



Development and reprotuction of *Spodoptera eridania* on natural hosts and artificial diet

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Abstract

The development and reproductive potential of *Spodoptera eridania* (Stoll, 1782) (Lepidoptera, Noctuidae) was compared on natural and artificial diets. Its biology was determined under laboratory conditions, providing an artificial diet, leaves of white clover and the peach cultivar ‘Chimarrita’ and fruits and leaves of the apple cultivar ‘Gala’. Larvae of *S. eridania* could not complete their biological cycle when fed on apple fruits or peach leaves. The artificial diet provided the shortest development time, with a cycle of 28.5 ± 0.14 days from egg to adult and 37.3% total viability. Apple leaves led to a longer development time from egg to adult (62.8 ± 1.22 days) and lower total viability (23%) and white clover provided the highest total viability (54.3%). The results showed that *S. eridania* can use white clover and apple leaves to develop normally in apple orchards. The reduction of food sources such as white clover used by the larvae results in infestation and damage to fruits from “test bites”.

Keywords: southern armyworm, life-cycle, reproduction, *Prunus persica*, *Malus domestica*, *Trifolium repens*.

Desenvolvimento e reprodução de *Spodoptera eridania* em hospedeiros naturais e dieta artificial

Resumo

O desenvolvimento e o potencial reprodutivo de *Spodoptera eridania* (Stoll, 1782) (Lepidoptera, Noctuidae) foi comparado em dietas naturais e artificial. A biologia de *S. eridania* foi determinada em condições de laboratório, comparando-se uma dieta artificial com folhas de trevo branco e de pêssego da cultivar ‘Chimarrita’ e frutos e folhas de maçã da cultivar ‘Gala’. As larvas de *S. eridania* não puderam completar seu ciclo biológico quando alimentadas com frutas de maçã ou folhas de pêssego. A dieta artificial proporcionou o menor tempo de desenvolvimento, com um ciclo completo de $28,5 \pm 0,14$ dias de ovo a adulto e 37,3% de viabilidade total. As folhas de maçã levaram maior tempo de desenvolvimento de ovo a adulto ($62,8 \pm 1,22$ dias) e menor viabilidade total (23%). O trevo branco apresentou a maior viabilidade total (54,3%). Os resultados mostraram que *S. eridania* pode usar trevo branco e folhas de maçã para se desenvolver normalmente em pomares de maçã. A redução de fontes alimentares, como o trevo branco usado pelas larvas, resulta em infestação e danos aos frutos através das “mordidas de prova”.

Palavras-chave: lagarta da vagem, ciclo evolutivo, reprodução, *Prunus persica*, *Malus domestica*, *Trifolium repens*.

1. Introduction

The southern armyworm *Spodoptera eridania* (Stoll, 1782) (Lepidoptera: Noctuidae) is a native species that is widespread in tropical regions of the Americas (Pogue, 2002). It has been recorded from 202 host species belonging to 58 families (Montezano et al., 2014). In the last few years, *S. eridania* has been recognized as a pest of annual crops, such as cotton *Gossypium hirsutum* L. and soybean *Glycine max* (L.) (Favetti et al., 2015; Santos et al., 2005, 2010), tomato *Lycopersicon esculentum* Mill. (Miranda et al., 2005), and strawberry *Fragaria x ananassa* Duch.

(Bortoli et al., 2012); and of perennial crops, such as fruit trees of temperate climates, e.g., apple (Nora and Reis Filho, 1988) and peach *Prunus persica* L. (Botton et al., 2012), and grapevines *Vitis vinifera* L. (Bortoli et al., 2012). This polyphagy causes important losses and contributed to the rapid adaptation of this pest to different agroecosystems (Bortolotto et al., 2014).

