealybug, *Planococcus citri* Risso (Homoptera: Pseudococcidae), is a polyphagus species known from all zoogeographical regions (Williams and Watson, 1988). It is one of the most common pests in nearly all greenhouses and nurseries, where it attacks a wide range of ornamental, citrus, and orchard crops in many temperate and tropical regions (McKenzie, 1967; Blumberg et al., 1995). In greenhouses, the citrus mealybug is the most common pest on bulbs, coleus, ferns, and gardenias; however, it may become abundant on other ornamentals as well (Blumberg and van Driesche, 2001). The nymphs and females cause damage to

host plants with their piercing-sucking mouthparts, which they use to suck sap and remove nutrients. As a result, the plants often become stunted, distorted, or yellowed and show reduced vigor. They excrete honeydew, which provides a medium for the growth of black sooty mold fungi (Al-Ali, 1969; Smith et al., 1997; Heinz et al., 2004). Black sooty mold fungi are detrimental to plants because they cover leaves, thus reducing photosynthesis and inducing plant stress (Malais and Ravensberg, 1922). The citrus mealybug is also known as a vector of some important plant viruses (Al-Ali, 1969; Bartlett, 1978; Rosciglione and Castellano, 1985; Lockhart and Olszewski, 1993; Su, 1998, 2000; Kubiriba et al., 2001; Watson and Kubiriba, 2005). The average number of eggs laid per female is strongly dependent on temperature. Each female lays fewer than 100 eggs at temperatures above 30°C, but can lay over 400 eggs at 18°C (Copland et al., 1985). Thus, they have a high reproductive potential and must be controlled before reaching injurious levels (McKenzie, 1967).

Temperature has profound effects on insect life history parameters such as development, survival, and reproduction. The response of insects to temperature can be important in predicting the potential geographical range of a species and in developing phenological models to predict population dynamics and the timing of various stages for planning control or survey programs (Keena, 2006). Based on demographic studies, we can estimate extinction probabilities, predict life history evolution, anticipate outbreaks of pest species, analyze population stability, and examine the dynamics of colonizing or invading species (Vargas et al., 1997).

The biology of citrus mealybug has been studied on different host plants, including citrus, coffee, pumpkin, and coleus (Copland et al., 1985; Yang and Sadof, 1995; Maphi and Radjabi, 1987; Martines and Sruis, 1987a, 1987b; Arai, 1996; Malleshaiah et al., 2000; Laflin and Parrella, 2004; Hogendorp et al., 2006). However, the temperature-dependent responses of citrus mealybug were not studied over a wide range of constant temperatures. Thus, the main objective of this study was to determine the effect of selected constant temperatures on survival, development, longevity, reproduction, and population growth parameters of P. citri on coleus. The results obtained in this study may provide useful information for designing a comprehensive pest management program for the citrus mealybug.

MATERIALS AND METHODS

Plants

The red variegated coleus, *Solenostemon scutellarioides* (L.) Codd [previously *Coleus blumei* (Bentham)], was used for this study because it is very susceptible to citrus mealybug, and most greenhouse coleus producers have to deal with it. The red coleus plants used in the study were originally obtained from greenhouses in Mahallat, Iran in spring and were maintained in a greenhouse of Arak Islamic Azad University under natural daylight conditions. Growth was at temperatures of $26\pm2^{\circ}$ C by day and $24\pm2^{\circ}$ C at night and relative humidity of $65\pm5\%$.

Citrus mealybug colony

The citrus mealybug population was originally collected from coleus plants infested in greenhouses of the Mahallat region in spring of 2006. Infested leaves were transferred to the laboratory and placed on all of the coleus plants. Three to four infested leaves per coleus plant were used. Each leaf was infested with about 25 individuals of first-instar nymphs. The infested leaves remained on the coleus plants until the advent of desiccation, which caused the firstinstar nymphs to move from the desiccated leaves onto the coleus plants. This transferring procedure minimized handling of immature stages of mealybugs (Sadof et al., 2003; Hogendrop et al., 2006). Ten coleus plants were inoculated with citrus mealybugs and kept under conditions of 24±2°C, 60-70% RH, and a photoperiod of 16:8 h (L: D) (Yang and Sadof, 1995).

