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BIOLOGY, THERMAL REQUIREMENTS, AND ESTIMATION OF THE NUMBER OF GENERATIONS OF *ZAPRIONUS INDIANUS* (DIPTERA: DROSOPHILIDAE) FOR THE MAIN FIG PRODUCING REGIONS OF BRAZIL

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ABSTRACT

The African fig fly, *Zaprionus indianus*, was first recorded in fig orchards in Brazil in 1999, at the Valinhos region, in the State of São Paulo. In view of the scarcity of biological data on this insect in Brazil, the objective of this research was to study the biology, temperature requirements, and to estimate the number of *Z. indianus* annual generations for the main fig producing regions of Brazil, aimed at controlling the pest. The insects were reared on artificial diet at constant temperatures of 18, 20, 22, 25, 28, 30, and 32°C, relative humidity 80 ± 10%, and 14-h photophase. Duration of the development stages was variable with temperature, being longer at the lower temperatures. Temperature also influenced viability during the larval and pupal stages and the egg-adult biological cycle, with significant differences between 32°C and the other temperature conditions. The lower temperature development threshold (TT) and thermal constant (K) values for the egg, larval, and pupal stages were 9.7 and 10.5; 9.2 and 148.6, and 10.7°C and 66.25 DD, respectively, for a total thermal constant of 262.2 DD for the egg-adult biological cycle. Based on a lower temperature development threshold of 7.9°C., *Z. indianus* may have up to 16.6, 12.4, and 12.7 annual generations in the regions of Valinhos-SP, Pelotas-RS, and São Sebastião do Paraíso-MG, respectively, which are traditionally known as fig producing regions in the country.

Key Words: fig fly, temperature requirements, exotic pest, bioecology

RESUMO

A mosca-africana-do-figo *Zaprionus indianus*, foi registrada, pela primeira vez, em pomares de figo no Brasil em 1999, na região de Valinhos, São Paulo. Tendo em vista a escassez de dados biológicos sobre o inseto no Brasil, o objetivo da pesquisa foi estudar a biologia e estimar, a partir das exigências térmicas, o número de gerações anuais de *Z. indianus* para as principais regiões produtoras de figo do Brasil, com vistas ao seu controle. Foram criados insetos em dieta artificial e nas temperaturas constantes de 18, 20, 22, 25, 28, 30 e 32°C, umidade relativa de 80 ± 10% e fotofase de 14 h. A duração das fases do desenvolvimento foi variável com a temperatura, sendo maior nas temperaturas menores. A temperatura também influenciou a viabilidade das fases de larva e pupa e do ciclo biológico ovo-adulto, havendo diferenças significativas entre 32°C e as demais condições térmicas. O limiar térmico inferior de desenvolvimento (Tb) e a constante térmica (K) para as fases de ovo, larva e pupa foram de 9,7 e 10,5; 9,2 e 148,6; 10,7°C e 66,25GD, respectivamente, totalizando para o ciclo biológico (ovo-adulto) uma constante térmica de 262,2GD, a partir de um limiar térmico inferior de desenvolvimento de 7,9°C. *Z. indianus* poderá dar até 16,6; 12,4 e 12,7 gerações anuais para as regiões de Valinhos-SP, Pelotas-RS e São Sebastião do Paraíso-MG, respectivamente, tradicionalmente conhecidas como produtoras de figo no país.

Translation provided by the authors.

The African fig fly, *Zaprionus indianus* (Diptera: Drosophilidae) was first reported on fig plants (*Ficus carica* L., Moraceae) in Valinhos, Brazil in 1999, causing yield losses of up to 50% (Vilela 1999). This drosophilid measures between 2.5 and 3.0 mm in length, and is brown in color,

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with red eyes. The dorsal region of the head and thorax has two longitudinal silvery-white stripes, between which run narrow black stripes. The eggs are milky and contain a filament, and generally are laid in small masses by more than one female into the ostiole of figs near maturation (Raga 2002).