In apple and peach orchards, the occurrence of lepidopteran insects is limited to a group of species that use annual crops such as soybean and maize *Zea mays*

L. as a primary source of food. These insects migrate to orchards when they cannot obtain food from their normal primary hosts (Fonseca, 2006; Santos et al., 2010; Nunes et al., 2013). In orchards, *S. eridania* is most frequent on cultivation systems that maintain cover vegetation with spontaneous plants, including clover *Trifolium* spp., chaptalia *Chaptalia nutans*, and dandelion *Taraxacum* spp. (Capinera, 1999). However, in many cases, fruit are damaged only when the preferred spontaneous plants are not available due to larval consumption or by the use of herbicides (Nora and Reis Filho, 1988; Capinera, 1999; Botton et al., 2012; Efrom et al., 2014).

One fundamental step in implementing a pest-management program is the knowledge wild plants such as white clover *Trifolium repens* L. that occur spontaneously in orchards. Moreover, there is no information on artificial diets for rearing this species in the laboratory for management and control purposes. The present study determined biological parameters of *S. eridania* fed on leaves and fruits of apple and on leaves of white clover and peach, comparing its performance to insects fed on artificial diet, under laboratory conditions.

2. Material and Methods

The biology of *S. eridania* was determined in the Entomology Laboratory at Embrapa Uva e Vinho, Bento Gonçalves, Rio Grande do Sul, in climate chambers regulated at a temperature of $25 \pm 1^\circ\text{C}$, relative humidity of $60 \pm 10\%$ and photophase of 14 hours. Eggs and larvae of *S. eridania* were collected in a vineyard located in the municipality of Bento Gonçalves, transported to the laboratory, placed in plastic containers and reared on the artificial diet of Burton and Perkins (1972). The study of the biology began after three generations in which insects were kept under laboratory conditions.

This study was conducted to compare the development of *S. eridania* on an artificial diet described by Greene et al. (1976), with development on natural hosts present in orchards: leaves and fruits of apple *Malus domestica* 'Gala', leaves of peach *Prunus persica* 'Chimarrita', or leaves of white clover *Trifolium repens*. Also, neonate larvae were fed up to the third instar with the artificial diet, and subsequently fed on *M. domestica* 'Gala' fruits. The leaves used in the experiments were harvested in the early hours of the day in orchards where no insecticides had been used, transported to the laboratory, and immersed for 1 min in a solution containing 1% sodium hypochlorite for disinfection and washed under water.

The traits evaluated were: embryonic period and egg viability; duration and viability of larval and pupal stages; number of instars in the larval stage; pupal weight of males and females at 24 hours of age; sex ratio; adult longevity; pre-oviposition period; and female fecundity. A fertility life table was constructed from the data obtained.

2.1. Embryonic period

To determine the duration and viability of the embryonic period, when there were, all the eggs laid at the second oviposition of each female were counted, separated, and kept on moistened filter paper in Petri dishes of 6.5 cm

diameter, and the number of larvae hatched were counted daily.

2.2. Larval stage

To determine the biological parameters of the immature stage, 150 newly hatched larvae were placed in individual flat-bottom glass tubes (8.5 cm long x 2.5 cm diameter) containing artificial diet or leaves of the natural hosts evaluated. Other larvae were fed the artificial diet in a glass tube until the third instar, when they were transferred to transparent plastic containers of 250 ml capacity containing an apple fruit. Duration and viability of the larval and pre-pupal stages were calculated based on observation of these 150 individuals.

2.3. Pupal stage

Individuals at the pupal stage were transferred to flat-bottom glass tubes (8.5 cm x 2.5 cm) containing moistened filter paper and buffered with hydrophilic cotton, where they remained in a heated chamber (Temperature: $25 \pm 1^\circ\text{C}$, RH $60 \pm 10\%$ and photophase: 14 hours) until the adults emerged. The pupae were sexed according to Butt and Cantu (1962) and weighed after 24 h.

2.4. Adult stage

To obtain data on the pre-oviposition period, longevity, and fecundity of females, 20 couples of the same age were formed whenever possible, placed in individual PVC cages (10 cm diameter x 10 cm high) covered with office paper on the sides, voile fabric on the top, and filter paper at the bottom. The couples were fed daily with a 15% honey solution, provided on cotton tufts in plastic containers (2 cm diameter x 1 cm high).

