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Oviposition Behavior of *Grapholita molesta* Busck (Lepidoptera: Tortricidae) at Different Temperatures

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Abstract

Cultivation of temperate-climate fruits is economically important for Brazil. *Grapholita molesta* Busck is a pest that causes damage to apples, peaches, plums, and pears growing in different micro-regions of southern Brazil, and understanding its reproductive behavior is essential to develop control strategies. The objective of this study was to ascertain the influence of different temperatures (13, 16, 19, 22, and 25ºC) on the oviposition behavior of *G. molesta*. Females of *G. molesta* were placed in individual plastic containers, and the pre-oviposition period and the number of eggs laid were assessed until adult death. Temperature influenced the pre-oviposition period, and females kept at 22° were the first to lay their eggs. Oviposition occurred over a longer period of time at 13°C than at the higher temperatures. The highest total number of eggs was obtained at 19°C, with the mean daily oviposition being directly proportional to the temperature. There was a negative interaction between the pre-oviposition period and the total number of eggs laid by females. The most suitable temperature for oviposition of *G. molesta* was 19°C.

Introduction

In Brazil, cultivation of temperate-climate fruits, especially apples, peaches, and plums, is mostly concentrated in the southern areas. The main apple-producing regions, Vacaria (Rio Grande do Sul state), Fraiburgo, São Joaquim and Lages (Santa Catarina state), and Lapa and Porto Amazonas (Paraná) have different climates because of their geographical locations. The environmental conditions in these regions influence the behavior of pests, including the oriental fruit moth *Grapholita molesta* (Busck), one of the key pests of temperate-climate fruit-growing regions in Brazil (Monteiro & Hickel 2004).

A population dynamics study showed that *G. molesta* is most abundant in the warmer months of the year (Hickel *et al* 2003), which coincide with the fruit-ripening period, resulting in high potential for economic damage. Although temperatures during the winter in southern Brazil are unsuitable for the physiological activity of the insect, adults of *G. molesta* can still be found in most of the fruit-producing regions. The exception is São Joaquim, a region situated at 1,200 a.m.s.l., where minimum monthly temperatures range from 3°C to 6°C and maximum temperatures from 18°C to 21°C (INMET 2007).

Studies evaluating the effects of temperature on the development and reproduction of *G. molesta* were carried out by Dustan & Armstrong (1933) and Chaudhry (1956), and their data guided several behavioral studies on *G. molesta*, in addition to serving as a basis for other ecological studies on this species. Understanding how temperature affects insect bioecology is a key process in developing efficient control strategies (Price 1997) and improving monitoring strategies, besides being an important factor when analyzing the insect's geographical distribution.

Very little is known on the influence of temperature on *G. molesta* in Brazil, and available information mostly describes the effects of temperature on the development of immatures (Grellmann *et al* 1992). Therefore, more information is required on the effects of temperature on *G. molesta* performance, especially in temperatures lower than those tested in previous studies (Chaudhry 1956, Grellmann *et al* 1992), in order to represent the natural conditions for the species in some of the major fruit-growing regions in southern Brazil.

The objective of this study was to assess the influence of temperature on the oviposition behavior of *G. molesta* under laboratory conditions.

Material and Methods

Insect origin

Pupae of *G. molesta* were obtained from a laboratory colony initiated from insects collected in apple orchards in Vacaria, RS (28º26'S and 50º48'W, altitude 950 m), and maintained for two years on an artificial diet (Guennelon *et al* 1981).

Oviposition behavior assessment

Insects were sexed during pupal stage (Beeke & Jong 1991), and insect couples were isolated in plastic containers (200 ml) closed with tulle fabric. Adults were fed with a solution of 13% honey and 0.13% nipagin impregnated on a cotton swab that was replaced every three days until the end of the experiment.

The oviposition performance of *G. molesta* was observed at 13, 16, 19, 22, and 25°C ± 1ºC by placing 50 couples in climate chambers with a 14:10 h (L:D) photoperiod and 70 ± 10% RH, in a fully randomized experimental design. The following parameters were assessed daily: the pre-oviposition period, the total number of eggs, and the time when the eggs were laid.