Citrus mealybugs were reared on coleus plants in the greenhouse for three generations before they were used in the experiments.

Experiments

This study was carried out under conditions of 12 constant temperatures (10, 12, 15, 18, 20, 23, 25, 28, 30, 32, 35, and 37°C), 70±10% RH, and a photoperiod of 16: 8 h (L: D) in the Entomology Laboratory of Arak Islamic Azad University, Iran. Adult females from the stock culture were placed to lay eggs on excised coleus leaves. After 24 h, all females were removed, while the eggs were kept. This procedure allowed standardizing the age of eggs under study. At the beginning of experiments for each temperature, newly laid eggs of citrus mealybug (0-24 h old) were individually transferred to detached coleus leaves. The petioles of detached leaves were placed separately in a glass vial filled with water. The vials were sealed with parafilm to prevent water evaporation. They were placed in transparent plastic cylindrical tubes (6×20 cm) covered with a micromesh

screen on the lid for ventilation and then transferred to growth chambers. A fine camel's hair brush was used for transferring eggs to the coleus leaves. At all temperatures, whenever the leaves became discolored, they were replaced with fresh ones.

Throughout the entire experiment, 100 citrus mealybugs eggs were examined at 10°C, 100 at 12°C, 93 at 15°C, 89 at 18°C, 95 at 25°C, 88 at 23°C, 81 at 25°C, 89 at 28°C, 87 at 30°C, 95 at 32°C, 100 at 35°C, and 100 at 37°C. Development times of individuals were determined for each nymphal instar. They were monitored daily, and the presence of exuviae was used to identify each stage. Daily monitoring was continued until maturity. Upon emergence of adult females, one male was added with a fine hair brush. The onset of oviposition was ascertained from the presence of an elongated white cottony egg sac extending beneath and behind the female. During the reproductive period, newly laid eggs were counted and removed daily until all females died. Different parameters such as the pre-reproduction period, reproduction period, post-reproduction period, pre-adult mortality, adult longevity, and life span were determined.

Life tables

From the fertility and survival schedules, several population growth parameters including the net reproductive rate (R_0), finite rate of increase (λ), mean generation time (T), doubling time (D_T), and intrinsic rate of natural increase (r_m) were calculated using the formulas suggested by Carey (2001):

$$\sum_{\alpha}^{p} l_{x} m_{x} e^{-r_{x}x} = 1$$
(1)
$$R_{0} = \sum_{\alpha} l_{x} m_{x}$$
(2)
$$\lambda = e^{r}$$
(3)
$$T = \frac{l_{n} R_{0}}{r}$$
(4)
$$D_{T} = \frac{\ln(2)}{r}$$
(5)

where x is the age of individuals in days, l_x is the age-specific survival, and m_x is the age-specific number of female offspring.

After population parameters such as r_m were computed from the original data $(r_{m(all)})$, the jackknife method was applied to evaluate the differences in r_m values by estimating the variances (Meyer et al., 1986). The jackknife pseudo-value $r_{m(j)}$ was estimated for *n* samples using the following equation (Maia et al., 2000):

$$r_{m(j)} = n \times r_{m(all)} - (n-1) \times r_{m(i)}$$
(6)

where $r_{m(i)}$ is the value for (n-1) females. The mean values of jackknife pseudo-values for each treatment were subjected to analysis of variance (ANOVA). Similar procedures were used for the other population parameters.

Statistical analysis

For statistical analysis, each mean value is given with its standard error (±SEM). The effect of temperature on the development time, oviposition period, and adult longevity and the jackknife estimates of various population growth parameters of *P. citri* were analyzed using one-way ANOVA. If significant differences were detected, multiple comparisons were made using the Student-Newman-Keuls (SNK) test ($P_{value} < 0.05$). A *t*-test was run to compare the development times of male and female individuals within each constant temperature. All analyses were conducted using SPSS (SPSS, 2004) and SAS (SAS, 2001) statistical softwares.