2.5. Statistical analysis

Results for the different stages were subjected to exploratory analyses to assess the assumptions of normality of residuals (Shapiro and Wilk, 1965), and non-normal data were transformed by ARSEN (SQUARE ROOT (X)). The means for each stage were subjected to analysis of variance and compared by Tukey's test at 5% probability, using the program SPSS 24.0 (SPSS Inc., Chicago, IL, U.S.A.).

Using the life table, the values of the different reproductive parameters of *S. eridania* were calculated: net reproductive rate (R_0), given by the ratio between the number of females in two successive generations; mean generation time (T), the mean number of days from the birth of the parents to the birth of the offspring; daily intrinsic rate of increase (r_m); and daily finite rate of increase (λ). The means and standard errors of the life-table parameters were estimated based on Jackknife resampling (Maia et al., 2000) and were compared by using a bilateral t-test ($\alpha = 0.05$) (SAS Institute, 2000).

3. Results

3.1. Embryonic period

The duration of the embryonic period and egg viability of *S. eridania* did not differ when larvae were reared on the artificial diet (4.0 days and 83.39%) and white clover

(4.0 days and 84.39%) (Table 1), but the duration differed ($P < 0.05$) from the period observed for apple leaves (5.5 days and 49.9%).

3.2. Larval stage

Neonate larvae of *S. eridania* did not develop when fed on peach leaves cv. 'Chimarrita' and apple fruits cv. 'Gala'. Similarly, when the larvae were maintained on the artificial diet until the 3rd instar and transferred to apple fruits, no individual reached the pre-pupal stage. The duration of the larval stage was shorter when reared on artificial diet (14.1 days) and white clover (16.7 days) in comparison to apple leaves (48.6 days) (Table 1).

3.3. Pupal stage

The duration of the pupal stage (Table 1) was influenced by the host and was longer in pupae from larvae fed on apple leaves (15.1 days). There were no significant differences between the artificial diet (9.8 days) and white clover (9.9 days), and the viability of the pupal stage on

white-clover leaves was higher than that on the artificial diet and apple leaves, i.e., 81.7, 67.0 and 65.7%, respectively.

The mean weight of pupae 24 hours old (Table 2) did not differ between male and female pupae when the larvae were fed on apple leaves, which can be attributed to the small number of experimental units. Pupae (males and females) from larvae fed on the artificial diet gained more weight than the pupae of larvae fed on white-clover and apple leaves, i.e., 0.34, 0.22 and 0.16 g, respectively. The sex ratio was similar among hosts, i.e., close to 0.50; females were significantly heavier than males in the treatments with artificial diet and white-clover leaves. The viability of the complete cycle of *S. eridania* for white-clover and apple leaves was higher than the viability on the artificial diet (54.3%), which suggests that this pest is less adapted to artificial diet than to white-clover and apple.

3.4. Adult stage

The mean longevity of males and females did not differ significantly among the diets (Table 3). However, when the mean values for males and females were compared among

Table 1. Duration (mean \pm SE) and viability (%) of *Spodoptera eridania* egg, larva, pupa and total cycle (egg to adult) kept on the artificial diet, white-clover leaves, and apple leaves cv. 'Gala'. Temperature: $25 \pm 1^\circ\text{C}$, RH $60 \pm 10\%$ and photophase: 14 hours.

Food source\periods		Egg	Larva	Pupa	Cycle (egg to adult)
Artificial diet	Duration (days)	4.0 \pm 0.0b* (17) ¹	14.1 \pm 0.2c (158)	9.8 \pm 0.1b (59)	28.5 \pm 0.14c (59)
	Viability (%)	83.0	55.7	67.0	37.3
White clover leaves	Duration (days)	4.0 \pm 0.0b (24)	16.7 \pm 0.1b (119)	9.9 \pm 0.09b (87)	30.4 \pm 0.16b (87)
	Viability (%)	84.4	91.5	81.5	54.3
Apple leaves	Duration (days)	5.5 \pm 0.7a (6)	48.6 \pm 1.3a (52)	15.1 \pm 1.3a (34)	62.8 \pm 1.2a (34)
	Viability (%)	49.9	67.3	65.7	23.0
CV (%)		9.56	12.11	7.49	9.94
DF		35	324	178	178
F		13.67	2,063.85	70.62	1,959.39

*Means followed by the same letters in the row do not differ by Tukey's test at 5% significance; ¹Values in parentheses refer to the number of observations.