Statistical analysis

The data collected on the number of eggs laid were subjected to Anova ($F < 0.05$) and means were compared by the Tukey test ($P < 0.05$), using the freely available software R (R Development Core Team 2005) and Statgraphics Centurion XV, version 15.1.02 (StatPoint®). The distribution of times of egg laying was fitted to the Gaussian model using the Matlab 7.0 Program (MathWorks®). The interaction between the pre-oviposition period and the total number of eggs laid was defined. The pre-oviposition period was estimated by classes, based on the quartiles and the total egg number in each class, calculated using the program Excel (Microsoft, San Francisco, USA).

Results and Discussion

Pre-oviposition period (POP)

The POP of *G. molesta* was represented by cumulative frequency curves (Fig 1), and two behavioral tendencies were observed. A longer POP was observed at temperatures below 19°C, with means of 11.4 and 7.5 days at 13°C and 16°C, respectively. Females started to lay eggs earlier at temperatures higher than 16°C, on average 4.4, 3.6, and 5.9 days, respectively, for 19, 22, and 25°C. The POP observed at these temperatures was on average 27%

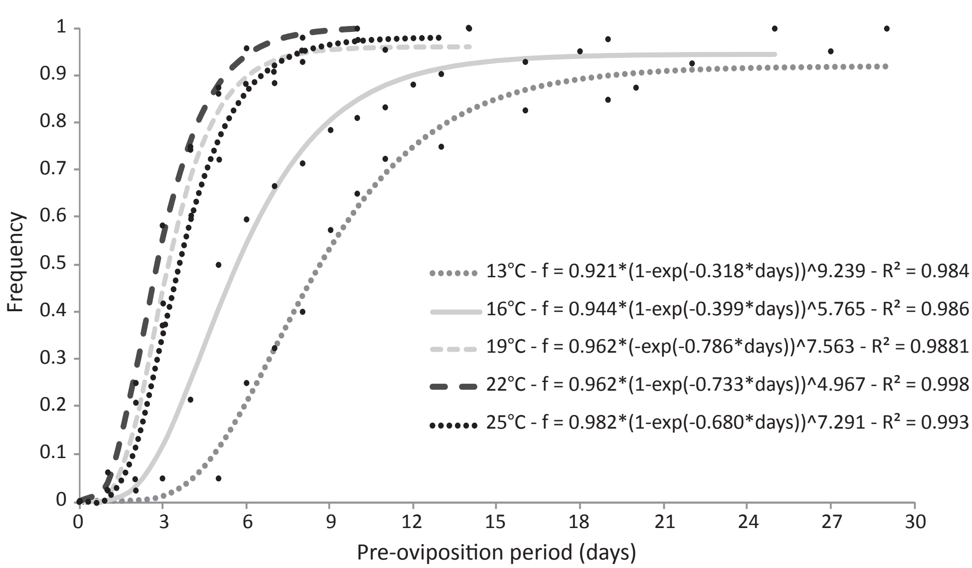

Fig 1 Cumulative frequency distribution of the pre-oviposition period (POP) of *Grapholita molesta* at 13, 16, 19, 22, and 25 ± 1°C.

lower than those at 13°C and 16°C. The relationship between temperature (t) and POP was established as POP = 56.158 - 4.9372 t + 0.1167 t^2 ($P < 0.05$; $R^2 = 0.985$). The second-degree equation predicts that higher temperatures will be unfavorable for *G. molesta* reproduction, resulting in an increase in POP in the areas of Brazil with temperatures higher than 22°C.

The POP was relatively uniform at 19, 22, and 25°C (respectively, σ = 2.8, 1.7, and 2.3 days); significantly lower ($P < 0.05$) than those at 13 and 16°C (respectively, σ = 6.7 and 4.7 days).

