horticultura brasileira

Research

ANDZEIEWSKI, S; LERIN, S; BORTOLI, LC; NONDILLO, A; BERNARDI, D; BOTTON, M. 2018. Thermal requirements and estimates of the annual number of generations of Lobiopa insularis on strawberry crop. *Horticultura Brasileira* 36: 054-058. DOI - http://dx.doi.org/10.1590/S0102-053620180109

Thermal requirements and estimates of the annual number of generations of *Lobiopa insularis* on strawberry crop

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ABSTRACT

Lobiopa insularis is one of the main pests of strawberry crop, causing direct damage to fruit, making them unfeasible for consumption and commercialization. This study aimed to estimate, under laboratory conditions, base temperature and thermal requirements of the eggs, larvae, pupae and the biological cycle (from egg to adult) of *L. insularis.* Insects were reared on artificial diet based on strawberry fruits, at temperatures of 16, 19, 22, 25, 28 and 31°C, using air-conditioned chambers (70±10% R.H.; 12 h photophase). The number of annual generations of *L. insularis* was estimated for eight municipalities belonging to the main strawberry growing regions in Brazil, considering base temperature and thermal constant. Development time of *L. insularis* was proportional to the temperature increase. The best development rate was obtained when insects were reared at 22 and 25ºC. Based on thermal requirements of *L. insularis*, the number of annual generations was estimated between 5 and 7 per year, according to the studied region.

Keywords: *Fragaria x ananassa*, strawberry sap beetle; biological cycle, base temperature.

RESUMO

Exigências térmicas e estimativas do número de gerações anuais de *Lobiopa insularis* em morangueiro

Lobiopa insularis é uma das principais pragas da cultura do morangueiro, provocando danos diretos nos frutos, inviabilizando--os para o consumo e comercialização. Neste trabalho, foi estimada a temperatura base e as exigências térmicas das fases de ovo, larva, pupa e do ciclo biológico (ovo a adulto) de *L. insularis.* Os insetos foram criados em dieta artificial à base de frutos de morango maduros, nas temperaturas de 16, 19, 22, 25, 28 e 31°C, utilizando câmaras climatizadas (70±10% U.R.; fotofase de 12h). O número de gerações anuais de *L. insularis* foi estimado para oito municípios pertencentes às principais regiões produtoras de morango no Brasil, por meio da temperatura base e da constante térmica. A velocidade de desenvolvimento de *L. insularis* aumentou com a elevação da temperatura. A faixa mais adequada para o desenvolvimento da espécie foi entre 22 e 25ºC. Com base nas exigências térmicas de *L. insularis*, o número de gerações anuais foi estimado entre cinco e sete de acordo com a região estudada.

Palavras-chave: *Fragaria x ananassa*, broca-do-morangueiro; ciclo biológico; temperatura base.

Received on June 29, 2016; accepted on May 5, 2017

In Brazil, strawberry (*Fragaria x ananassa*) is the species which belongs to the group of most widely cultivated and consumed small fruits, and which also shows greater economic expression (Tazzo *et al*., 2015). The estimated production of this fruit in Brazil is about 105,000 t, in an area of approximately 4,000 ha (Reisser Júnior *et al*., 2014), highlighting the States of Minas Gerais (1,790 ha), Paraná (535 ha), Rio Grande do Sul (400 ha) and São Paulo (331.4 ha) (Carvalho *et al*., 2013).

Among the arthropods pests associated with the strawberry crop, able to cause significant losses in the crops and in the quality of fruits, strawberry sap beetle (Coleoptera: Nitidulidae) is the one which stands out (Guimarães *et al*., 2009; Botton *et al*., 2014; Rondon *et al*., 2014). Popularly known as strawberry sap beetle, this cosmopolitan insect has the capability to feed on different hosts such as banana, fig, corn, vine and strawberry (Rondon *et al*., 2014). In strawberry crop, insects are able to infest green, semi-ripe and ripe fruits (Fornari *et al*., 2013), which after infestation, become unsuitable for consumption and marketing (Guimarães *et al*., 2009; Carvalho *et al*., 2013; Bortoli *et al*., 2014; Botton *et al*., 2014). In addition to causing direct damage to fruits, adults can facilitate the dispersal of pathogens that cause fruit rot, increasing crop production losses (Rondon *et al*., 2014). Damage is caused both by larvae and adults, occurring mainly during the fruiting period (Williams *et al*., 1995; Rondon *et al*., 2014).