Table 2. Pupal weight of males and females (Mean \pm SE), sex ratio and number of instars of *Spodoptera eridania* kept on an artificial diet, white-clover leaves, and apple leaves cv. 'Gala'. Temperature: $25 \pm 1^\circ\text{C}$, RH $60 \pm 10\%$ and photophase: 14 hours.

Host	Weight (mg)			Sex ratio	Instars
	Males	Females	Males and females		
Artificial diet	323.8 \pm 4.72aB* (43) ¹	364.4 \pm 7.80aA	345.7 \pm 5.01a (87)	0.51	6
White-clover leaves	200.0 \pm 6.37bB (58)	233.8 \pm 4.34bA (61)	217.3 \pm 4.11b (119)	0.51	6
Apple leaves	156.7 \pm 5.05cA (18)	155.3 \pm 7.03cA (17)	156.0 \pm 4.22c (35)	0.49	6
CV %	8.98	7.26	8.72		
DF	118	120	239		
F	139.09	219.88	295.59		

*Means \pm SE followed by the same letters in the line do not differ by Tukey's test at 5% significance; ¹Values in parentheses refer to number of observations.

the diets, *S. eridania* showed a pre-oviposition period of 5.3 and 4.8 days when fed on the artificial diet and apple leaves, respectively, which was significantly longer than the period on white-clover leaves (2.2 days). Mean fecundity was significantly higher in females reared on white-clover leaves compared to those reared on the artificial diet and apple leaves, i.e., 1,560.6, 840.6 and 310.0 eggs per female, respectively (Table 3).

The net reproductive rate (R_0) indicated that the population of *S. eridania*, when white clover was the host, can increase 509.4±35 times in each generation. On the artificial diet, R_0 was 336.9±33 times, and on apple leaves, only 34.9±16.3 times (Table 4). The mean generation time (T) was approximately 36.1±0.3 days for the artificial diet and 35.1±0.13 days for white-clover leaves, which was shorter than for apple leaves (69.9±1.24 days) in this study. The intrinsic growth rate (r_m) and finite rate of increase (λ) were 0.159 ± 0.001 and 1.172±0.002 on the artificial diet, different from the values for white-clover ($r_m = 0.177\pm0.002$ and $\lambda = 1.194\pm0.002$) and apple leaves ($r_m = 0.053\pm0.007$ and $\lambda = 0.008\pm0.001$).

4. Discussion

S. eridania larvae did not feed on peach leaves and apple fruits. Even when fed on these diets until the third instar and transferred to apple fruits, the larvae only damaged the fruit and were unable to molt further. These

results demonstrate the occurrence of host resistance to the development of *S. eridania* in these structures, where the observed reduction in development, as with larvae of *S. eridania* that feed on peach leaves or apple fruits, may be related to poor nutritional quality and the presence of allelochemicals, as observed in annual crops, which mostly affect ecdysis, preventing them from reaching the pupal stage (Reynolds et al., 1984).

Our results showed that the development of *S. eridania* was more strongly affected when used apple as host compared to the other food sources, which showed increases in the duration of the larval and pupal stages and consequently the total cycle duration, as well as a reduction in the weight of pupae. According to Panizzi and Parra (2009), the ingestion of food in the early stages of development is an important factor for polyphagous insects, because some nutrients present in the hosts can supply the nutritional needs of the insect, supporting normal development until the adult stage. Thus, inadequate food sources delay development, as observed on bracinga *M. scabrella* (Mattana and Foerster, 1988), strawberry and grape (Bortoli et al., 2012), and an artificial diet (López and López, 2010). However, when the insect feeds on a suitable food source, the insect/host response is better, with a shorter development period and greater viability, as observed for other food sources, such as cotton *Gossypium hirsutum*, morning-glory *Ipomoea grandifolia* and soybean *Glycine max* (Santos et al., 2005). Thus,

Table 3. Longevity of males and females, pre-oviposition (days) (mean ± SE) and eggs per female (mean ± SE) of *Spodoptera eridania* kept on the artificial diet, white clover leaves, and apple leaves cv. 'Gala'. Temperature: 25±1°C, RH 60±10% and photophase: 14 hours.

