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Original Article

Effect of a short-cycle apple tree cultivar on oriental fruit moth (Lepidoptera: Tortricidae) development and larval behavior

Efeito de uma cultivar de macieira de ciclo curto no desenvolvimento e comportamento larval da mariposa oriental (Lepidoptera: Tortricidae)

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Abstract

The oriental fruit moth, *Grapholita molesta* (Busch, 1916) has historically been a major problem in traditional apple-growing regions in Rio Grande do Sul and Santa Catarina; however, a lower occurrence of *G. molesta* has been observed in early variety ('Eva') than long cycles cultivar 'Gala' in Paraná State. The objective of this study was to evaluate the performance of immature and adult *G. molesta* in apple cultivars with short and long cycles, in addition to elucidating whether there is adaptive potential for development and fertility from the first generation to the fourth. The experimental treatments consisted of larvae fed 'Eva' and 'Gala' fruit and a commercial diet. Immature development time, head capsule width, longevity and reproductive parameters were compared across the treatments. The larvae of the fourth generation exhibited better performance than those of the first on 'Eva' and 'Gala'. Immature oriental fruit moth development occurred via four or five instars. A greater number of larvae with four instars occurred on 'Eva' than on 'Gala'. The head capsule width was narrower on 'Gala' than on 'Eva' for larvae with four instars. Females reared on 'Eva' also laid more eggs than those reared on 'Gala'. The larvae that were more adapted to the feeding conditions showed reduced cycles/instars, and the females exhibited better performance when new generations originated from the same substrate. The high fluctuation of *G. molesta* in 'Gala' orchards is not due to the nutritional condition of the fruits.

Keywords: *Grapholita molesta*, apple, larval feeding behavior, reproduction, biology.

Resumo

A mariposa oriental, *Grapholita molesta* (Busch, 1916), tem sido historicamente um grande problema nas regiões tradicionais de cultivo de maçã no Rio Grande do Sul e em Santa Catarina; entretanto, observou-se menor ocorrência de *G. molesta* na variedade precoce ('Eva') no Paraná do que na cultivar tardia 'Gala'. O objetivo deste estudo foi avaliar o desempenho larval e adulto de *G. molesta* em cultivares de macieira com ciclos curtos e longos, além de elucidar se há potencial adaptativo para o desenvolvimento e fertilidade da primeira à quarta geração. Os tratamentos experimentais consistiram em larvas alimentadas com frutas 'Eva' e 'Gala' e uma dieta comercial. Tempo de desenvolvimento imaturo, largura da cápsula cefálica, longevidade e parâmetros reprodutivos foram comparados entre os tratamentos. As larvas da quarta geração exibiram melhor desempenho do que as da primeira em 'Eva' e 'Gala'. O desenvolvimento imaturo da mariposa oriental ocorreu em quatro ou cinco instares. Um maior número de larvas com quatro instares ocorreu em 'Eva' do que em 'Gala'. A largura da cápsula da cabeça foi mais estreita em 'Gala' do que em 'Eva' para larvas com quatro instares. As fêmeas criadas em 'Eva' também colocam mais ovos do que aquelas criadas em 'Gala'. As larvas mais adaptadas às condições de alimentação apresentaram instares reduzidos, e as fêmeas exibiram melhor desempenho quando as novas gerações se originaram do mesmo substrato. A alta flutuação de *G. molesta* nos pomares 'Gala' não se deve ao estado nutricional dos frutos.

Palavras-chave: *Grapholita molesta*, macieira, comportamento alimentar larval, reprodução, biologia.

1. Introduction

The Oriental fruit moth, *Grapholita molesta* (Busch, 1916) (Lepidoptera: Tortricidae) (GM), is an oligophagous invasive species from Asia that reproduces on plants within the Rosaceae family, producing several generations per year in Brazil on both peach and apple trees (Monteiro and Hickel, 2004; Silva et al., 2010). The majority of apple orchards in Brazil consist of 'Gala' and 'Fuji' varieties, adapted below the 27th parallel south; however, in warmer regions such as Paraná (Brazil), producers plant 'Eva' (Hauagge and Tsuneta, 1999). 'Eva' is an early, high-yielding apple cultivar that produces good-quality fruit. It was a generated from cross between 'Anna' and 'Gala'

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and requires the accumulation of 300-450 chill units to break bud dormancy (Hauagge and Tsuneta, 1999). In the Porto Amazonas and Lapa municipalities (Paraná State), fruits from "Eva" are harvested in early December, while 'Gala' apples are harvested 45 days later, in mid-January (personal information).

The host-associated tolerance status of GM in the early maturing cultivar of apple and the fluctuations of GM in Paraná State is not available in the literature. Evidence suggests that there is a lack of synchrony between the occurrence of the pest and the maturation period of the fruits (personal information). This pest exhibits population peaks under warmer temperatures (Hickel et al., 2003; Monteiro and Hickel, 2004; Silva et al., 2010). Between October and January, monthly average temperatures of 16.9, 18.3, 19.6 and 20.6° C have been reported in Porto Amazonas (http: //en.climate-data.org/location/43733/). Abiotic conditions influence insect populations (Carroll and Quiring, 1993); however, the nutritional status of hosts also plays a determining role (Scriber and Slansky Junior, 1981; Najar-Rodriguez et al., 2013).

GM exhibits less of a preference for attacking the fruits of early apple varieties (Silva et al., 2010) as well as less of a preference for oviposition and larval development on early varieties (Myers et al., 2006a, b). In opposite situation, oviposition behavior in hot-temperature regions is of particular interest because late fruits are more attractive and it is subject to great damage caused by immature of GM (Myers et al., 2006a, b; Najar-Rodriguez et al., 2013). This may be due to host quantitative and qualitative traits, such as the physical traits of tissues and secondary metabolite concentrations (phenols, sugars and acids) specific to each variety (Paganini et al., 2004; Hecke et al., 2006). Many studies have been conducted on the development of GM larvae on apple cultivars such as 'Golden Delicious', 'Gala' and 'Fuji', all of which are highly demanding cultivars in the cold (Myers et al., 2006a, c; Silva et al., 2010; Bisognin et al., 2012), but there have been few studies on the development of GM on short-cycle cultivars in hot regions. Additionally, knowledge about the behavior of the larvae can provide important information about the susceptibility of cultivars and the efficacy of control tactics.

The greater capture of GM adults in 'Gala' orchards may be due to the better nutritional characteristics and susceptibility of this cultivar in relation to 'Eva'. The objectives of the study were to verify the performance of immature and adult GM in apple cultivars with short and long cycles and to understand whether there is adaptive potential for development and fertility from one generation to the next, simulating the variations that occur during the growing season.