About 60% of the females kept at 16°C initiated egg-laying activity on the fifth day after emergence, while 90% of females kept at 19°C or above were already actively laying eggs after this period (Fig 1). This behavior of females at low temperatures may be related to the need to accumulate energy to maintain vital functions, and has also been observed in another insect species, *Plutella xylostela* (Lepidoptera: Yponomeutidae) (Crema & Castelo Branco 2004).

Total number of eggs (TNE)

The influence of temperature on *G. molesta* TNE was significant and showed a symmetrical trend, with the largest mean number of eggs per female at 19°C (Fig 2), about twice as large as the TNE obtained at the extreme temperatures. The maximum number of eggs per female was 272, at 19°C, more than the 262 observed by Reichart & Bodor (1982). At 22°C and 25°C, more eggs were produced than was observed by Chaudhry (1956), for temperatures between 21°C and 26.6°C. The relationship between TNE and temperature (t) can be expressed by TNE = -1605.9244 + 243.8651 t -11.0668 t^2 + 0.16 t^3 ($P < 0.29$; $R^2 = 0.943$) (Fig 2).

The variance of the total number of eggs laid per female was similar ($P = 0.26$) in all treatments. That is, the TNE was homoscedastic regardless of the experimental conditions.

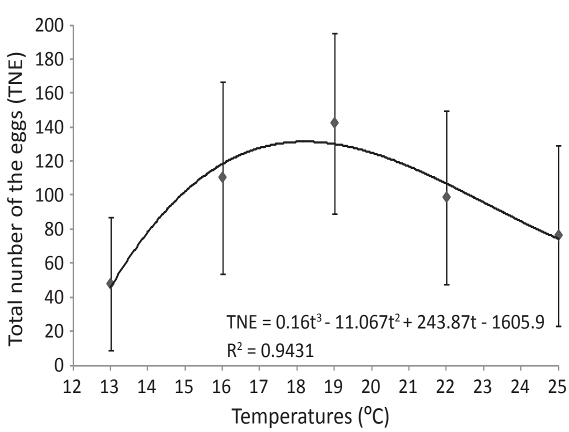

Fig 2 Mean of the total number of eggs (TNE) laid per *Grapholita molesta* female at 13, 16, 19, 22, and 25 ± 1°C.

Oviposition daily rhythm (ODR)

Females maintained at 13°C (Fig 3a) distributed their eggs homogeneously over a total of 51 days of oviposition. At temperatures higher than 13°C, eggs were laid during up to 50% of the ODR period (Fig 3b, c, d, e), and the lowest ODR occurred at 22°C (Fig 3 d).

The average duration of egg-laying activity (DELA) was similar at 13, 16, and 19°C (Fig 4a), and was nearly 45% longer than at the other temperatures tested. This difference was shown by analyzing the probability function of the accumulated DELA (Fig 4b) that distinguishes these two groups.

The variance in DELA was larger at temperatures lower than 22°C, and decreased as the temperature increased (Fig 4a). The variance showed that part of the females prolonged their egg-laying activity at low temperatures. In nature, this extension of the egg-laying period may increase reproductive success by distributing egg production over a wider range of conditions.

The ratio between total number of eggs and average duration of egg-laying activity (TNE/DELA) showed that the largest mean daily egg production (DEP) occurred at 22°C (Fig 5), suggesting that females in this temperature were more efficient in producing/laying eggs, although females at 19°C laid a larger number of total eggs. In low temperatures, mean DEP was reduced 50% compared to the number of eggs produced at 22°C; a similair reduction (56%) was observed at 13°C compared to 16°C (Fig 5). The ratio between temperature (t) and DEP can be defined by the equation DEP = -4.45667 + 0.728667 t ($P < 0.05$; $R^2 = 0.850$) (Fig 5).