In studies carried out in laboratory, strawberry sap beetle showed five stages of larval development when fed with artificial diet based on strawberries, with adult longevity of approximately 271±20.7 days for males and 318±14.9 days for females, at a temperature of 25°C (Bortoli *et al*., 2014). Under field conditions, in order to overcome hard weather conditions during off-season period or during the winter season, mainly in cold regions such as the South of Brazil, the larvae and adults show the ability to go into diapauses, sheltering in areas adjacent to crops, under the undergrowth, stones and pieces of wood (Fornari *et al*., 2013; Rondon *et al*., 2014).

Thus, understanding the insect behavior is one of the main steps to be studied for Integrated Pest Management (IPM) (Kogan, 1998). Temperature is an important biotic factor which can interfere with adaptation and development of a species (Bursell, 1974). Therefore, mathematical models which use thermal units (degrees-day) can be used to describe development rates and predict occurrence of insect population peaks in the field, helping define times to use control measures (Woodson & Edelson 1988; Cividanes, 2000).

Due to the importance of strawberry sap beetle for strawberry crop, associated to its occurrence in all Brazilian regions where this crop can be grown (Guimarães *et al*., 2009; Fornari *et al*., 2013), the aims of this study were to verify the effect of different temperatures on the development of strawberry sap beetle and determine the thermal requirements of the insect, estimating the annual number of generations in different strawberry producing regions in Brazil.

MATERIAL AND METHODS

The experiment was developed in a laboratory. Adults of strawberry sap beetle were collected in commercial strawberry fields, in the municipality of Caxias do Sul-RS (29º11’48”S, 50º57’15”W) and, in the laboratory, the insects were packed in 300 mL-plastic boxes and kept under controlled conditions (temperature of 25±1ºC, relative humidity of 70±10% and 12 h photofase), according to the method proposed by Bortoli *et al*. (2014). The authors used as substrate chemical residue-free ripe strawberry fruits, cultivar Aromas (Bortoli *et al*., 2014).

In order to study basal temperature and thermal requirements for different development phases (egg, larvae and pupae) and biological cycle (from egg to adult), the postures were removed from the plastic container lids with the aid of a fine-tipped brush, then individualized and placed in Petri dishes (7-cm diameter and 2-cm height) sealed with plastic film (Magipack) and put in air-conditioned BOD type chambers, regulated at constant temperature of 16, 19, 22, 25, 28, 31±1ºC, relative humidity of 70±10% and 12 h photofase. After hatching, the larvae were fed with an artificial diet based on strawberry fruits (Bortoli *et al*., 2014). After the addition of the artificial diet (10 g diet per plate), the plates were closed with plastic lid wrapped with a plastic film tape to prevent larvae from leaving. The artificial diet was renewed every two days. For all the treatments (temperatures), the authors evaluated duration and viability of eggs, larval, pupal stages and biological cycle (egg to adult), through daily observations.

Thermal requirements for immature stages of eggs, larvae and pupae and for the biological cycle (egg to adult) of strawberry sap beetle were estimated calculating the inferior thermal threshold or base temperature (Tb) and thermal constant (K). Based on the values of Tb, K and accumulation of degree-days (GD), the authors estimated annual number of generations of strawberry sap beetle for the main Brazilian regions where strawberry is grown, using average monthly historical temperatures of each region. The probable number of annual strawberry sap beetle generations was estimated for eight municipalities of the main strawberry producing regions in the country: Caxias do Sul-RS, Pelotas-RS, Jaboti-PR, São José dos Pinhais-PR, Atibaia-SP, Piedade-SP, Datas-MG and Pouso Alegre-MG. For Pouso Alegre, Datas, Jaboti and São José dos Pinhais, the authors used the data obtained in the municipalities of Itajubá-MG, Diamantina-MG, Joaquim Távora-PR and Curitiba-PR respectively, since these municipalities have similar weather conditions, and they also have weather stations. The monthly average temperatures used were obtained at Universidade Federal de Itajubá (data used for Pouso Alegre), Instituto Agronômico do Paraná (for the municipality of Jaboti), at Centro Integrado de Informações Agrometeorológicas (for the municipalities of Atibaia and Piedade), and at Instituto Nacional de Meteorologia for the other cities.