2. Material and Methods

2.1. Study area

The orchard was located in Porto Amazonas (25°32'42" S, 49°53'24" O, 793 m) and consisted of two plots of 15 ha each containing the apple cultivars 'Eva' and 'Gala' separated by 500 meters. The average fluctuations of GM on the two cultivars over the last seven years were determined by installing ten delta-style pheromone traps (BioControle, Indaiatuba, SP, Brazil), hung in the center and at the borders of the blocks. The catch of male moths was recorded twice weekly. The number of GM collected in the 'Gala' plots was higher than in the 'Eva' plots, particularly in periods without insecticide application (February to October) (Figure 1).

2.2. Fruit sampling and insect rearing

GM larvae were collected from apple ripe fruits in a commercial orchard and reared on 'Eva' and 'Gala' fruit and an artificial diet (Guennelon et al., 1981) at the Integrated Pest Management Laboratory (LAMIP) in Curitiba at 21 ± 1°C, 70% RH and 16: 8 photoperiod (L: D). Egg laying was performed in plastic tubes (8 cm in diameter, 20 cm long), after the eggs were deposited on fruits or a commercial diet in a plastic container (30 x 14 x 10 cm) for larval development. The larvae pupated in gauze strips in plastic containers.

GM reproduced on the substrates for four generations. The first larvae obtained in the laboratory were referred to as the first generation: those reared on 'Eva', they were referred to as EVA1, on 'Gala', as GALA1, and on the commercial diet, as DIET1. After they had reproduced for four generations, the larvae were referred as EVA4, GALA4 and DIET4. In addition, a subset of the larvae that had reproduced on 'Eva' and 'Gala' for three generations was reared on the commercial diet the fourth generation, which were referred to as the EVA4-DIET and GALA4-DIET, respectively. The standard commercial diet for this bioassay was Lepidoptera larvae (Stonefly Industries Ltd, Rochester, NY).

Ripe 'Eva' and 'Gala' fruits were collected from the study orchard, in which phytosanitary control was reduced, applying mating disruption and no insecticides were applied 40 days before harvest. The fruits were stored in a controlled atmosphere chamber (0° ± 2°C, 90% RH) located at the producer's facility. The fruits were used for rearing larvae until the 4th generation (from January to August). These fruits were washed with soap and warm water, rinsed, and allowed to air dry. Fruits weighing from 78 g to 100 g were individually placed in 0.3 L clear plastic containers with the peduncle side down. Two gauze pieces measuring 2.0 cm^2 were placed under and over the fruit to provide sites for pupation and easy observance of this activity.

2.3. Biological parameters on the diet

The following biological parameters were assessed: immature development period (IDP), adult development period (ADP), pre-oviposition period (POP), total number of eggs (TNE), number of eggs per female (NEF), oviposition daily rhythm (ODR), daily egg production (DEP) and duration of egg-laying activity (DELA). Biological evaluations were carried out in both first- and 4th-generation GM. Immature development (1st, 2nd, 3rd, 4th and 5th instars) took place in a container (2 x 2 x 2 cm) with the commercial diet, which was covered with a plastic lid. Neonates of the 1st- and 4th-generations were observed every eight hours to

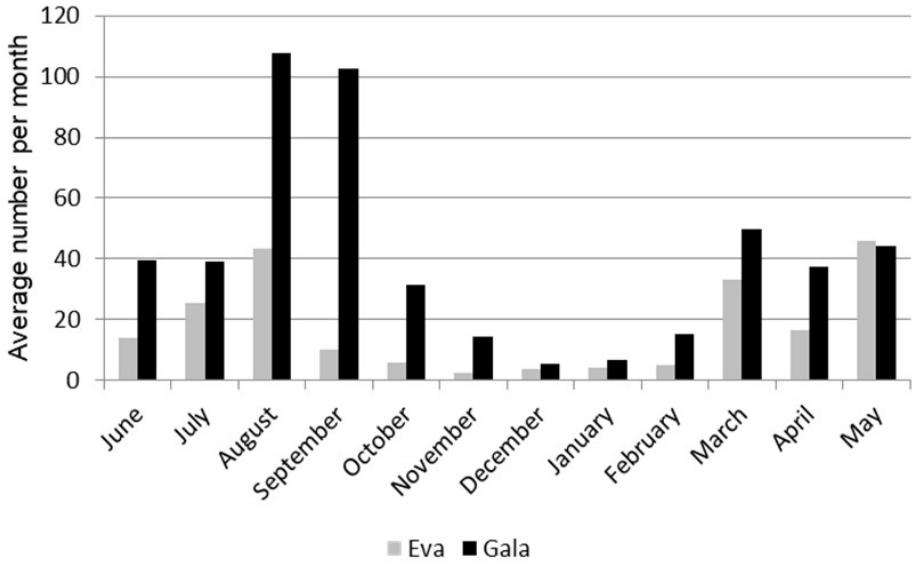

Figure 1. Average number of *Grapholita molesta* males captured monthly by pheromone-bait traps in 'Eva' and 'Gala' apple orchards during seven years in Porto Amazonas, Paraná, Brazil.

record the duration of each instar (Silva et al., 2011). The larvae were transferred to the commercial diet by using a fine camel hair brush. Neonates with a maximum age of one hour penetrated the diet and formed a gallery. In each instar, the head capsule was retracted at the opening of the entry hole. The treatment included 100 repetitions distributed in a chamber at 21 ± 1°C under 70% RH and 16: 8 (L: D) h photoperiod.

2.3.1. Measurement of the head capsule

The head capsules of larvae of the 4th-generation reared in the DIET4, GALA4-DIET and EVA4-DIET were stored in acrylic containers. The width of the head was measured by using a micrometric ocular under a stereoscopic microscope for each instar. The number of repetitions in each treatment depended on the mortality in the prior instar and the integrity of the head capsule.

2.3.2. Immature and adult development on fruit

One neonate was placed on each fruit on the calyx of the apples arranged in containers covered with PVC film. The larvae developed inside of both types of fruits until approximately the 19th day, when they exited the fruits and traveled to the provided gauze for pupation. Each treatment ('Eva', 'Gala' and commercial diet) included 100 repetitions distributed in a chamber at 21 ± 1°C under 70% RH and a 16: 8 (L: D) h photoperiod. The larvae were transferred to fruit by using a fine camel hair brush.

The pupae were sexed according to Beeke and Jong (1991) and placed individually in containers (2 x 2 x 2 cm) covered with plastic lids. They were observed to score the stage duration. GM females and males were mated according to the emergency sequence in each treatment and then placed individually in 300 mL plastic bottles covered with gaze strips. They were kept in a conditioned chamber (21 ± 2°C, 70% RH and 16: 8 (L: D) h photoperiod) and fed a solution of honey (13%) and nipagin (0.13%) (Arioli et al., 2007) in water, which was impregnated in cotton balls. The food was replaced every two days. The number of eggs laid was registered daily. POP, NEF, DEP and longevity were evaluated according to Silva et al. (2011).