Z. indianus is a semicosmopolitan fly which, apparently by human mediation, has spread across tropical regions in recent decades (Vilela et al. 2000; Bächli 1999-2005; van der Linde et al. 2006). It is probably of African origin, where it has been recorded in fruits of 74 plant species (Vilela 1999). In Brazil it has been recorded in several hosts (Souza Filho et al. 2000), and it became a pest in fig and required the use of chemical control by fig growers. The vast majority of drosophilid species are associated with bacteria and yeasts; *Candida tropicalis* has been identified in figs infested with *Z. indianus* collected in Valinhos, SP. This yeast causes increasing fig depreciation during production and trade, and attracts adults of the pest for feeding and oviposition (Raga 2002).

Chemical control was initially adopted but proved to be ineffective and brought about a significant increase in production costs. In addition, products registered for this pest are nonexistent, and there are barriers imposed by importing countries, especially from Europe, preventing import of fruits that present residues of unregistered chemical products (Vilela et al. 2000).

Within this context, several other management techniques are being adapted and developed, such as the use of fig ostiole protectors, which make it difficult for *Z. indianus* to oviposit and feed (Raga et al. 2003), use of attractive traps, and elimination of food substrates in the area and surroundings (Raga & Souza Filho 2003). Additionally, the collection and identification of natural enemies may facilitate the development of applied biological control (Marchiori & Silva 2003).

An important measure to improve pest control effectiveness is to establish prediction models for their occurrence based on temperature because it directly influences insect biology, metabolism, reproduction, and longevity (Sharpe & DeMichele 1977). Ecological zoning, based on temperature requirement values, indicates the most favorable regions for development of the biological potential of pests. In turn, population monitoring of the pest in the regions that are most favorable and with the fastest development rates increases the chance of determining when a pest outbreak will occur (Cividanes 2000).

The goals of this work were to study the development of *Z. indianus* at different temperatures, determine its temperature requirements in the laboratory, and estimate the number of generations for the main fig producing regions of Brazil, which may facilitate the establishment of pest management strategies.

Materials and Methods

Stock Rearing

Zaprionus indianus started from insects that came from the Entomology laboratory at Instituto Agronômico de Campinas. One-liter glass cages were used for rearing, with lids adapted with a nylon screen to allow aeration. The diet used for larval development and adult feeding consisted of brewer's yeast (7.5 g), banana (205 g), agar (5 g), nipagin (methyl parahydroxybenzoate) (1 g), Tetrex® (tetracycline) (0.05 g), and water (500 mL), and was adapted from diets for drosophilids (Singh 1977). The insects were maintained in the laboratory at a temperature of 27 ± 2°C, relative humidity of 80 ± 10%, and 14 h photophase.

Biology at Different Temperatures

In order to obtain eggs, approximately 300 adults were placed in a plastic cage (50 × 40 × 60 cm). An acrylic plate (2 × 7 cm) was placed inside the cage containing liquefied mango pulp to serve as substrate for oviposition. Every 15 min, the plate was replaced with a fresh one, and the substrate was inspected with a stereoscopic microscope to remove the eggs, which were transferred to Petri dishes (2 × 10 cm). In order to retain moisture and facilitate egg visualization on the plates, a piece of black filter paper (dyed with Guarany® fabric dye) was placed on the bottom of the Petri dish and moistened as needed. Twenty eggs were transferred to each Petri dish, for a total of 200 eggs. Each set of eggs was placed in climatic chamber (Fanem®, Camara Incubadora BOD. Mod. 347 CD, São Paulo, Brasil) held at 18, 20, 22, 25, 28, 30, and 32°C, 80 ± 10% relative humidity, and 14-h photophase. Evaluations were performed hourly (12 h), and duration and viability were recorded by counting the number of larvae hatched at each temperature.

Vilela's diet (1999) inside glass vials (2 × 8 cm) was used to study development at the larval stage. Ten larvae with ages up to 30 min after hatching were transferred to each vial, totaling 300 larvae at each temperature. Evaluations were performed daily, and the duration of the larval stage was recorded.

Acrylic plates (6.0 × 2.0 cm) containing moistened filter paper on the bottom were used for the development of the pupal stage. Pupae up to 24 h of age were used, totaling 200 insects at each temperature.

Bioassays for the egg, larval, and pupal stages were conducted in a completely randomized design, constituted of 7 treatments (temperatures) with 20, 30, and 20 replications, for egg, larval

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and pupal stages, respectively. The duration and viability data were submitted to analysis of variance (ANOVA) and the means were compared by Tukey test ($P \leq 0.05$).