RESULTS

Development, mortality, and sex ratio

Citrus mealybug females and males successfully developed into adults at temperatures ranging from 15 to 32°C and from 18 to 32°C, respectively. The percentage of egg hatching at 10, 12, 35, and 37°C, was 7, 33, 69, and 58%, respectively. At 35°C, the survival rate of the second-instar nymphs was determined to be 5%, but all of them died during the third instar. All first-instar nymphs died at 10, 12, and 37°C. At 15°C, none of the females were successful in oviposition. The effect of temperature on the development of *P. citri* is presented in Table 1. Development time of all stages of citrus mealybug

Darameter	Temperature (°C)							
	15	18	20	23	25	28	30	32
Female								
Egg	13.14 ± 0.26^{a}	$9.68 {\pm} 0.26^{b}$	$9.28{\pm}0.20^{b}$	5.70±0.13 ^c	2.96 ± 0.07^{f}	4.2 ± 0.14^{d}	3.6±0.09 ^e	4.3 ± 0.17^{d}
Instar I	16.14 ± 0.40^{a}	10.87 ± 0.030^{b}	$10.68 {\pm} 0.18^{b}$	6.73±0.16 ^c	4.02 ± 0.07^{f}	5.12 ± 0.14^{e}	5.21±0.15 ^e	6.40 ± 0.21^{d}
Instar II	$20.85{\pm}0.050^a$	11.65 ± 0.33^{b}	12.20 ± 0.20^{b}	8.02±0.21 ^c	5.42 ± 0.10^{e}	5.94±0.19 ^e	6.81 ± 0.17^{d}	8.72±0.25 ^c
Instar III	24.57 ± 0.75^{a}	12.81 ± 0.49^{b}	13.64±0.27 ^b	9.26±0.25 ^c	6.22±0.11 ^e	6.82±0.21 ^e	8.03 ± 0.21^{d}	9.48±0.31 ^c
Overall nymph	61.58 ± 1.45^{a}	35.34 ± 1.07^{b}	36.52 ± 0.27^{b}	24.02±0.55 ^c	15.65 ± 0.21^{f}	17.89±0.49 ^e	20.06 ± 0.50^{d}	$24.6 \pm 0.66^{\circ}$
Total	74.71 ± 1.57^{a}	45.03 ± 1.26^{b}	45.8 ± 0.60^{b}	29.73±0.56°	18.61±0.22 ^e	22.17 ± 0.55^{d}	23.66 ± 0.53^{d}	28.96±0.66 ^c
Male								
Egg	-	9.50 ± 0.24^{a}	8.25 ± 0.5^{a}	5.37±0.8 ^c	3.03±0.2 ^e	4.16 ± 0.4^{d}	$3.95 {\pm} 0.8^{d}$	4.23 ± 0.0^{d}
Instar I	-	10.66 ± 0.33^{a}	10.25 ± 0.7^{a}	6.93 ± 0.28^{b}	$4.63 \pm 0.10^{\circ}$	$5.50 \pm 0.20^{\circ}$	4.95±0.25 ^c	$4.84 \pm 0.10^{\circ}$
Instar II	-	13.16 ± 0.30^{b}	$14.50 {\pm} 0.4^{a}$	7.93±0.60 ^c	5.85 ± 0.11^{d}	6.66±0.25 ^{cd}	6.61±0.9 ^{cd}	6.30 ± 0.17^{d}
Pre-pupae	-	9.50 ± 1.06^{a}	9.25±1.3ª	5.18 ± 0.27^{b}	$2.85 \pm 0.08^{\circ}$	3.16±0.4 ^c	3.04 ± 0.4^{c}	3.61±0.1 ^c
Pupae	-	9.83 ± 1.14^{b}	10.75 ± 1.03^{a}	4.25±0.31 ^c	2.40 ± 0.09^{d}	2.66 ± 0.11^{d}	2.52 ± 0.13^{d}	$3.23 {\pm} 0.12^{d}$
Overall nymph	-	$23.83 {\pm} 0.47^{a}$	24.75 ± 0.85^{a}	14.87 ± 0.84^{b}	10.48±0.19 ^c	12.16±0.41 ^c	$11.57 \pm 0.50^{\circ}$	11.15±0.19 ^c
Total	-	52.67 ± 1.73^{a}	53.5 ± 2.33^{a}	26.69 ± 1.1^{b}	18.77±0.37 ^c	22.16±0.48 ^c	$21.09 \pm 0.82^{\circ}$	$22.23 \pm 0.28^{\circ}$
Sex ratio (%±SE)	65.18 ± 1.76^{b}	65.65 ± 2.12^{b}	70.12±0.69ª	$70.91 {\pm} 0.70^{a}$	62.04 ± 0.48^{bc}	$52.83 {\pm} 0.52^{d}$	59.18±0.51°	46.46±0.40 ^e