2.4. Statistical analyses

The data were tested for normality using the Shapiro and Wilk (1965) test and for homoscedasticity using the Bartlett (1937) test. Data on the stage duration, lifespan, and fecundity that did not show a normal distribution or homogeneity of variance were x + 0.5 root transformed. The collected data 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 Statgraphics Centurion XV, version 15.1.02, software (StatPoint®). 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).

3. Results

3.1. Immature Development Period (IDP) on the diet

No statistically significant differences of IDP's were detected among Larva 1, 2, 3 and 4 reared in DIET-1, regardless of sex. The IDP from the 1st, 2nd and 3rd instars of the first generation reared in the DIET1 was 3.5, 2.7 or 2.8 days, respectively (Table 1). Other group still exhibited the larval development of larval development including five instars (4th + 5th group); on average, the 4th instar

Table 1. Immature development period (day) (±se) of *Grapholita molesta* in first and fourth generation feeding on commercial diet (22 ± 1°C; 70 ± 10% RH; 16 h photophase). Larval development when completes in five or only four instars.

Larval stage	First generation		Fourth generation					
	n	DIET-1	n	DIET-4	n	EVA4-DIET	n	GALA4-DIET
Larva 1 female	17	3.4 ± 0.48 a	34	3.0 ± 0.49 b	33	3.1 ± 0.60 b	27	3.2 ± 0.51 b
Larva 1 male	50	3.5 ± 0.62 a	20	3.0 ± 0.47 b	42	3.2 ± 0.69 b	36	3.1 ± 0.61 b
Larva 1 mean	67	3.5 ± 0.59 a	54	3.0 ± 0.48 b	75	3.2 ± 0.66 b	63	3.2 ± 0.57 b
Larva 2 female	17	2.6 ± 0.62 a	34	2.5 ± 0.69 ab	33	2.3 ± 0.56 b	27	2.2 ± 0.54 b
Larva 2 male	42	2.8 ± 0.65 a	20	2.5 ± 0.51 b	42	2.3 ± 0.65 b	36	2.4 ± 0.62 b
Larva 2 mean	59	2.7 ± 0.65 a	54	2.5 ± 0.64 b	75	2.3 ± 0.62 b	63	2.3 ± 0.60 b
Larva 3 female	14	3.0 ± 0.79 a	34	2.8 ± 0.71 a	33	2.9 ± 0.79 a	27	2.7 ± 0.69 a
Larva 3 male	32	2.7 ± 0.89 a	20	2.6 ± 0.58 a	42	2.8 ± 0.79 a	36	2.5 ± 0.62 a
Larva 3 mean	45	2.8 ± 0.87 a	54	2.7 ± 0.68 a	75	2.8 ± 0.79 a	63	2.6 ± 0.66 a
Larva 4 female	5	2.9 ± 0.80 a	9	2.9 ± 0.76 a	2	2.3 ± 1.03 a	13	2.5 ± 1.36 a
Larva 4 male	15	2.5 ± 0.94 a	2	2.5 ± 0.00 a	7	1.6 ± 0.44 b	11	2.7 ± 0.75 a
Larva 4 mean	20	2.7 ± 0.92 a	11	2.8 ± 0.74 a	9	1.7 ± 0.71 b	24	2.6 ± 0.85 a
Larva 4 Bis female[1]	9	7.9 ± 0.61 a	25	6.6 ± 0.85 b	30	6.3 ± 0.74 b	12	6.5 ± 0.94 b
Larva 4 Bis male[1]	14	7.8 ± 0.80 a	18	6.3 ± 0.98 b	33	5.9 ± 0.85 b	25	5.9 ± 0.65 b
Larva 4 Bis mean[1]	23	7.8 ± 0.74 a	43	6.5 ± 0.91 b	63	6.1 ± 0.81 c	37	6.1 ± 0.82 c
Larva 5 female[2]	5	7.4 ± 1.03 a	9	5.2 ± 1.67 a	2	6.8 ± 1.03 a	14	5.2 ± 1.17 a
Larva 5 male[2]	10	7.4 ± 1.00 a	2	6.0 ± 1.19 ab	7	5.7 ± 0.80 b	11	5.0 ± 1.14 b
Larva 5 mean[2]	15	7.4 ± 0.99 a	11	5.4 ± 1.59 b	9	5.9 ± 0.90 ab	25	5.1 ± 1.15 b
Immature female[1]		16.9		14.7		14.6		14.6
Immature male[1]		16.8		14.4		14.2		13.9
Immature female[2]		19.3		16.4		17.4		15.8
Immature male[2]		18.9		16.6		15.6		15.7

[1]Larval development with four instars. [2]Larval development with five instars. Means between generations with the same letter are not significantly different (Tukey's test; P<0.05).

was completed in 2.7 days, and the 5th instar in 7.4 days. The larval group 4th Bis presented only four instars, which 4th instar lasted 7.8 days until pupation. This behavior of the larvae in the 4th instar observed in DIET1 was similar in the 4th-generation (Table 1). The average IDPs in the 4th Bis group in DIET1 and DIET4 were 22.8% and 19.8% lower, respectively, than those in the 4th + 5th group, which was also observed in the EVA4-DIET and GALA4-DIET larvae (20.0%). The average cycle of the female/male larvae in the 4th Bis group was less than 11.8% of that one of the 4th + 5th group (Table 1). The average numbers of larvae in the 4th Bis group were approximately 1.2; 3.9; 7.0 and 1.5 more than in the 4th + 5th group in DIET1, DIET4, EVA4-DIET and GALA4-DIET, respectively. In addition, the number of larvae was higher in the 4th-generation than in the 1st-generation.

In general, the IDP of GM in DIET1 was significantly longer than that in DIET4 (Table 1): 1st-instar female and male larvae and the mean development of both sexes (respectively F= 33.84, p= <0.0001; F= 31.43, p= <0.0001; F= 70.79, p= <0.0001), 2nd-instar male larvae and the mean development of both sexes (F= 8.74, p= 0.0044; F= 12.32, p= 0.0006), 4th Bis female and male larvae and the mean development of both sexes (F= 13.87, p= <0.0001; F= 26.91, p= <0.0001; F= 38.40, p= <0.0001), and 5th-instar mean development for both sexes (F= 8.27, p= 0.0083).

The IDPs of the 1st, 2nd, 3rd, 4th Bis and 5th instars in DIET4 were lower by 14.2%, 7.4%, 3.6%, 16.7% and 27.0%, respectively, than those of GM that reproduced in the DIET1 group (Table 1). The IDP did not differ among EVA4-DIET, GALA4-DIET and DIET4, except for 4th-instar male larvae in the EVA4-DIET group.