Cyclical oviposition peaks were identified (Fig 3a-f) at approximately 3-day intervals, but in different quantities among treatments. Thus, females at 13°C required 34 days after emergence to complete 90% oviposition, which was 212% longer than the time needed at 16°C and 19°C (16 days). An increase of 3°C, compared to 19°C, resulted in a four-day decrease in the period needed to carry out 90% of the oviposition; there was no difference between 22°C and 25°C (12 days). Fig 3f shows the general oviposition performance, regardless of temperature, and in this case, 19 days were needed to carry out 90% of the oviposition.

Relationship between the pre-oviposition period (POP) and the total number of eggs (TNE)

As seen in Table 1, the TNE tended to be higher in the first three quartiles in all treatments. Females were more fecund when the POP was lower, a relationship observed at 19°C and 22°C.

The combined results of all treatments allowed a statistical analysis as a single observation, from which it was ascertained that the first two classes (frequency of eggs) represented about 76% of the total clutches (Table

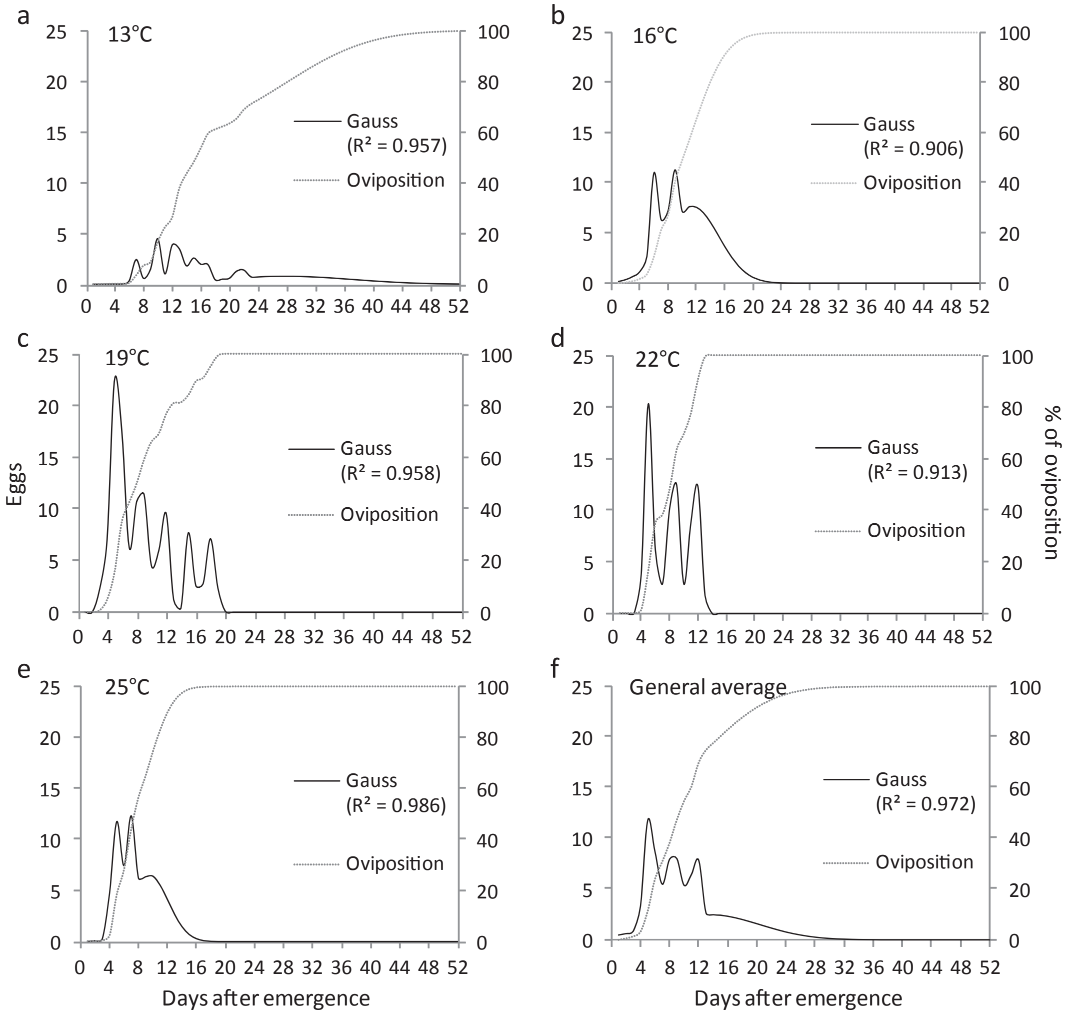

