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PLANT PROTECTION
Article

Biological parameters of the American grapevine moth on artificial diets

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ABSTRACT: The São Francisco Submedium Valley in Brazil is the country's most important table grape exportation region. In 2015, a new pest was reported in the area, the American grapevine moth *Lasiothyris luminosa* (Razowski & Becker) (Tortricidae), causing significant damage from the bud flowers until the grape berries in the harvesting period. The present work aimed to evaluate artificial diets to guarantee the rearing of *L. luminosa* in laboratory conditions. Two artificial diets used to rear *Anticarsia gemmatalis* Hübner and *Helicoverpa* zea (Boddie) were compared to the species' natural host (grape berries). It was evaluated the larva, pupal, and larval-pupal period, longevity, fecundity, larval and pupal viability, and sex ratio. *L. luminosa* completed its cycle in both artificial diets, making it possible to use both to maintain the species in the laboratory. The larval and larval-adult period were shorter for the treatment with *A. gemmatalis* artificial diet. Also, this diet provided greater pupal viability and higher fecundity. Thus, the *A. gemmatalis* diet is more indicated to rear *L. luminosa* in the laboratory than the *H. zea* diet and grape berries.

Key words: *Lasiothyris luminosa*, *Vitis*, Tortricidae, insect rearing.

INTRODUCTION

Lepidopteran species from the Tortricidae family are important grapevine pests in different countries, such as *Lobesia botrana* (Den. & Schiff.), *Eupoecilia ambiguella* (Hubn.), and *Paralobesia viteana* (Clemens) (Loeb et al. 2011, Ioriatti et al. 2012, Thiéry et al. 2018). These species can bore into flowers, berries, rachis, and peduncles, causing direct damage (Ioriatti et al. 2012) and increasing losses by fungal damage (Cozzi et al. 2006). *Cryptoblabes gnidiella* (Millière) and *Argyrotaenia sphaleropa* (Meyrick) are also grapevines pests, but these tortricids feed externally on berries and other grapevine structures (Bentancourt et al. 2003, Lucchi et al. 2019).

In 2015, another tortricid species was identified as a grape pest in northeast Brazil, the American grapevine moth *Lasiothyris luminosa* (Razowski and Becker), causing losses in grapevines around U$ 5.150/ha (Costa-Lima et al. 2016). In Brazil, the São Francisco Submedium Valley (SFSV) is the most important table grape-producing region, whereas 99% of the fruit exportation originated in the country. The pest behavior was similar to *L. botrana, E. ambiguella*, and *P. viteana*, with reported damages in buds, flowers, and berries, in which the larvae penetrate all structures to feed internally. Until now, the species occurrence is restricted to Brazil, in the northeast (SFSV) and south region (Brusque, SC) (Razowski and Becker 1983, Costa-Lima et al. 2016, Costa-Lima et al. 2021). Due to its distribution and potential damage in 2017, the European and Mediterranean Plant Protection Organization included *L. luminosa* on its alert list (EPPO 2017). In 2019, the species was incorporated as a quarantine pest for the MERCOSUL countries (MERCOSUL 2019).

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The *L. luminosa* management is improving, with results including biological control with *Trichogramma pretiosum* Riley, already adopted by growers in SFSV (Costa-Lima et al. 2021). Other methods have been also recommended, such as cultural methods and chemical control (Costa-Lima 2020). However, management alternatives must be developed considering its difficulty to control. Thus, it is important to establish a rearing system for the species to aid further research in obtaining monitoring and control methods for *L. luminosa*. The present work aimed to evaluate two artificial diets for *L. luminosa* larvae development and their effect on the tortricid's biological parameters.