The average head capsule width in DIET4 was 20.2% larger in the 4th Bis group than in the 4th-instar larvae of 4th + 5th group (Table 2). The head capsules of the larvae in GALA4-DIET were significantly narrower than those in EVA4-DIET in the 3rd and 4th Bis instars (F= 28.50, p= <0.0001; F= 10.34; p= <0.0001, respectively); however, in the 5th instar, there was no difference. The average head capsule growth rate between the diets ranged from 1.5 to 1.7; however, it presented the lowest ratio (1.1 - 1.2) in the 4th instar (Table 2).

Table 2. Average width of cephalic capsule (CC) of *Grapholita molesta* larvae and growth rate during larval development in 'Gala' and 'Eva' apple fruits and in commercial diet.

Instar	EVA4-DIET			GALA4-DIET			DIET4		
	n	CC[1]	r[2]	n	CC	r	n	CC	r
First	32	252.8 ± 4.06b		39	262.1 ± 3.61a		75	249.5 ± 3.92b	
Second	85	374.6 ± 5.04a	1.5	87	375.9 ± 4.14a	1.4	71	374.3 ± 4.82a	1.5
Third	76	649.3 ± 5.69a	1.7	75	608.4 ± 7.67b	1.6	64	661.1 ± 5.73a	1.8
Fourth	9	687.6 ± 9.54c	1.1	25	745.0 ± 7.63b	1.2	11	800.8 ± 7.99a	1.2
Only fourth	52	983.0 ± 7.57a	1.5	32	922.7 ± 8.31b	1.5	30	962.2 ± 7.05a	1.5
Fifth	9	1116.1 ± 6.18a	1.6	25	1159.3 ± 9.23a	1.6	11	1120.7 ± 6.41a	1.4

[1]Width of cephalic capsule. [2]Growth rate between instars: for larvae with only four instars, the ratio was calculated in relation to the 3rd instar; for larvae that reached 5th instar, it was calculated in relation to the 4th instar. Means followed by the same letter in each instar are not significantly different (Tukey's test; P<0,05).

3.2. Immature Development Period (IDP) in fruit

In general, the IDPs of GM in the first generation reared were longer than the fourth generation for both cultivars. The average larval cycle when GM was reared in GALA1 was 10.3% longer (Table 3) than that in GALA4, and the difference was significant for both female and male larvae F= 7.37; p= 0.0086 and F= 5.95; p= 0.0192, respectively; the same was true for female/male pupae (F= 41.73; p= <0.0001 and F= 75.02; p= <0.0001), female/male adults (F= 34.12; p= <0.0001 and F= 12.45; p= 0.0012) and the female/male larva-adult cycle (F= 45.06; p= <0.0001 and F= 36.60; p= <0.0001). A similar situation occurred for female EVA1 larvae in relation to EVA4 (F= 4.73; p= 0.0364) for female pupae (F= 123.50; p= <0.0001), male pupae (F= 31.70; p= <0.0001), female larva-adult cycle (F= 13.05; p= 0.0016) and male larva-adult cycle (F= 4.69; p= 0.0413). The average IDPs in EVA1 and GALA1 fruits were 50.7% and 66.2% longer than in DIET1, respectively, while that in EVA4 and GALA4 was 51.7% longer than that in DIET4 (Table 3). The average IDP in EVA4 and GALA4 was 52.2% longer than that in EVA4-DIET and GALA4-DIET (Table 3). The development of the 4th-generation populations that reproduced on the commercial diet did not differ.

3.3. Pre-oviposition Period (POP), Oviposition Daily Rhythm (ODR) and Daily Egg Production (DEP)

The adult development period (ADP) did not differ, except on 'Gala' in the 4th-generation, which was 26.4% shorter. The GM POP and ODR were represented by cumulative frequency curves (Figure 2). The POP was significantly shorter in 'Eva' and 'Gala' in the 1st-generation (F= 4.74, p= 0.015) and in 'Eva' in the 4th-generation (F= 3.51; p= 0.0107). Nearly 75.0%, 100.0% and 96.0% of the females started to lay eggs earlier than the third day in EVA1, EVA4 and EVA4-DIET, respectively, whereas the corresponding values in GALA1, GALA4 and GALA4-DIET were 62.0%, 37.0% and 41.0%, respectively (date not shown). The POP of the GM populations in DIET1 and DIET4 remained intermediate, at approximately 54.0% and 64.0%, respectively.

The total number of eggs per female (NEF) throughout life did not differ within the same generation (Table 4). Females in EVA4 and GALA4 showed oviposition rates that were approximately 41.0% and 51.0% higher, respectively, than in the first generation, but only females in GALA4 exhibited a significant gain (F= 5.74; p= <0.0001). The NEF in EVA4-DIET and GALA4-DIET was lower, corresponding to approximately 14.6% and 4.4% of those in EVA4 and GALA4, respectively. The daily egg production (DEP) showed no differences in either generation, but DIET1 presented the lowest DEP.

Females in EVA4 exhibited the longest duration of egg-laying activity (DELA) in fruits, which was approximately 59.0% longer than in GALA4 (Figure 2A-2D). The ODR ranged from 24 to 26 days in the 1st-generation to 22 to 35 days in the 4th-generation in 'Gala' and 'Eva', respectively, as represented by cumulative frequency curves (Figure 2). The second-degree equation (Figure 2) predicted that GM required approximately 6.3 days in GALA1/GALA4 and 7.5 and 5.6 in EVA1 and EVA4, respectively, to oviposit 50.0% of total eggs, while 8.2 days were needed in DIET4 (Figure 2A). The ODR was not homogeneous as a function of DELA, and the females in fruits oviposited 90.0% of their eggs (DELA 90.0%) between 12.8 and 15.8 days, as shown by the probability function (Figure 2). The remaining 10.0% of eggs were oviposited in the last 22 days in EVA4, whereas in GALA4, oviposition was performed in nine days.

The smallest TNE was observed in GALA1 (F= 5.82; p= 0.0186) (Table 4), and no difference was observed in the 4th-generation. The ratio between TNE and DELA 90.0% showed that there was an increase in the mean daily egg production (DEP) from the first to the 4th-generation, which was approximately 41.2% and 59.0% for EVA and GALA, respectively (Figure 3, Table 4). There was a tendency of the females that reproduced solely in fruits to be more fertile (Figure 3).