Determination of Temperature Requirements and Number of Generations

Based on the duration data for the egg, larval, and pupal stages and for the egg-adult period, the lower temperature development thresholds (TT) and thermal constants (K) were determined by the hyperbole method (Haddad & Parra 1984). Duration at 32°C was not taken into consideration, since it was not statistically different from duration at 30°C ($P \leq 0.05$), thus avoiding the linearity of the hyperbolic curve that represented duration × temperature.

The likely number of annual *Z. indianus* generations was estimated based on the number of degree-d required for development of the insect as determined in the laboratory, and the temperature normals of the main fig producing regions of Brazil (Valinhos—SP, Pelotas—RS, and São Sebastião do Paraíso—MG).

Results and Discussion

The biological cycle of the insect (egg-adult) lasted less than 1 month at the 7 temperature conditions studied, creating conditions for the occurrence of several generations of the pest throughout the year. It was observed that the duration and viability of the different stages of the biological cycle varied according to the temperature (Tables 1 and 2). During the embryonic stage, there was a shortening of duration with increase in temperature from 18°C (1.23 d) to 30°C (0.49 d); at 32°C there were no changes in the incubation stage, showing a tendency of unsuitability of the egg stage to temperatures higher than 30°C although this value was statistically similar for 28°C, 30°C and 32°C. A high viability was observed at all temperatures except at 32°C, with a much lower value than the values recorded at the other temperatures (Table 2).

The tendency for negative effect at higher temperatures was observed in the larval stage. The duration at 32°C (9.3 d) was longer than at 30°C (8.6 d) and at 28°C (7.8 d), although it was only statistically different from 28°C and not from 30°C (Table 1). The negative effect of higher temperatures on larval development can be observed in the viability as 71.1% of the insects died at 32°C (Table 2).

For the pupal stage, a variation from 9.7 d at 18°C to 3.2 d at 32°C was observed, and the duration was shorter in the range from 28 to 32°C, although no significant differences were observed. Possibly, temperatures higher than 32°C are harmful to *Z. indianus* development as the viability at this temperature was significantly lower than the other temperatures, which showed values higher than 88.6%.

The total duration of the cycle (egg-adult) increased with the decrease in temperature, varying from 28.8 d at 18°C to 12.7 d at 28°C (Table 1). At 30°C (13.3 d) and 32°C (13.0 d), the duration of the cycle was longer than at 28°C (12.7 d), suggesting that high temperatures are harmful to development although there were no significant differences observed (Table 1). For total viability,

Table 1. Mean duration and variation interval (between parentheses) for the egg, larval, and pupal stages and biological cycle (egg-adult) of *Z. indianus* on artificial diet and at different temperatures, 80 ± 10% RH, and 14-h photophase.

Temperature (°C)	Duration			
	Egg stage (d)	Larval stage (d)	Pupal stage (d)	Biological cycle (d)
18	1.23 ± 0.07 a (2.05-0.83)	17.9 ± 0.41 a (19.6-13.7)	9.7 ± 0.08 a (10.5-6.6)	28.8 ± 0.68 a (30.7-23.1)
20	1.00 ± 0.04 b (1.17-0.53)	13.0 ± 0.38 b (15.5-10.8)	6.9 ± 0.19 b (8.1-4.2)	20.9 ± 0.66 b (25.5-16.1)
22	0.84 ± 0.03 c (1.01-0.58)	11.4 ± 0.20 c (13.5-10.6)	5.4 ± 0.07 c (6.4-4.2)	17.6 ± 0.23 c (22.3-15.7)
25	0.73 ± 0.03 d (0.88-0.35)	9.9 ± 0.20 d (11.3-8.2)	4.8 ± 0.12 c (5.5-3.4)	15.4 ± 0.26 d (18.3-13.1)
28	0.60 ± 0.03 e (0.76-0.35)	7.8 ± 0.16 e (8.6-6.8)	4.3 ± 0.07 d (5.0-3.7)	12.7 ± 0.21 e (15.2-11.0)
30	0.49 ± 0.03 e (0.69-0.22)	8.6 ± 0.28 de (10.00-7.30)	3.2 ± 0.11 d (3.6-3.0)	13.3 ± 0.47 e (14.1-10.9)
32	0.51 ± 0.04 e (0.74-0.17)	9.3 ± 0.29 d (10.0-7.3)	3.2 ± 0.07 d (3.4-3.0)	13.0 ± 0.53 de (15.0-10.6)

Means followed by the same letter in the columns are not statistically different by the Tukey test ($P \leq 0.05$).