Table 1. Development time (means±SE) of *Planococcus citri* reared on coleus at various constant temperatures.

Mean values followed by different letters in the rows are significantly different (P < 0.05, SNK after one-way ANOVA). Sex ratio is female: male.

females and males reared at various temperatures differed significantly (*F* = 372.32, df = 243, *P* < 0.01; and F = 174.64, df = 104, P < 0.01, respectively). The longest stages for females and males were the third and second nymphal instars, respectively. The total development times of females and males at 18, 20, and 32°C were significantly different (*t*-test, P <0.01), while no significant differences were observed at the other temperatures (t-test, P > 0.05). Total development times of females were longer than males at all experimental temperatures except 25°C. The lower temperatures (10, 12, and 15°C) caused higher egg mortality than the higher temperatures examined (32, 35, and 37°C). At all temperatures, the percentage of nymphal mortality was higher in the first instar than in subsequent instars. Prepupae and pupae were quite resistant between 15 and 32°C. The lowest percentages of mortality for first and second instars were obtained at 25°C. Third instars had no mortality at 23 and 25°C; therefore the optimal temperature for survival of nymphs in all instars seems to be 25°C. The sex ratio, expressed in terms of the proportion of females, differed significantly at various temperatures (F = 59.87, df = 39, P < 0.01) (Table 1). The maximum female ratio occurred at

23°C and the minimum at 32°C. The sex ratio was female-biased between 15 and 30°C, while slightly greater numbers of males occurred at 32°C.

Longevity and reproduction

Temperature had a significant effect on longevity of both females (F = 30.63, df = 161, P < 0.01) and males (F = 6.52, df = 104, P < 0.01) (Table 2). Females and males had the highest average longevity at 18 and 20°C, respectively. The lowest longevity of both females and males occurred at 25°C. Mean longevity of adult females decreased with increasing temperatures, but this was reversed with a slight difference at 28 and 32°C. The pre-oviposition period, oviposition period, and post-oviposition period differed at various temperatures (F = 13.34, df = 104, P < 0.01; F = 17.53, df = 161, P < 0.01; and F = 9.74, df = 161, P < 0.01, respectively). Reproduction was highest on days 70, 70, 44, 33, 42, 37, and 47 at 18, 20, 23, 25, 28, 30, and 32°C, respectively. The highest number of eggs laid per female was recorded at 23°C.

Population growth parameters

Values of the net reproductive rate (R_0) , intrinsic

Table 2. Oviposition period and adult longevity of *Planococcus citri* reared on coleus at various constant temperatures (means±SE).