The presentation of the TNE in quartiles showed the ODR in detail for each diet (Table 5). In the females that reproduced in the EVA1 or EVA4 fruits, 65.8% or 79.1% of the TNE, respectively, was concentrated in the first quartile, while the females on 'Gala' showed a broader distribution of their eggs among the first three quartiles. The most long-lived females came from the 1st-generation (F= 4.82; p= 0.0001), while those from the 4th-generation

Table 3. Adult and immature development period (day) (±se) of *Grapholita molesta* in first and fourth generation, by rearing larvae in 'Gala' and 'Eva' apple fruits and in commercial diet (22 ± 1°C; 70 ± 10% RH; 16 h photophase).

Stage	Fruits and artificial diet	n	First generation in fruit and diet	n	Fourth generation in fruit and diet	n	Fourth generation in diet[1]
Larva female	Eva	24	23.5 ± 1.63 aA	13	21.6 ± 1.48 bA	32	14.7 ± 1.0 cA
	Gala	17	24.5 ± 1.81 aA	44	22.4 ± 1.58 bA	26	15.3 ± 0.99 cA
	Diet	14	17.4 ± 1.15aB	34	15.3 ± 1.27 bB		
Larva male	Eva	26	21.2 ± 1.62 aB	13	22.6 ± 1.61 aA	40	14.4 ± 0.95 bA
	Gala	13	24.2 ± 1.83 aA	29	21.1 ± 1.49 bA	36	14.5 ± 1.05 cA
	Diet	23	17.0 ± 1.32 aC	20	15.0 ± 1.09 bB		
Larva cycle mean	Eva	50	22.3 ± 1.69 aB	26	22.3 ± 1.55 aA	72	14.5 ± 0.99 bA
	Gala	30	24.6 ± 1.80 aA	73	22.3 ± 1.54 bA	62	14.8 ± 1.0 cA
	Diet	37	14.8 ± 1.26 aC	54	14.7 ± 1.22 aB		
Pupa female	Eva	17	11.0 ± 0.84 aA	13	7.3 ± 1.05 cC	29	9.0 ± 0.98 bA
	Gala	14	10.9 ± 1.15 aA	41	9.0 ± 0.88 bA	24	8.4 ± 0.68 cA
	Diet	15	11.2 ± 0.72 aA	31	8.5 ± 0.84 bB		
Pupa male	Eva	22	10.3 ± 1.15 aA	9	6.9 ± 1.39 cB	35	9.2 ± 0.94 bA
	Gala	11	10.5 ± 0.72 aA	31	9.0 ± 0.90 bA	31	8.6 ± 0.76 bA
	Diet	14	10.9 ± 0.84 aA	20	9.1 ± 0.76 bA		
Pupa cycle mean	Eva	39	10.6 ± 1.07 aA	22	7.1 ± 1.21 aA	64	9.1 ± 0.96 aA
	Gala	25	10.8 ± 1.03 aA	72	9.0 ± 0.89 bA	55	8.5 ± 0.73 cA
	Diet	29	11.1 ± 0.79 aA	51	8.8 ± 0.84 bA		
Adult female	Eva	12	24.4 ± 2.28 aA	11	21.4 ± 2.29 aA	23	20.7 ± 2.25 aA
	Gala	10	23.5 ± 2.41 aA	32	16.9 ± 1.96 bB	17	19.1 ± 2.56 bA
	Diet	15	26.7 ± 2.5 aA	21	21.3 ± 2.40 bA		
Adult male	Eva	16	24.0 ± 2.04 aA	8	21.1 ± 2.56 abAB	25	19.0 ± 2.19 bA
	Gala	8	23.9 ± 2.21 aA	27	17.6 ± 1.99 bB	26	19.8 ± 2.47 abA
	Diet	14	21.0 ± 2.83 aA	12	21.4 ± 2.48 aA		
Adult cycle mean	Eva	28	24.2 ± 2.33 aA	19	21.3 ± 2.78 abA	48	19.8 ± 2.22 bA
	Gala	18	23.7 ± 2.29 aA	59	17.9 ± 1.97 bB	43	18.9 ± 2.50 bA
	Diet	29	22.1 ± 2.78 aA	33	22.2 ± 2.43 aA		
Cycle larva-female	Eva	12	58.7 ± 2.39 aA	11	50.3 ± 2.32 bA	23	44.3 ± 2.26 cA
	Gala	10	59.4 ± 2.50 aA	36	47.4 ± 2.15 bAB	17	42.9 ± 2.63 cA
	Diet	13	54.3 ± 2.51 aB	21	45.4 ± 2.47 bB		
Cycle larva-male	Eva	16	55.7 ± 2.56 aA	8	49.7 ± 2.51 bA	24	42.1 ± 2.21 cA
	Gala	8	57.8 ± 1.91 aA	28	48.3 ± 1.98 bA	26	42.9 ± 2.45 cA
	Diet	14	48.6 ± 2.94 aB	12	46.5 ± 2.34 aA		
Cycle mean of larva-adult	Eva	28	57.0 ± 2.50 aA	19	50.0 ± 2.38 bA	47	43.2 ± 2.25 cA
	Gala	18	58.7 ± 2.28 aA	64	47.8 ± 2.08 bAB	43	42.9 ± 2.51 cA
	Diet	27	51.4 ± 2.83 aB	33	45.8 ± 2.41 bB		

[1]Larval development during three generations in fruit and during the fourth generation on the diet. Means followed by the same lowercase letter in the lines and uppercase letter in the columns for each stage are not significantly different (Tukey's test; P <0.05).

were the shortest lived. The average female longevity in DIET1 was significantly higher in GALA1 (F= 5.67; p= 0.0073) (Table 4), whereas the females that reproduced in GALA4 exhibited the lowest longevity in the fourth generation, but the difference was not significant (F= 2.18, p = 0.0775).

Table 4. Oviposition and longevity of *Grapholita molesta* in first and fourth generation after rearing immature phase in 'Gala' and 'Eva' apple fruits and in commercial diet (21 ± 2°C, 70% RH and 16 h photophase).

Generation/ diet	n	TNE	NEF	DEP	POP (day)	Longevity of female (day)
First generation						
EVA1	12	201.0 ± 6.79 aAB	8.7 ± 1.74 aBC	8.8 ± 2.90 aAB	2.3 ± 0.80 bB	24.3 ± 2.28 abAB
GALA1	13	155.5 ± 7.51 bB	9.0 ± 1.87 aBC	6.5 ± 2.80 aBC	2.2 ± 0.96 bAB	19.0 ± 2.70 bBC
DIET1	13	182.1 ± 5.42 abAB	7.1 ± 1.47 aC	6.0 ± 2.64 aBC	2.9 ± 0.85 aAB	26.7 ± 2.23 aA
Fourth generation						
EVA4	12	230.2 ± 7.12 aA	12.3 ± 2.16 aAB	8.9 ± 3.09 aAB	2.0 ± 0.63 bAB	20.3 ± 2.48 abABC
EVA4-DIET	26	207.5 ± 6.85 abAB	10.5 ± 1.79 aABC	7.4 ± 2.66 aBC	2.2 ± 0.71 bAB	20.9 ± 2.25 aABC
DIET4	24	186.3 ± 7.66 abAB	9.5 ± 2.07 aABC	7.0 ± 2.15 aBC	2.5 ± 1.21 abAB	21.5 ± 2.48 aABC
GALA4	17	228.0 ± 7.83 aA	13.6 ± 1.92 aA	10.3 ± 2.94 aA	3.1 ± 1.01 aA	17.2 ± 1.87 bC
GALA4-DIET	11	217.3 ± 8.26 abAB	13.0 ± 2.37 aAB	7.4 ± 2.74 aBC	2.8 ± 0.87 abAB	18.7 ± 2.69 abBC