Fig 3 Daily and cumulative oviposition rhythm of *Grapholita molesta* according to the Gaussian model at different temperatures (a to e). General daily and cumulative oviposition rhythm for all treatments (f).

1). Thus, females that had the smallest POP were those that laid the largest TNE, shown by the equation TNE = $185.3e^{-0.14\ POP}$ ($P < 0.05$; $R^2 = 0.880$) (Fig 6).

As a conclusion, females of *G. molesta* with a shorter pre-oviposition period were the most fecund. As temperature affected the pre-oviposition period, the temperature of 19°C was be the most favorable for *G. molesta* reproduction (expressed as egg-laying activity).

Egg-laying peaks occurred cyclically, every three days, in the oviposition behavior of *G. molesta*, regardless of

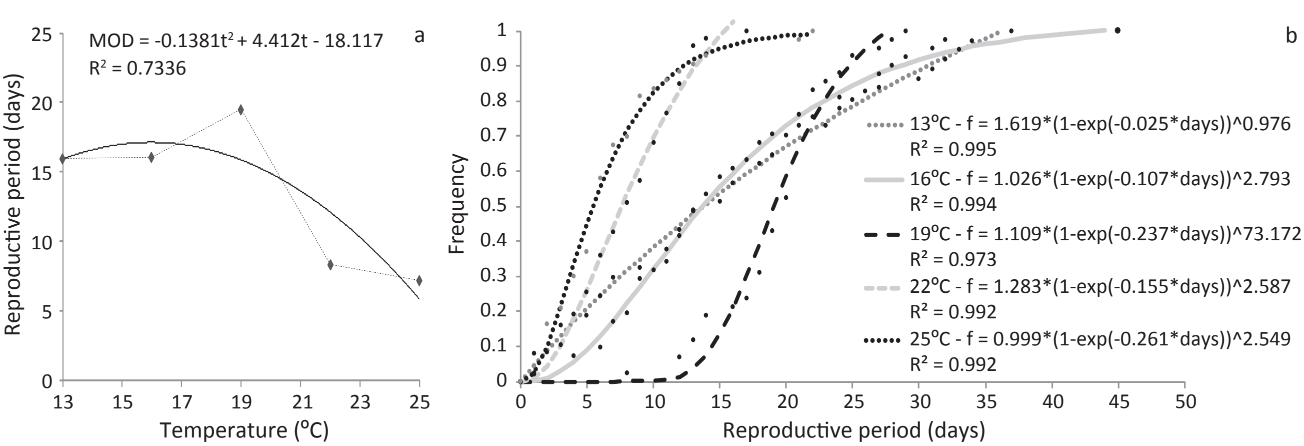

Fig 4 Reproductive period of *Grapholita molesta* at different temperatures (a). Cumulative distribution frequency of *Grapholita molesta* reproductive periods at different temperatures (b). MOD - reproductive period.

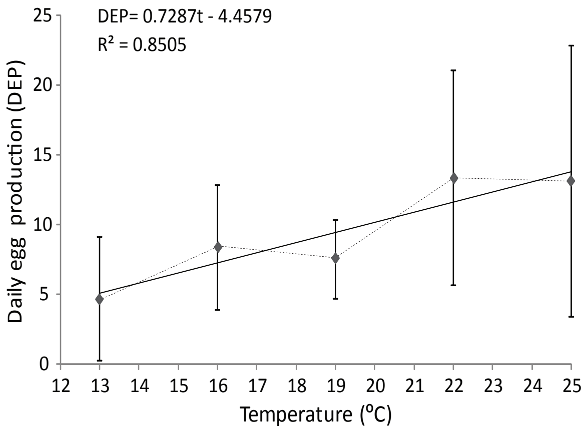

Fig 5 *Grapholita molesta* mean daily egg production at different temperatures.