		Temperature (°C)						
Parameters	15	18	20	23	25	28	30	32
Pre-oviposition	-	19.38±0.81ª	18.75±0.59 ^a	12.52±0.77 ^b	11.13±0.77 ^b	11.96±0.84 ^b	13.60±1.47 ^b	13.90 ± 0.81^{b}
Oviposition	-	23.71±0.68 ^a	21.55±0.94 ^a	18.13 ± 0.82^{b}	17.60 ± 0.67^{b}	17.89 ± 0.84^{b}	15.30±0.76 ^{bc}	13.45±0.66 ^c
Post-oviposition	-	4.19 ± 0.37^{b}	3.45 ± 0.53^{bc}	1.87 ± 0.18^{d}	3.87 ± 0.31^{b}	2.29±0.25 ^{cd}	3.05±0.44 ^{cd}	5.35±0.51ª
Female longevity	-	47.29±0.81ª	43.75±1.22 ^b	32.52±1.14 ^c	$32.07 \pm 1.04^{\circ}$	32.14±1.22 ^c	31.95±1.47 ^c	32.70±0.89 ^c
Male longevity	-	$2.17 {\pm} 0.30^{ab}$	$2.50{\pm}0.80^{a}$	2.06 ± 0.17^{ab}	1.41 ± 0.09^{d}	1.50±0.12 ^{cd}	1.67 ± 0.10^{bc}	1.23 ± 0.12^{d}

Mean values followed by different letters in the rows are significantly different ($P \le 0.05$, SNK, after one-way ANOVA).

Table 3. Population growth parameters of *Planococcus citri* reared on coleus at various constant temperatures (means±SE).

Temperature (°C)	R_{o}	r _m	λ	D_t	Т
18	24.90±1.84 ^{de}	$0.058 {\pm} 0.001^{\rm f}$	$1.06 {\pm} 0.001^{\rm f}$	11.87±0.34ª	55.12±1.17 ^b
20	84.04±3.29 ^c	0.07 ± 0.000^{e}	1.07 ± 0.001^{e}	9.18±0.11 ^b	58.72±0.69ª
23	$144{\pm}12.80^{ab}$	0.12±0.001 ^c	1.13±0.004 ^c	5.41±0.17 ^b	38.89±0.93°
25	154±6.94ª	$0.17 {\pm} 0.004^{a}$	1.19±0.005ª	3.97±0.10 ^e	28.91±0.74 ^e
28	123.60 ± 10.10^{b}	$0.14 {\pm} 0.005^{b}$	1.15 ± 0.005^{b}	4.70±0.16d ^e	32.71 ± 0.90^{d}
30	36.42±3.62 ^d	$0.10 {\pm} 0.003^{d}$	1.11 ± 0.006^{d}	6.49±0.34 ^c	33.74 ± 1.44^{d}
32	8.83±1.06 ^e	$0.055 {\pm} 0.003^{\rm f}$	$1.05 {\pm} 0.003^{\rm f}$	12.43±0.1ª	39.32±0.1°

Mean values followed by different letters in the columns are significantly different (P < 0.05, SNK, after one-way ANOVA).

rate of natural increase (r_m) , finite rate of increase (λ) , mean generation time (T), and doubling time (D_T) were significantly different at various constant temperatures $(F = 58.82, \text{ df} = 161, P < 0.01; F = 122.59, \text{ df} = 161, P < 0.01; F = 116.75, \text{ df} = 161, P < 0.01; F = 136.55, \text{ df} = 161, P < 0.01; \text{ and } F = 125.50, \text{ df} = 161, P < 0.01, respectively) (Table 3). The net reproductive rate was highest at 25°C, with 154 eggs/female/generation, and lowest at 32°C, with 8.83 eggs/female/generation. The intrinsic rate of natural increase <math>(r_m)$ of *P. citri* rose with temperature to reach a maximum at 25°C and then declined at 28 to 32°C. The highest values of r_m and λ and lowest values of D_T and *T* occurred at 25°C, suggesting that this is the optimal temperature for *P. citri*.

DISCUSSION

The development time from egg to adult female of *P. citri* decreased between 15 and 25°C (with a slight increase at 20°C). It increased at temperatures higher than 25°C (Table 1). These data are in agreement with the results of Walton and Pringle (2005), who studied *Pseudococcus ficus* (Signoret) on Waltham Cross grapevines. Cloyd (1999) reported 33.7 days for development of *P. citri* on red variegated coleus.