Means followed by the same lowercase letter on the line and uppercase letter between generation are not significantly different (Tukey's test; $P < 0.05$). TNE - total number of eggs per female; NEF - number of eggs per female; DEP - daily egg production; POP - pre-oviposition period

4. Discussion

The initial hypothesis was that immature GM would exhibit better performance on the late cultivar 'Gala' and, thus, greater fluctuation of adults than in the 'Eva' orchard. To show these differences, larvae were collected from fruits of early and late cultivars and reproduced in 'Eva' and 'Gala' fruits and a commercial diet for four generations.

Our data showed adaptation of immature GM to the commercial diet, represented by a shorter IDP, mainly in the fourth generation (Table 1). The same phenomenon occurred in larvae that fed only on fruits for three generations and then on the commercial diet in the fourth generation (EVA4-DIET and GALA4-DIET), which showed 13.6% and 17.5% reductions in the IDP, respectively (Table 1). We can infer that immature performance may be related to the better quality of the commercial diet, which was balanced in nutrients for the production of *Heliothis*, thus potentially explaining the faster development observed (Scriber and Slanski Junior, 1981; Rosenthal et al., 1994).

In addition, the immature fruits exhibited good development regarding the nutritional characteristics of the fruits (Table 3). The average IDPs of females and males in fruits were 8.1% and 3.7% shorter, respectively, in the fourth generation in relation to the first generation. The average IDP was significantly longer in fruits than on the commercial diet, approximately 23.3% and 30.0% in the first and fourth generations, respectively (Table 3). These results follow those obtained by Najar-Rodriguez et al. (2013) (25.0% shorter IDP on apple fruits than in artificial diet). Apple fruits are considered an intermediate substrate for GM (Myers et al., 2006c; Myers et al., 2007; Najar-Rodriguez et al., 2013) because they produce substances containing complexes that inhibit the digestive and nutritional processes of the larvae (Scriber and Slanski Junior, 1981; Bauerfeind and Fischer, 2005; Davis et al., 2013). The presence of allelochemicals, proteins and vitamins makes the fitness cost of full development higher, influencing fertility and longevity (Panizzi and Parra, 1991; Arioli et al., 2007). In both cultivars, the IDP represented 40.2% and 45.0% of the biological cycle, while on the commercial diet, it corresponded to only 33.8% and 33.0% of the biological cycle in the 1st- and 4th-generation, respectively.

In apple orchards in Brazil during winter, GM larvae are in diapause (Hickel et al., 2003) or continue their development while feeding on burrknots (Silva et al., 2014; Bisognin et al., 2012). These are undifferentiated structures that contain cyanogenic glycosides, which are toxic to insects at high concentrations (Zagrobelny et al., 2004), causing symptoms associated with larval tissue deterioration (LTD) (Silva et al., 2014). This type of food influences the biology of GM in relation to fruits (Silva et al., 2014; Bisognin et al., 2012) but allows the perpetuation of the species in winter, when no other preferred sources of food, such as new shoots and fruits, are available.

Following the spring and the growing season, the new generation (postdiapause or postwinter) of GM can find better conditions for development on the pointers of peach and apple (Monteiro and Hickel, 2004; Piñero and Dorn, 2009); however, these fruits are still not an ideal source of insect food (Silva et al., 2010), which leads us to believe that the improvement of biological parameters will be gradual.

Against this background, we can infer that better feeding of larvae may shorten the cycle (Nylin and Gotthard, 1998) and promote behavioral changes, such as alteration of the number of instars. All treatments in this study included individuals that went through five instars; however, among the total individuals, only 12.5% in EVA4-DIET, approximately 20.0% in DIET4, 39.0% in GALA4-DIET and 47.0% in DIET1 reached the 5th instar (Table 1), suggesting that the fifth instar is not necessary when the requirements associated with antibiosis and food quality are met (Slansky Junior and Rodriguez, 1987). Thus, it is possible that the occurrence of the largest number of 4th Bis larvae in EVA4-DIET (Table 1) was related to nutritional adaptation, and that the same phenomenon occurred between the first and