Host	Longevity (days)		Pre-oviposition (days)	Fertility
	Males	Females		
Artificial diet	17.2±0.98a* (17) ¹	14.8±1.14a (17)	5.3±1.08a (8)	840.6±236.7b (17)
White clover leaves	16.5± 0.87a (24)	15.44±0.86a (24)	2.2±0.09b (25)	1560.6±107.8a (27)
Apple leaves	7.33±1.30b (6)	9.83 ±1.51a (6)	4.83±1.01a (6)	310.0±145.3b (6)
CV (%)	24.41	15.71	16.53	48.84
DF	46	46	38	49
F	15.96	2.97	16.79	11.37

*Means ± SE followed by the same letters in the line do not differ by Tukey's test at 5% significance; ¹Values in parentheses refer to the number of observations.

Table 4. Mean generation time (T), net reproductive rate (R_0), intrinsic rate of population increase (r_m) and daily finite rate of increase (λ) for *Spodoptera eridania* kept on the artificial diet, white-clover leaves, and apple leaves cv. 'Gala'. Temperature: 25±1°C, RH 60 ± 10% and photophase: 14 hours.

Diets	T (days)	R_0 (♀ / ♀)	r_m (♀ / ♀*day)	λ (individuals/♀/day)
Artificial diet	36.11±0.30b*	336.92±33.2b	0.16±0.001b	1.17±0.002b
White-clover leaves	35.13±0.13a	509.39±34.9a	0.18±0.002a	1.19±0.002a
Apple leaves	69.9±1.24c	34.9±16.3c	0.05±0.007c	0.01±0.001c
P value	<0.0001	< 0.0001	< 0.0001	<0.0001

*Values represent mean ± SEM obtained with the Jackknife method through the SAS program. For each parameter evaluated, values followed by the same letter in the column do not differ statistically from each other (P> 0.05).

although apple leaves can support the development of the larval stage, it is not a suitable host for complete development of *S. eridania*, such as the diet developed by Greene et al. (1976), Montezano et al. (2014), which showed that the larvae had a poor development.

The duration of the pupal stage was influenced by the host, in agreement with the results for *S. eridania* fed on strawberry and grape leaves (Bortoli et al., 2012). However, *S. eridania* had a shorter pupal stage when fed on velvet bean *Stizolobium aterrimum*, pigeon pea *Cajanus cajan*, jack bean *Canavalia ensiformes*, oilseed radish *Raphanus sativus* and sunn hemp *Crotalaria juncea* at ambient temperatures (Jesus et al., 2013). The mean pupal weight of males and females did not differ when the larvae were fed on apple leaves, which can be attributed to the low number of pupae evaluated. Pupae (males and females) from larvae fed on the artificial diet gained more weight than the pupae of larvae fed on white-clover and apple leaves. The lower weight of pupae from larvae fed on apple leaves may be related to a non-preference of larvae for the food or ingestion of substances in the leaves (allelochemicals), causing antibiosis and impeding their development, or even to both factors combined, which fosters host resistance to the attack of defoliators (Santos and Boiça-Júnior, 2001).

Several studies have shown that the development of *S. eridania* is influenced by the host, especially when it uses food sources from perennial crops, where it shows lower viability and increased duration of the entire cycle, as seen on apple and peach leaves in this study and by Bortoli et al. (2012) for grapes, compared to short-cycle (annual) crops such as cotton, where the viability is higher and the total cycle is shorter (Santos et al., 2005). However, larvae may be less viable on these annual crops, as observed for soybean by Santos et al. (2005). Thus, the effects on the biological parameters observed for peach leaves and fruits and leaves of apple may reflect the expression of some protective mechanism (resistance), in which the insect feeds on the plant and its development is affected. This resistance mechanism would have an adverse effect at some stage of development, which may cause high mortality in the immature stages, reduction in size or weight, change the sex ratio, and shorter life span (Smith, 2005).