The experimental design was completely randomized, with six treatments (temperatures) with 100 replicates per treatment. The data related to duration and viability of egg, larval, pupal stages and biological cycle (egg to adult) were submitted to the analysis of variance using F-test. When significant, the averages were compared using Tukey test, at 5% probability (SAS Institute, 2000). In order to verify quantitative effect of the treatments (temperatures) on the studied biological parameters, regression equations were estimated, calculating the respective coefficients of determination (R^2) and graphics which better adjusted the regression curves were elaborated (SAS Institute, 2000). Thermal requirements of different stages of the development of strawberry sap beetle were estimated through the method of hyperbole (Haddad *et al*., 1999), calculating the inferior thermal threshold or base temperature (Tb) and thermal constant (K) (SAS Institute, 2000), and, then, estimating the probable number of annual strawberry sap beetle generations based on the average annual temperature of different municipalities of four different regions, as previously described.

RESULTS AND DISCUSSION

Temperatures affected all the stages of development of strawberry sap beetle, which showed an inverse relationship between increase in temperature and duration of development (Table 1; Figure 1). In the embryonic period, the longest duration was recorded at temperature of 16ºC (12.0±0.10 days) and the shortest at temperatures of 28ºC (3.8±0.04 days) and 31ºC (3.8±0.04 days), which differed (F= 2154.10; $d.f$= 5; 468; P<0.0001) from the other temperatures studied. Significant differences were also observed (F= 14.60; $d.f$= 5; 594; P<0.0001) for the viability of the egg, considering that the lowest viability observed occurred at temperature of 19ºC (55%) and the highest at temperature of 28ºC (98%)

Table 1. Duration (average ± SE) of the eggs, larvae, pupae and biological cycle (egg to adult) of *Lobiopa insularis* under different temperatures (70±10% RH; 12 h photophase. Bento Gonçalves, Embrapa Uva e Vinho, 2014.

Temperature (°C)	Duration (days)			
	Egg	Larvae	Pupae	Egg - adult
16	12.0 ± 0.10 a (76)[1]	72.0 ± 5.50 a (6)	*	*
19	7.60 ± 0.08 b (55)	39.7 ± 8.94 b (20)	14.0 ± 0.11 a (18)	61.3 ± 0.89 a (15)
22	5.0 ± 0.06 c (72)	34.7 ± 0.78 c (53)	8.9 ± 0.18 b (54)	48.06 ± 1.05 b (40)
25	4.8 ± 0.06 c (90)	33.1 ± 0.54 c (53)	7.7 ± 0.24 b (48)	45.6 ± 0.65 b (43)
28	3.8 ± 0.04 d (95)	28.6 ± 0.82 d (37)	5.9 ± 0.27 c (32)	38.3 ± 0.65 c (30)
31	3.8 ± 0.04 d (86)	32.6 ± 2.37 c (10)	4.2 ± 0.20 d (5)	40.6 ± 2.2 c (5)

*Temperature not used for statistical analysis; Averages followed by the same letter in the column did not differ significantly from each other, Tukey, p<0.05. [1]Values in parentheses indicate the number of observations.

(Table 2).