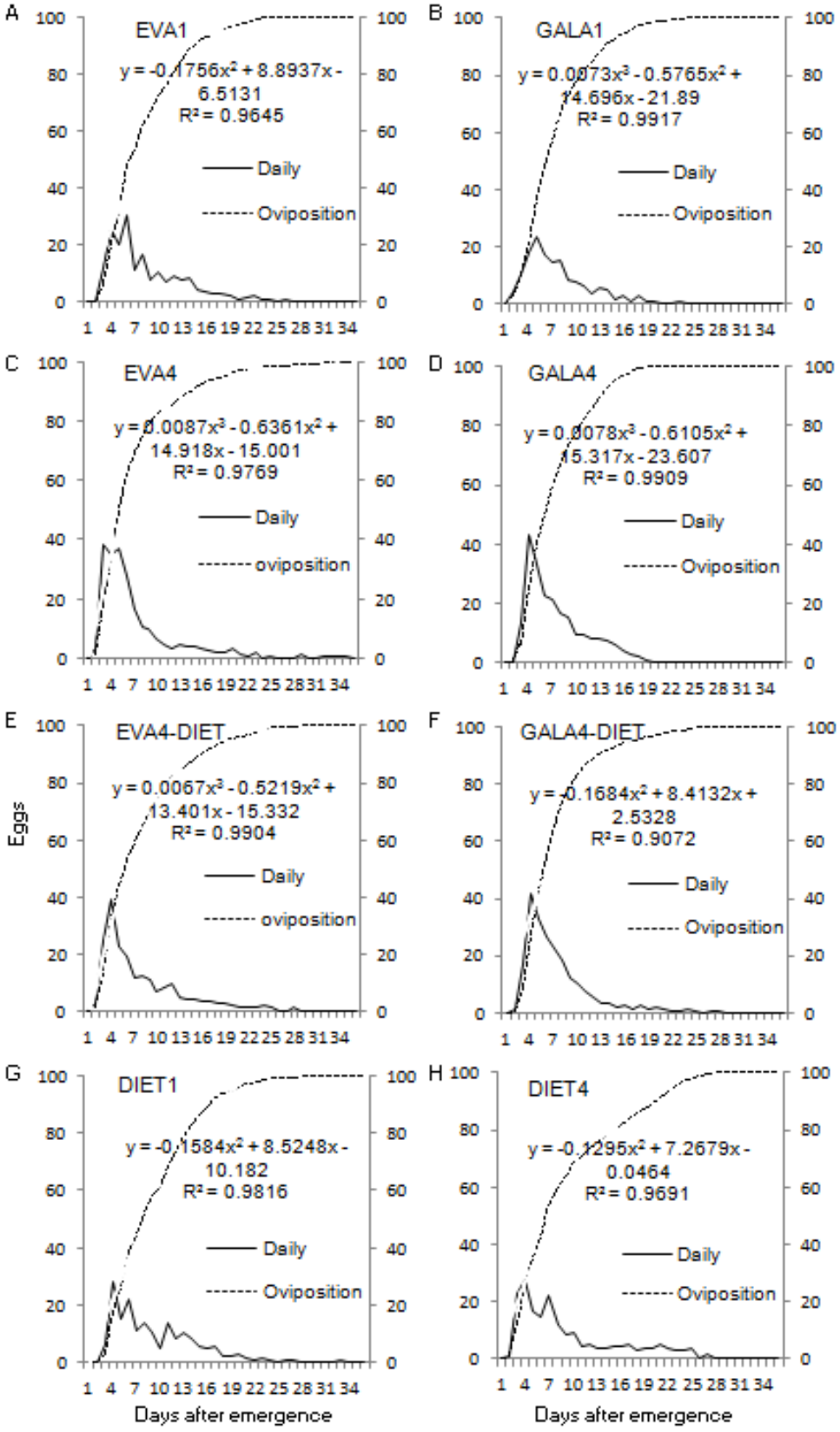

Figure 2. Daily and cumulative oviposition rhythm of *Grapholita molesta* according to Gaussian model (A to H).

Table 5. Relationship between duration of egg-laying activity (DELA) and total number of eggs (TNE) of *Grapholita molesta* after rearing immature phase in 'Gala' and 'Eva' apple fruits and in commercial diet, arranged by quartiles.

Treatment	Quartile[1]	N[2]	DELA[3] (day)	NEF[4]		σ	CV	TNE[5]	Frequency of eggs[6]
EVA1	1st	12	<8.0	18.8	a	7.19	38.20	131.70	0.65
	2nd	7	8.0 - 15.0	7.6	b	2.02	26.48	53.38	0.29
	3rd	7	15.0 - 22.0	2.1	bc	0.84	40.11	14.69	0.08
	4th	7	> 22.0	0.2	c	0.24	129.10	1.31	0.01
GALA1	1st	13	<6.0	14.8	a	7.43	50.17	74.08	0.48
	2nd	6	6.0 - 12.0	9.4	ab	4.70	49.99	56.38	0.36
	3rd	6	12.0 - 18.0	3.3	bc	1.87	56.39	19.85	0.13
	4th	6	> 18.0	0.7	c	0.33	47.01	4.23	0.03
DIET1	1st	13	<9.0	15.2	a	7.41	48.73	106.38	0.58
	2nd	9	9.0 - 18.0	7.1	b	3.34	46.91	64.00	0.35
	3rd	8	18.0 - 26.0	1.3	c	0.81	62.92	10.31	0.06
	4th	7	> 26.0	0.2	c	0.27	136.31	1.38	0.01
EVA4	1st	12	<9.0	22.3	a	13.79	61.80	178.54	0.79
	2nd	9	9.0 - 18.0	3.9	b	1.41	36.51	34.85	0.15
	3rd	8	18.0 - 26.0	1.2	b	1.00	86.85	9.23	0.04
	4th	9	> 26.0	0.4	b	0.36	101.64	3.23	0.01
GALA4	1st	17	<6.0	23.3	a	17.39	74.63	116.52	0.51
	2nd	5	6.0 -11.0	14.4	ab	5.04	35.08	71.89	0.31
	3rd	5	11.0 - 16.0	6.7	b	1.80	26.95	33.41	0.15
	4th	5	>16.0	1.3	b	1.20	90.15	6.63	0.03
EVA4-DIET	1st	26	<8.0	18.8	a	11.69	62.03	131.88	0.65
	2nd	7	8.0 - 15.0	6.9	b	2.74	39.85	48.21	0.24
	3rd	7	15.0- 22.0	2.4	b	0.87	35.42	17.13	0.08
	4th	8	>22.0	0.7	b	0.64	90.75	5.67	0.03
GALA4-DIET	1st	11	>8.0	22.9	a	12.94	56.43	160.59	0.74
	2nd	7	8.0 - 15.0	6.2	b	3.68	59.80	43.12	0.20
	3rd	7	15.0 - 22.0	1.6	b	0.68	43.56	10.94	0.05
	4th	7	>22.0	0.4	b	0.48	108.77	3.06	0.01
DIET4	1st	24	>8.0	16.8	a	8.73	52.04	117.45	0.60
	2nd	7	8.0 - 15.0	5.6	b	2.35	42.16	39.09	0.20
	3rd	7	15.0 - 22.0	4.0	b	0.81	20.44	27.73	0.14
	4th	7	>22.0	1.8	b	1.30	72.07	12.64	0.06
FRUIT	1st	29	<7.0	19.4	a	12.41	64.10	483.88	0.61
	2nd	27	7.0 - 14.0	8.0	b	4.89	61.00	216.49	0.27
	3rd	26	14.0 - 21.0	3.0	c	2.39	80.55	77.17	0.10
	4th	27	>21.0	0.6	c	0.68	119.44	15.40	0.02
DIET	1st	33	<8.0	18.4	a	10.27	55.69	516.30	0.65
	2nd	30	8.0 - 16.0	6.5	b	2.99	46.20	194.42	0.24
	3rd	29	16.0 - 23.0	2.3	c	1.30	57.00	66.10	0.08
	4th	29	>23.0	0.8	c	0.95	121.51	22.75	0.03

[1]Quartiles calculated by the program Excel 2007 (Microsoft, USA). [2]Number of females. [3]DELA: duration of egg-laying activity. [4]NEF: number of eggs per female. [5]TNE: total number of eggs. [6]Frequency of eggs in relation to the total eggs laid in each treatment. Means followed by different letters within the treatment are not significantly different by Tukey's test (P <0.05).