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TABLE 2. MEAN VIABILITY AND VARIATION INTERVAL (BETWEEN PARENTHESES) FOR THE EGG, LARVAL, AND PUPAL STAGES AND BIOLOGICAL CYCLE (EGG-ADULT) OF *Z. INDIANUS* ON ARTIFICIAL DIET AND AT DIFFERENT TEMPERATURES, 80 ± 10% RH, AND 14-H PHOTOPHASE.

Temperature (°C)	Viability (%)			
	Egg stage	Larval stage	Pupal stage	Biological cycle
18	86.8 ± 4.26 a (100.0-40.0)	74.6 ± 4.6 a (90.0-30.0)	91.4 ± 3.40 a (100.0-80.0)	57.1 ± 2.61 a (64.8-46.7)
20	87.8 ± 3.12 a (100.0-70.0)	76.0 ± 4.12 a (100.0-40.0)	90.0 ± 5.8 a (100.0-60.0)	65.3 ± 7.5 a (80.0-36.0)
22	90.3 ± 2.01 a (100.0-70.0)	66.0 ± 3.62 a (100.0-50.0)	91.4 ± 2.60 a (100.0-80.0)	59.3 ± 4.8 a (75.0-43.2)
25	82.5 ± 3.3 a (93.0-50.0)	72.0 ± 4.3 a (100.0-30.0)	88.6 ± 3.40 a (100.0-70.0)	49.3 ± 9.1 a (81.0-18.0)
28	84.0 ± 2.7 a (100.0-66.7)	84.6 ± 4.47 a (100.0-50.0)	93.3 ± 4.22 a (100.0-70.0)	62.0 ± 7.7 a (90.0-32.0)
30	84.2 ± 4.0 a (100.0-20.0)	67.0 ± 7.61 a (100.0-30.0)	91.7 ± 3.07 a (100.0-80.0)	46.0 ± 8.8 ab (72.9-18.9)
32	25.3 ± 3.22 b (36.0-3.0)	28.9 ± 4.2 b (100.0-50.0)	66.7 ± 8.43 b (40.0-10.0)	15.3 ± 4.8 b (80.0-30.0)

Means followed by the same letter in the columns are not statistically different by Tukey test ($P \leq 0.05$).

it was observed that temperatures higher than 32°C are harmful to the development (Table 2).

In general, it can be said that temperatures near to 28°C is the thermal optimum to egg-adult development of *Z. indianus*, allowing shorter development time and high viability. This temperature is similar to the findings by Silveira Neto et al. (1976), who reported that temperatures within the range of 22 and 28°C are optimal for insect development in tropical conditions. Furthermore, the duration and viability results indicate that, although the diet employed was simple and consisted of components easily purchased in the domestic market, it was suitable for rearing this pest in laboratory conditions.

In Brazil, Stein et al. (2003) studied the biology of the insect at 25°C, and obtained a duration of 17.1 d for the egg-adult period. In other countries (Saudi Arabia), Amoudi et al. (1993) verified a biological cycle of 23 d at 20°C for *Z. indianus*, higher than the value obtained in the present research (Table 1). Amoudi et al. (1991) recorded values of 8.1 and 6.9 d at 25° and 30°C for the larval stage and 6.9 and 4.7 d for the pupal stage of this species. These small variations from results obtained in the present work are probably due to the different diet used and to different photoperiod conditions. The authors in Saudi Arabia reared the insect with a 24-h photophase, while in the present research the insect was reared with a 14-h photophase; in addition, different populations were studied. It is convenient to highlight that variations during the egg stage may be related to the interval between observations, which was short in the present research (1 h) and longer in the other studies (Amoudi et al. 1991).