In relation to the duration of larval stage of strawberry sap beetle, the authors noticed a significant reduction considering days (F= 103.06; $d.f$= 5; 163; P<0.0001) with an increase in temperature (Table 1; Figure 1). The increase in the stage of larval development influenced directly on larval viability, considering that at temperatures of 16 and 31ºC, the authors verified the lowest larval viabilities (inferior to 10%), differing from the other temperatures (F= 25.61; $d.f$= 5; 594; P<0.0001) (Table 2), which resulted in complete mortality of the larvae at a temperature of 16ºC (Table 1), resulting in the extinction of the population in this climatic condition (Table 2). However, for the other temperatures, values of pupal duration ranged significantly

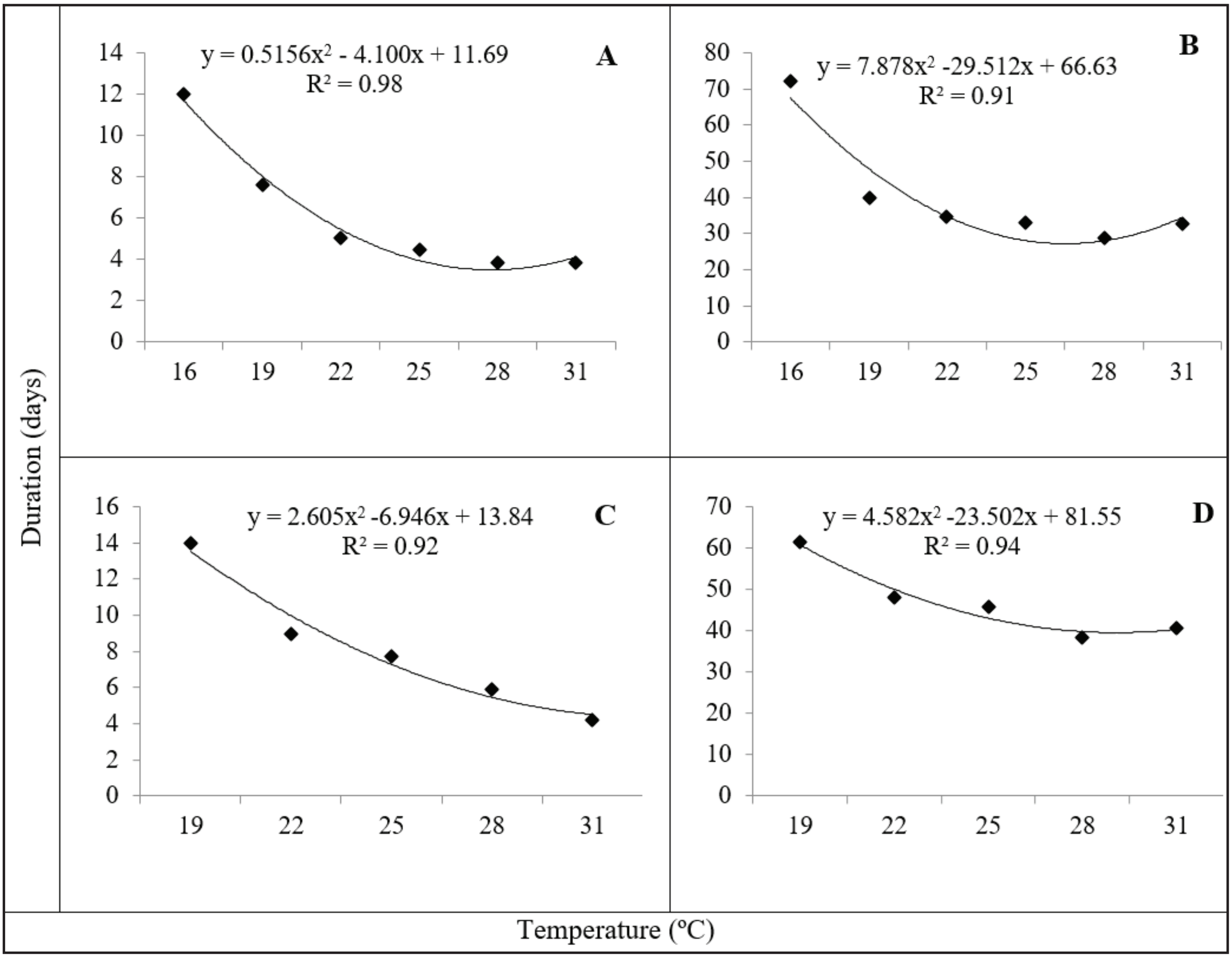

Figure 1. Regression curves adjusted for the duration of egg (A), larvae (B), pupae (C) and biological cycle (egg-adult) (D) periods of Lobiopa insularis under different temperatures (70±10% RH; 12 h photophase).