MATERIAL AND METHODS

To initiate the *L. luminosa* rearing, grape berries with larvae were collected in three grapevine farms in Petrolina (PE) and Lagoa Grande (PE), Brazil. The fruits were positioned in trays with vermiculite and covered with a fine mesh cloth. The emerging adults were transferred to cages (10 cm diameter and 20 cm height) made with PVC pipe with lateral openings (8 × 10 cm) closed with a fine mesh cloth. A Petri dish was positioned in the bottom, covered with a paper towel. In each cage, the adults were offered a sugar solution (5%) and distilled water by capillary in glass falconettes. Four to six grape berries (~40 days after pruning) were provided over the paper as an oviposition substrate. In pre-tests, it was observed that *L. luminosa* females oviposited in the paper with a preference for the cage's inferior surface. However, the number was inferior compared to the grape berries, and the egg viability was lower. Daily, the grape berries and the paper towel were removed, and new ones were added. The sugar solution and water were renewed every two days. The grape berries with *L. luminosa* eggs were transferred to plastic containers (9 cm diameter and 6 cm height) with filter paper on the bottom humidified with distilled water.

Two artificial diets were chosen to be evaluated for *L. luminosa* larvae development, considering their differences in composition and costs. The first one was developed for *Helicoverpa zea* (Boddie) (HZD) (Perkins et al. 1973) and was characterized by a low number of ingredients and low cost. The second is used for *Anticarsia gemmatalis* Hübner (AGD) (Greene et al. 1976), which shows a richer composition and higher cost than HZD (Table 1). For the control treatment, it used grape berries var. Sweet Jubilee (50–55 days after pruning).

Table 1. Artificial diet composition used to rear *Lasiothyris luminosa* larvae.

Composition	Artificial diets	
	Helicoverpa zea[1]	***Anticarsia gemmatalis***[2]
White bean (g)	-	28.12
Pinto bean (g)	85.00	-
Wheat germ (g)	39.60	22.50
Soybean protein (g)	-	11.25
Casein (g)	-	11.25
Brewer's yeast (g)	25.25	14.07
Vitamin solution* (mL)	-	3.37
Ascorbic acid (g)	2.55	1.35
Sorbic acid (g)	0.82	0.67
Nipagin (g)	1.57	1.12
Tetracyclene (mg)	-	42.37
Formaldehyde (40%) (mL)	-	1.35
Formaldehyde (10%) (mL)	6.25	-
Agar (g)	10.25	8.75
Distilled water (mL)	597.50	450.00

*Niacinamide (1 g); calcium pantothenate (1 g); riboflavin (0.5 g); thiamine HCl (0.25 mg); pyridoxine HCl (0.25 g); folic acid (0.25 g); biotin (0.02 g); vitamin B12 (350 µg/2mL); distilled water (1 L); [1]based in Perkins et al. (1973); [2]based in Greene et al. (1976).

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Two separate bioassays were conducted to evaluate the diets' effects on *L. luminosa*. The first one evaluated the effect over the larva, pupa, and larva-adult period, and the second assessed the adult's longevity and fecundity.

Plastic containers (30-mL volume, 5-cm diameter) were used to add 15 mL of each diet closed with a transparent lid. A recipient (9-cm diameter and 6-cm height) was used for the control treatment, with filter paper on the bottom and four grape berries. To each one, artificial diets and control treatment, three *L. luminosa* larvae were added (less than 24 h). A fine brush transferred the recently hatched larvae to the recipients. In pre-tests, it was observed that some larvae died during this process, considering the larva's small size (~1 mm). Thus, the data analyses did not consider larval mortality observed in less than 12 h. Daily observations were conducted to evaluate the larvae development stage and survivorship. The rotten grapes were discarded once per week, and new ones were added for the control treatment. The duration (days) and viability (%) for larva and pupa stages and the sex ratio were registered.

For the bioassay with adults, transparent plastic cups (7.5-cm diameter and 11-cm height) with lateral openings (4 × 5 cm) closed with fine mesh cloth were used as cages. The cage base was similar to that adopted for the rearing cage, as well as the feeding source and water (Fig. 1). One *L. luminosa* couple with less than 24 h was added per cage originated from larvae developed in two artificial diets (AGD and HZD) and grape berries (control). Daily, the grape berries and the paper towel were renewed. On a stereomicroscope (80x), the number of eggs per cage was counted. When the male died first, a new one was added to the cage to reduce the possible interference in the female oviposition. It was registered the male and female longevity and the female fecundity.