Our data indicate that 25°C is the optimal temperature for P. citri development among the tested temperatures. A higher female-biased sex ratio occurred at all temperatures except 32°C. More males at extreme temperature (32°C) can be seen as an adoptive response (Walton and Pringle, 2005) and a result of stress, producing greater genetic variability and increasing the probability of survival of the population (Margolies and Wrensch, 1996; Walton and Pringle, 2005). The adult sex ratio of Maconellicoccus hirsutus Green (Homoptera, Pseudococcidae) has been reported to be male-biased $(1.4 \stackrel{?}{\bigcirc}: 1 \stackrel{?}{\supseteq})$ (Persad and Khan, 2002). Walton and Pringle (2005) reported that the pre-oviposition period of P. ficus lasted for 45.87, 36.11, 3.44, 15.97, and 19.90 days at 18, 20, 25, 27, and 30°C, respectively, values which were higher (except at 25°C) than the data obtained in the current study for P. citri.

The highest total fecundity occurred at 20°C. Printz (1923) and Bodenheimer and Guttfeld (1929) found that *P. citri* produces 12 and 180 eggs per female at 17 and 21°C, respectively. According to the study of Copland et al. (1985), *P. citri* lays fewer than 100 eggs above 30°C, but can lay over 400 at 18°C. These data are lower than those obtained in the current study, except at 18°C, which is higher than our data. Mafi and Radjabi (1997) reported that the mean numbers of eggs per female of P. citri were 150, 180, and 195 on citrus leaves at 19, 23, and 27°C with 70, 75, and 80% RH, respectively, which are also lower than our results at similar temperatures. These differences may be due to different host plants and experimental conditions. The intrinsic rate of natural increase (r_m) of *P. citri* estimated in the current study ranged from 0.055 to 0.170 female/female/day. These values are similar to the values reported by Walton and Pringle (2005) for P. ficus at 20 (0.068), 25 (0.169), and 27°C (0.131), but they were higher at 30°C and very close at 18°C. The highest intrinsic rate of natural increase of P. citri occurred at 25°C and revealed high reproduction ability of citrus mealybug females at this temperature. Also, the mean generation time was shortest at 25°C. This result indicates that development of P. citri took place faster at this temperature than at the other temperatures. According to Yang and Sadof (1995), the intrinsic rate of natural increase for P. citri reared on red variegated coleus was 0.122 females/female/day. The intrinsic rate of natural increase is a composite statistic that takes into account life history parameters such as development rate, fecundity, longevity (survival), and sex ratio (Carey, 1993). Thus, it is one of the most important criteria used for evaluating the influence of temperature on life history of a pest. The values of population growth parameters of P. citri (except generation time) at 28°C in the current study were higher than data reported for M. hirsutus by Persad and Khan (2002).

Our results indicate that the development, reproduction, and survival of *P.citri* are influenced by temperature as an important physical factor, which should be taken into account in designing a routine sampling program (Zamani et al., 2006). Using the data obtained in the current study, we are able to calculate development and population growth rates of citrus mealybug over a wide temperature range. Consequently, the best time of pest control is predictable. However, although these results are valuable as a first step for establishment a IPM program to control citrus mealybug, more research needs to be conducted to determine the effect of fluctuating temperatures, various host plant cultivars, and other environmental factors on *P. citri* performance.

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УТИЦАЈ ТЕМПЕРАТУРЕ У РАЗВИЋУ И РАСТУ ПОПУЛАЦИЈЕ КОД *PLANOCOCCUS CITRI* (HOMOPTERA, PSEUDOCOCCIDAE) НА SOLENOSTEMON SCUTELLARIOIDES

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Развиће, животни циклус, репродукција и растење популације *Planococcus citri* Risso на *Solenostemon scutellarioides* (L.) (Codd.) проучавани су при температурама од 10-37°C, 70±10 RH и фотопериоду од 16:8 сати. Наша истраживања указују на огроман утицај температуре на развиће планококуса.

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