Table 2. Viability (%) (average ± SE) of eggs, larvae, pupae and biological cycle (egg-adult) of *Lobiopa insularis* under different temperatures (70±10% RH; 12 h photophase). Bento Gonçalves, Embrapa Uva e Vinho, 2014.

Development stage	Temperature (ºC)					
	16	19	22	25	28	31
Egg	76.0 ± 4.3 bc	55.0 ± 5.0 d	72.0 ± 4.5 c	91.0 ± 2.9 ab	95.0 ± 2.2 a	86.0 ± 3.5 ab
Larvae	7.0 ± 2.56 d	20.0 ± 4.0 c	53.0 ± 5.1 a	55.0 ± 5.0 a	37.0 ± 4.9 b	10.0 ± 3.0 d
Pupae	*	90.0 ± 3.1 b	98.2 ± 1.4 a	90.6 ± 2.9 ab	86.5 ± 3.5 b	37.5 ± 5.2 c
Cycle (egg-adult)	0.0 ± 0.0 e	9.9 ± 3.0 c	37.4 ± 1.4 ab	45.1 ± 2.9 a	30.2 ± 3.4 b	3.2 ± 1.1 d

*Temperature not used for statistical analysis; Averages followed by the same letter in the line did not differ significantly from each other, Tukey, p<0.05.

Table 3. Inferior thermal threshold (Tb), thermal constant (K), linear equation of development rate (1/D) and determination coefficient (R^2) of eggs, larvae, pupae, and biological cycle (egg-adult) period of *Lobiopa insularis* under different temperatures (70±10% RH; 12 h photophase). Bento Gonçalves, Embrapa Uva e Vinho, 2014.

Development stages	Tb (ºC)	K (GD)	Regression equation	R^2	F	*p*
Egg	7.9	80.1	y = - 0.099 + 0.0125x	0.93	55.2	0.002
Larvae	6.5	571.4	y = - 0.0113 + 0.0017x	0.91	31.7	0.011
Pupae	13.9	76.9	y = - 0.181 + 0.013x	0.95	61.4	0.004
Egg-adult*	5.1	833.3	y = - 0.006 + 0.001x	0.93	30.1	0.032

*For the biological cycle (from egg to adult) temperatures of 16 and 31°C were removed from the analysis.

Table 4. Strawberry producing municipalities in Brazil, annual average temperatures (°C), accumulated annual degree-days (GD) and probable number of *Lobiopa insularis* generations per year. Bento Gonçalves, Embrapa Uva e Vinho, 2014.

Municipality-state	Annual average temperature (ºC)	Accumulated degree-days (GD)	Probable number of generations/ year
Pelotas-RS	17.9	4658.2	5.58
Caxias do Sul-RS	16.6	4183.8	5.02
São José dos Pinhais-PR	17.2	4395.9	5.27
Jaboti-PR	21.0	5801.7	6.96
Dantas-MG	18.6	4906.3	5.88
Pouso Alegre-MG	20.2	5499.9	6.59
Atibaia-SP	20.6	5659.1	6.79
Piedade-SP	19.8	5358.5	6.43

(F= 107.62; $d.f$= 4; 151; P<0.0001) according to the temperature (Table 1), being the shortest pupal period (days) observed at temperature of 31ºC. The shortest period of pupal development resulted in low viability of the stage (<40%) (Table 2), differing from the other treatments (F= 14.11; $d.f$= 4; 498; P<0.0001) which showed pupal viability higher than 80%.