The artificial diet used in this work was prepared with yeast and banana and was suitable for development for *Z. indianus*, although 75% viability recognized by Singh (1983) as the percentage above which the artificial diet can be considered suitable was not achieved. The results obtained by C. P. Stein (pers. inform.), who maintains laboratory populations since 1999, demonstrated that the insect does not become adapted to the diet as verified with other laboratory species. In this case, such alterations might be associated with the effect of high temperatures, causing greater genetic variability in the population, while temperatures near the optimum reduce variability, increasing viability (David et al. 1983). In the present case, such effect was evident for the egg and pupal stages (Table 3). In this research viability ranged from 15.3% (32°C) to 65.3% (20°C) (Table 2). The highest mortality occurred in the larval stage in general, with low mortality values in the egg and in the pupal stage. Handling adjustments and even replacement of diet components might improve the insect's performance during the larval stage. Stein et al. (2003) also observed greater mortality in the larval stage when they reared the pest at 25°C; Amoudi et al. (1991) reared the insect at 35°C and obtained low larval viability (26%). They did not record pupal development at that condition and no adults of the species were obtained. The unsuitability of higher temperatures was clearly demonstrated for the egg and larval stages and for the entire cycle of *Z. indianus*.

The lower temperature development threshold (TT) and thermal constant (K) values for the egg, larval, and pupal stages were 9.7 and 10.5; 9.2

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TABLE 3. TEMPERATURE THRESHOLD (TT), THERMAL CONSTANT (K), REGRESSION EQUATION (1/D), AND COEFFICIENT OF DETERMINATION (R^2) FOR THE EGG, LARVAL, AND PUPAL STAGES AND FOR THE EGG-ADULT PERIOD OF *Z. INDIANUS*, UNDER LABORATORY CONDITIONS.

Stages/period	TT (°C)	K (DD)	Regression equation	R^2	*P*
Egg	9.7	10.5	$1/D = 0.095276\ t_i - 0.923945$	0.9742	0.05
Larva	9.2	148.6	$1/D = 0.006727\ t_i - 0.062075$	0.9804	0.05
Pupa	10.7	66.25	$1/D = 0.015094\ t_i - 0.161966$	0.9392	0.05
Egg-adult	7.9	262.2	$1/D = 0.003814\ t_i - 0.030169$	0.9754	0.05

DD = Growing degree-days.
D = Duration.

and 148.6; and 10.7°C and 66.25 DD, respectively. Therefore, the Tb and K values for the biological cycle (egg-adult) were 7.9°C and 262.2 DD (Table 3, Fig. 1). These TT values differ from those recorded for *Z. indianus* in Saudi Arabia, as 7.7°C for the egg stage, 11.7°C for the larval stage, and 8.0°C for the pupal stage, totaling 10.1°C for the biological cycle (egg-adult) (Amoudi et al. 1993). Probably the geographic distribution (Honék 1996) and/or food used in the larval stage (Zeiss et al. 1996) may have contributed toward these differences, in addition to the photoperiod and insect strain used.

These results may contribute to implement control measures aimed at fig exports. Adults lay their eggs into the ostiole, and these eggs will originate larvae in a very short period of time. Thus, depending on the condition, the larvae will hatch before arriving at the importing country. Placing figs at temperatures below the egg stage TT (9.7°C) may kill the embryos and prevent hatching and damage. According to C. V. Rombaldi (pers. comm.), the Roxo-de-Valinhos fig cultivar can withstand temperatures from 2 to 5°C, with a shelf life of 2 d. Research must be conducted in this direction, making fig storage compatible with the mortality of eggs laid, although due to the short embryonic development period, such measure only finds application for freshly-laid eggs.

The number of annual *Z. indianus* generations varied according to the mean monthly temperature of each region. Estimated values for the Valinhos-SP, Pelotas-RS, and São Sebastião do Paraíso-MG regions were 16.6, 12.4, and 12.7 generations, respectively (Table 4). Although a succession of generations may occur throughout the year, in some regions figs are only available during a certain season. In the other months, *Z. indianus* probably feeds on other hosts. Souza Filho et al. (2000) mentioned that like all drosophilids, the pest feeds on decaying fruits and plants, including acerola, banana, cashew, star fruit, citrus, guava, jaboticaba, mango, peach, purple mombin, tomato, persimmon, and others.