For the hosts evaluated here, the mean longevity of males did not differ from that of females. Bortoli et al. (2012) found no significant differences in the longevity of male and female *S. eridania* on strawberry and grape leaves. However, in the present study, adults originated from larvae reared on apple leaves had shorter life spans. Santos et al. (2005) also observed reduced longevity of adults from larvae fed on cotton, soybeans and morning-glory, compared to those observed in the present study for adults from larvae fed on the artificial diet and white clover. These results indicate that the artificial diet and white clover provide adequate nutrition, while improving the larval development and adults longevity. The greater longevity of adults may also be related to non-fertilization of females, or if they are fertilized later by males (Montezano et al., 2013, 2014).

The pre-oviposition period observed in the present study was longer on the artificial diet and the white-clover and apple leaves. These results may be associated with the host or with the reduced number of copulations at this development stage, as reported by Montezano et al. (2013), which is reflected in the increase in longevity. The shortest pre-oviposition period was observed by Mattana and Foerster (1988) for bracinga *Mimosa scrabella* and sweet potato *Ipomoea batatas*. López and López (2010) also found a long pre-oviposition period in *S. eridania* fed on the artificial diet, with longer periods than when it was fed on soybean leaves.

Females that were fed on white-clover in the larval phase showed higher fertility than females from individuals fed on apple leaves and the artificial diet. Santos et al. (2005) found fertility values similar to those observed in apple leaves and the artificial diet when females were fed on cotton. López and López (2010) found similar values to other results when females were reared on the artificial diet in the larval stage. The mean fertility observed for females reared on white-clover was similar to that observed on soybean (Parra et al., 1977), sweet-potato (Mattana and Foerster, 1988), strawberry and grape (Bortoli et al., 2012). These results show that white-clover has a higher nutritional value than apple for the development of *S. eridania*. In this case, differences in the amount of food ingested or physical or chemical differences among the foods may have been responsible for these effects on reproduction (Johansson, 1964).

The net reproductive rate (R_0) of *S. eridania* varied widely, and the response also varies between cultivated plants and spontaneous plants (Santos et al., 2005). The R_0 also differed between strawberry and grape in trials by Bortoli et al. (2012). The R_0 indicated that the population of *S. eridania*, when white-clover was the host, can increase 225.71 times in each generation. The R_0 was 118.18 times for the artificial diet, and only 48 times for apple leaves (Table 4). The mean generation times (T) observed with the artificial diet and white clover were shorter than those observed by Bortoli et al. (2012) in larvae fed on strawberry (T = 59.1) and grape (T = 48.8 at 22° C), but similar to values obtained by Montezano et al. (2013) for the artificial diet (35.8 days at 25° C).

The intrinsic rates of increase (r_m) and finite rates of increase (λ) on white-clover and the artificial diet were similar to those observed by Montezano et al. (2013), and higher than those observed on apple leaves. These values were also similar to those reported by Bortoli et al. (2012) in strawberry and grape. However, one must consider that the values for the same species vary widely, especially as a function of temperature, host plant, or rearing on an artificial diet (Parra et al., 1977; Mattana and Foerster, 1988; Bavaresco et al., 2002; Santos et al., 2005).

The present results showed that white-clover, when present as a cover crop in apple and peach orchards, is a suitable host for the development of *S. eridania*. In the case of commercial orchards, *S. eridania* cannot grow on peach leaves, while the viability of the pest on apple

leaves is low and unsuitable for the development of the species, although it can complete its life cycle. In this case, *S. eridania* can develop on suitable species such as white-clover present in apple and peach orchards. The damage caused by *S. eridania* is possibly due to “test bites” made in fruits, usually at night, even though the fruit is not a suitable host species for the larvae. Thus, inadequate management practices, such as herbicide application, elimination of cover crops, and reduction of the food source used by larvae, result in infestation and damage to fruit trees after these commercial crops have become established.

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