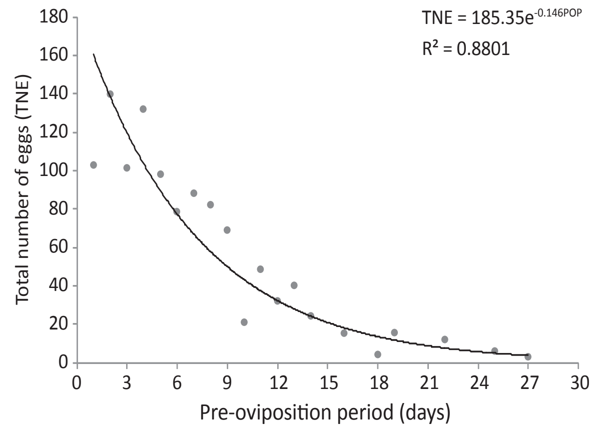

Fig 6 General relationship between the pre-oviposition period and total number of eggs laid by *Grapholita molesta*.

Table 1 Relationship between pre-oviposition period (POP) and total number of eggs (TNE) of *Grapholita molesta* at different temperatures, arranged by quartiles.

Temperature (°C)	Quartile[1]	N[2]	POP (days)	NTE[3]	σ	CV	Total number eggs	Frequency of eggs[4]
13	1st	10	<6	59.10 a	36.02	60.95	591	0.334
	2nd	13	6.0 – 9	61.62 a	49.65	80.58	801	0.453
	3rd	7	9 – 13.0	32.00 a	18.30	57.20	224	0.127
	4th	10	>13	15.10 b	15.81	104.69	151	0.085
16	1st	9	<4	132.56 a	59.28	44.72	1193	0.264
	2nd	12	4 – 5.0	121.58 a	47.12	38.75	1459	0.323
	3rd	12	5.0 – 9	129.67 a	41.18	31.76	1556	0.344
	4th	9	>9	34.44 b	23.96	69.56	310	0.069
19	1st	16	<3	157.00 a	31.70	20.19	2512	0.421
	2nd	16	3.0 – 4.0	163.19 a	46.83	28.70	2611	0.438
	3rd	5	4 – 5.0	107.80 ab	53.12	49.28	539	0.090
	4th	6	>5	50.33 b	43.98	87.39	302	0.051
22	1st	12	<2	104.50 a	49.07	46.96	1254	0.271
	2nd	16	2.0 – 3	83.75 a	52.46	62.64	1340	0.290
	3rd	8	3 – 4.0	132.75 a	46.28	34.86	1062	0.230
	4th	11	>4	88.18 a	48.69	55.22	970	0.210
25	1st	18	<3	102.94 a	50.59	49.15	1853	0.566
	2nd	8	3.0 – 4.0	67.00 ab	47.04	70.22	536	0.164
	3rd	12	4.0 – 6.0	51.25 b	41.51	81.00	615	0.188
	4th	5	>6.0	53.60 ab	64.30	119.96	268	0.082
Overall	1st	64	<3	112.89 a	52.72	46.70	7225	0.359
	2nd	69	3.0 – 5.0	117.06 a	58.80	50.23	8077	0.401
	3rd	31	5.0 – 7.0	81.13 b	56.31	69.40	2515	0.125
	4th	52	>7.0	44.83 c	42.94	95.79	2331	0.116

[1]Quartiles calculated by the program Excel 2007 (Microsoft, USA); [2]Number of females; [3]TNE - total number of eggs per female; [4]Frequency of eggs in relation to the total eggs laid in the treatment.
Means followed by different letters within the treatment were significantly different by the Tukey test (α = 0.05).

temperature. At 19°C, *G. molesta* females had the greatest potential for daily egg laying.

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