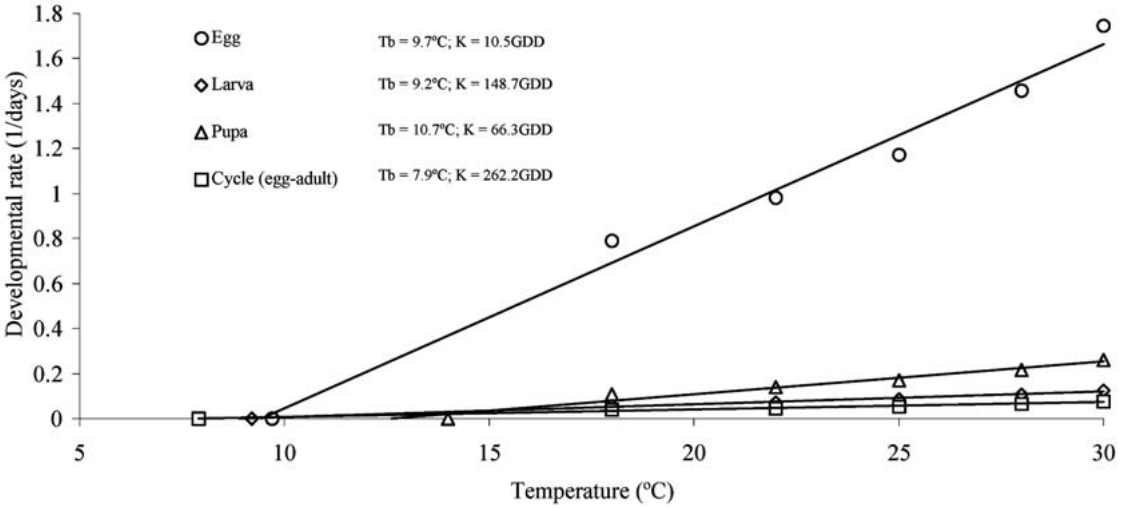


Fig. 1. Relation between developmental speed and temperature for the egg, larval, and pupal stages and biological cycle (egg-adult) of *Z. indianus* reared on artificial diet, RH: 80 ± 10%, and 14-h photophase. TT = Temperature thresholds; K = Thermal constant.

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TABLE 4. TEMPERATURE NORMALS FOR THREE FIG PRODUCING REGIONS IN BRAZIL, CUMULATIVE DEGREE-DAYS, AND ESTIMATES FOR THE NUMBER OF GENERATIONS OF *Z. INDIANUS* AS DETERMINED FROM TEMPERATURE REQUIREMENTS.

	Valinhos (SP)[1]		Pelotas (RS)[2]		São Sebastião do Paraíso (MG)[3]	
	Temperature (°C)	Cumulative degree-d	Temperature (°C)	Cumulative degree-d	Temperature (°C)	Cumulative degree-d
Jan	23.5	458.8	23.2	449.5	19.0	319.3
Feb	23.6	417.2	23.0	400.4	18.3	268.8
March	23.0	443.3	21.7	403.0	18.5	303.8
Apr	20.8	363.0	18.5	294.0	17.5	264.0
May	18.4	300.7	15.1	198.0	16.6	244.9
Jun	17.1	252.0	12.4	111.0	15.4	201.0
Jul	16.9	246.0	12.3	105.0	15.3	198.0
Aug	18.5	303.8	13.4	145.7	17.8	282.1
Sep	20.0	339.0	14.9	186.0	18.9	306.0
Oct	21.2	387.5	15.5	210.8	19.7	341.0
Nov	22.0	399.0	19.6	327.0	18.8	303.0
Dec	22.7	434.0	22.0	412.3	18.7	310.0
Total		4,344.3		3,242.7		3,341.9
Thermal constant for *Z. indianus*		262.2		262.2		262.2
Number of annual generations		16.6		12.4		12.7

Data obtained from [1]Departamento de Ciências Exatas, ESALQ/USP; [2]Embrapa Clima Temperado; [3]Epamig.

Therefore, local control measures, including orchard cleaning, with elimination of fallen fruit, may contribute to reduce the population of the pest. In regions where late prunings are performed, and production occurs almost throughout the year, problems with the pest are even greater, such as in the Valinhos region.

Nonetheless, the temperature requirement-related results obtained are a head start for studies on the prediction of occurrence of the pest, based on alert system to be installed in fig producing areas.

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