In relation to biological cycle (from egg-adult), the authors noticed that the higher the temperature, the lower the duration of development (Table 1). At temperature of 31°C, the value of the duration of egg-adult period showed a significant increase in days (F= 107.62; $d.f$= 174.4; P<0.0001) considering the temperature of 22 and 25ºC, being similar to the temperature of 28ºC., though. Similarly, the authors verified difference (F= 24.11; $d.f$= 4; 484; P<0.0001) in biological cycle viability (from egg to adult) in relation to temperature, ranging from 17, 50,5; 47; 32 and 4%, to temperatures of 19; 22; 25; 28; and 31ºC, respectively (Table 2), showing that the optimal range of development for biological cycle (from egg to adult) is between 22 to 25ºC, corroborating the results found by Bortoli *et al.* (2014) which verified the same biological performance at constant temperature of 25°C.

Considering the regression curves for each development stage of strawberry sap beetle (eggs, larvae and pupae and egg to adult) in relation to temperature, the data were adjusted to second degree equation (R^2>0.90) (Figure 1), showing significant inverse relationship between duration of development and increase in temperature in all development stages, showing that over 90% of the times the decreasing of time for strawberry sap beetle development is explained by an increase of temperature. Generally, extreme constant temperatures (16 and 31°C) are detrimental to the development of strawberry sap beetles. However, under field conditions, the authors conclude that the insect is able to survive in these thermal conditions since extreme temperatures for long periods are rare and they normally occur during the day (Haddad *et al.*, 1999). This factor and also the fact that embryonic and larval development of the insect occur inside the fruits, provide protection against unfavorable temperatures (Rondon *et al.*, 2014). Also, larvae and adults can go into diapauses, sheltering in areas adjacent to crops, as for example, under the undergrowth, stones and pieces of wood (Fornari *et al*., 2013; Rondon *et al*., 2014).

Based on the stages of the development of eggs, larvae and pupae and biological cycle (from egg to adult) of strawberry sap beetles, under different temperatures, the authors verified that basal temperature (Tb) of the different stages were: 7.98ºC for egg, 6.46ºC for larvae, 13.92ºC for pupae and 5.11ºC for biological cycle (from egg to adult), corresponding to thermal constant (K) of 80.1; 571.4; 76.9 and 833.3 degree-days (GD), respectively (Table 3). Considering data of thermal constant and climatological normal of each municipality, the authors estimated the number of generations of the insect per year in different strawberry growing regions in Brazil. For Pelotas-RS, Caxias do Sul-RS, São José dos Pinhais-PR, Jaboti-PR, Datas-MG, Pouso Alegre-MG, Atibaia-SP and Piedade-SP the number of generations year^{-1} was 5.58; 5.02; 5.27; 6.96; 5.88; 6.59; 6.79 and 6.43, respectively (Table 4), showing that the number of annual generations of this species can vary according to the region and local temperature. Even if the number of generations per year of strawberry sap beetle has been calculated on the basis of the thermal requirements (Tb and K), observed under laboratory conditions, this information is good to obtain inferences for different locations and climatic conditions, since the pest was reported in practically almost all the Brazilian regions where strawberries are grown (Guimarães *et al*., 2009; Botton *et al*., 2014; Zawadneak *et al*., 2014).

In studies in order to verify population fluctuation of strawberry sap beetle in strawberry crops with the aid of monitoring traps based on ripe strawberry, a significant increase of this pest populations was observed due to the increase of temperature, with higher population peaks in January, when the temperature ranged from 20 to 25ºC (Fornari *et al*., 2013), which is in accordance with the data found in this study, determining that the best thermal conditions for the insect development were 22 and 25ºC. These conditions associated with high biotic potential of strawberry sap beetles in strawberry fruits (Bortoli *et al*., 2014) and its ability to infest fruits in different degrees of ripeness (Fornari *et al*., 2013), may provide optimal conditions for the insect, concerning its development throughout the growing stages of strawberry under field conditions. Thus, determining thermal requirements and estimating the number of annual pest generations for different growing regions may contribute to predict pest occurrence in the field, which can help develop management strategies and control species in relation to the strawberry growing area and time of year.

ACKNOWLEDGEMENTS

To The National Council for Scientific and Technological Development (CNPq), Coordination of Improvement of Higher Education Personnel (CAPES), Foundation for Research Support of Rio Grande do Sul (FAPERGS) and Embrapa Grape and Wine (Embrapa Uva e Vinho) for providing equipment and structure.

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