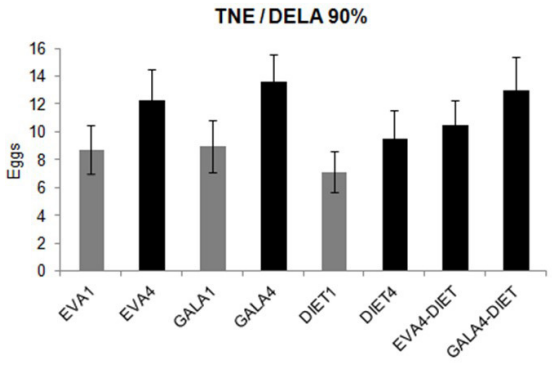

Figure 3. General relationship between the total number of eggs (TNE) and duration of egg-laying activity (DELA) after oviposition of 90% of eggs by *Grapholita molesta* in first and fourth (dark column) generation under different diets.

fourth generation. Roberts et al. (1978) considered that the field-living GM population may undergo five instars. Myers et al. (2007) assumed that four instars occurred in GM in studies on apple and peach. Other researchers have not reported this phenomenon on artificial diets (Arioli et al., 2007; Bisognin et al., 2012; Najar-Rodriguez et al., 2013). To visualize this division, it is necessary to evaluate the development of the larvae at eight-hour intervals at a minimum. The head capsule width in the 4th Bis ranged from 922.7 to 983.0 µm, and that in the fifth instar was greater than 1,116.0 µm (Table 2), which was greater than the values of 800.0 µm indicated by Roberts et al. (1978) for fourth-instar larvae.

The 4th Bis larvae were physiologically modified to reduce the number of instars, which explains why the head capsule width was 28.4% larger than in the 4th instar, at an average growth rate of 1.5, similar to the average obtained by Bisognin et al. (2012). The fourth-instar larvae that reproduced in fruits (EVA4-DIET and GALA4-DIET) exhibited a narrower head capsule width than those in DIET4 (800.8 µm), suggesting the occurrence of some biological damage (Roberts et al., 1978), as demonstrated by Fugi (2003) in studies of *Anticarsia gemmatalis* (Lepidoptera: Noctuidae) in the presence of unsuitable food.

This behavior of reducing the larval phase or the number of instars seems natural under ideal feeding conditions (Panizzi and Parra, 1991; Nylin and Gotthard, 1998; Davis et al., 2013) and will produce more vigorous, fertile and long-lived adults (Bauerfeind and Fischer, 2005). Although holometabolous insect diets and energetic needs change between life stages (Bauerfeind and Fischer, 2005), it appears that adults tend to improve upon the existing nutritional mechanisms as they multiply for several generations on the same food.

Reproductive adaptations have been observed in adults. The pre-oviposition periods (POPs) of the females reared on fruit were similar in the first generation (Table 4); however, the POP was shorter on 'Eva' in the fourth generation. Silva et al. (2011) showed that females with a short POP were more fecund, and this relationship is variable from fruit to fruit, depending on the stimuli and the efficiency of food conversion related to oviposition (Davis et al., 2013; Najar-Rodriguez et al., 2013). Studies on kairomone signaling related to oviposition in virgin and mated females have shown the oviposition rate is higher in 'Eva' than in 'Gala' (Strapasson et al., 2016) and that oviposition occurs earlier in peach (Piñero and Dorn, 2009). The females that fed on 'Gala' showed lower performance; it was similar to that obtained by Silva et al. (2010) and Silva et al. (2011).

As a result of the shorter POP of GM on 'Eva,' the analysis of the oviposition daily rhythm (ODR) showed that the cumulative oviposition frequency curve increased faster on 'Eva' than in the other treatments, and these females laid eggs earlier than the females on 'Gala'. The best performance on 'Eva' was evident in the 4th-generation, when 79.1% of the total number of eggs (TNE) were deposited in the first quartile (Table 5), compared with only 51.0% in GALA4. This suggests that GM on 'Gala' experiences higher fitness costs, perhaps as a consequence of the immature phase.

The precocity of the TNE parameter observed in 'Eva' is assumed to be related to the shorter duration of egg-laying activity (DELA), since herbivores tend to lay their eggs as quickly as possible under ideal conditions (Nylin and Gotthard, 1998; Silva et al., 2011; Najar-Rodriguez et al., 2013). The precociousness of oviposition did not lead to a reduction in DELA in EVA4; on the contrary, in some of the females in EVA4, DELA lasted 13.4 days longer than that on 'Gala' after DELA 90% was reached in both cultivars (data not shown). This female behavior may be related to the adaptive process of females in 'Eva' from the 1st-generation to the 4th-generation and their best performance in the immature phase, influencing their fertility and longevity (Panizzi and Parra, 1991; Arioli et al., 2007). The other females from the 'Gala' and commercial diet groups exhibited a more balanced TNE/DELA ratio, distributing their eggs in the first three quartiles (Table 5). NEF on 'Eva' was influenced by DELA progressed.

The TNE per female was greater on 'Eva' than on 'Gala' (Table 4) in both generations. This can be explained by the lower longevity of females on 'Gala' (Table 4). In general, the TNE and NEF on fruits were larger than on the diet (Figure 3), probably because commercial diets are not sufficiently balanced with the secondary nutrients found in fruits that are responsible for stimulating females (Bauerfeind and Fischer, 2005; Davis et al., 2013; Strapasson et al., 2016). In addition, NEF on 'Eva' was decreased by the greater DELA, while DEP increased with an increasing DELA. Silva et al. (2011) reported that DEP was 10.8 for females at 22° C, which is similar to that observed in this work considering the estimated error (Figure 3).

The ADP was influenced by the diet. Both sexes exhibited significantly shorter longevity on 'Gala', which was estimated to be close to 15.2 days by Silva et al. (2011). According to Najar-Rodriguez et al. (2013), longevity shows a positive tendency associated with fecundity, depending on the intraspecific host variety in which development occurs, as observed in EVA1. As suggested by Myers et al. (2006a), cultivar-dependent longevity estimates could be used as a parameter for calculating the action threshold based on population monitoring through the use of pheromone-baited traps. In addition, longevity can influence flight behavior. If adults live longer, they

can disperse over longer distances, affecting pest control (Piñero and Dorn, 2009; Silva et al., 2010).

Although some authors have considered that early varieties may be less attractive (Silva et al., 2010) or nonpreferred (Myers et al., 2006a, b), our studies showed that the lower occurrence of GM in 'Eva' orchards is not due to nutritional conditions (larval development, adult longevity and fertility were better on 'Eva' than on 'Gala'). Thus, the largest catch of GM on 'Gala' orchards may be related to other factors such as: i. the adaptive capacity of postdiapause generations to find good-quality food in sequence (burrknots, new pointers and fruit), or ii. the possibility of synchronizing available food sources to achieve the best performance of GM (after a generation on 'Eva') under ideal climatic conditions for its development and reproduction (Carroll and Quiring, 1993).

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