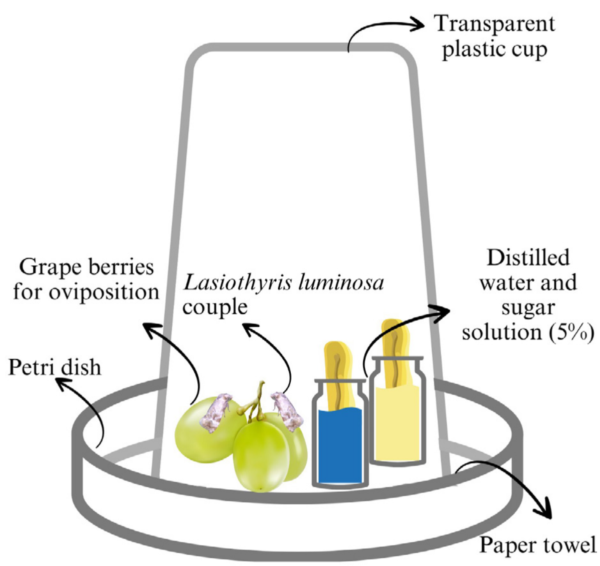

Figure 1. Description of the method used to evaluate *Lasiothyris luminosa* longevity and fecundity from adults originated from larvae developed in two artificial diets (*Anticarsia gemmatalis* diet and *Helicoverpa zea* diet) and grape berries (control).

All the experiments were conducted in climatic chambers, with 25 ± 1°C, 65 ± 10% relative air humidity, and 12-h photophase. The experimental design was completely randomized for both bioassays. For the immature development, each insect was considered a repetition. The elimination of dead larvae in the first 12 h led to a variable number of repetitions per development stage:larva (male: 41–87; female: 44–92), pupa (male: 43–87; female: 45–92), and larva-pupa (male: 42–99; female: 43–103). For the adult bioassay, each cage with an insect couple was considered a repetition, with variable numbers for survivorship (male: 24–30; female: 25–29) and fecundity (16–18). This variability was caused by insects that died from different causes, such as those attached to sugar solution.

All the statistical analyses and plots were performed with R software, version 4.1.0 (R Core Team 2023). Generalized linear models with quasi-binomial distributions were used to compare larval and pupal viability and sex ratio, and quasi-Poisson distribution for fecundity. The model distributions were determined using simulation envelopes with the hnp

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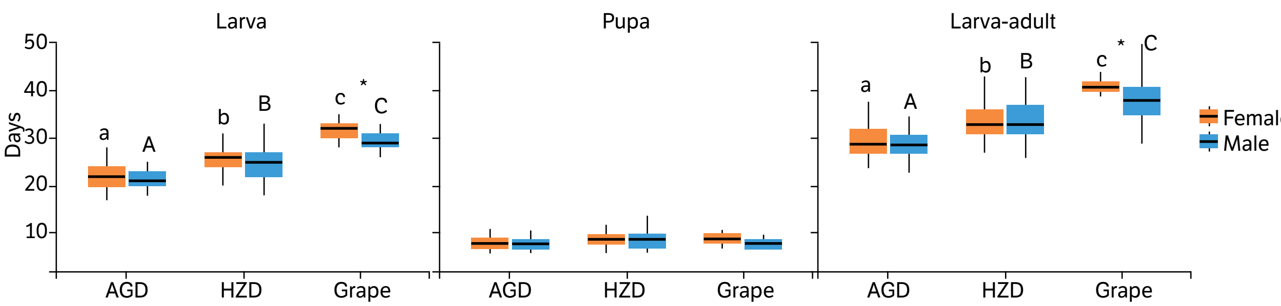
package (Moral et al. 2017). When there was a significant difference between treatments, multiple comparisons were made (Tukey test, $p < 0.05$) with the *glht* function from the multicomp package, with *p*-value adjustments. For the longevity and development stages (larva, pupa, and larva-pupa), the means and standard errors were computed by the Kaplan-Meier estimator (Kaplan and Meier 1958). Comparisons between groups were conducted using the log-rank test with the *pairwise_survdiff function* from the survminer package ($p < 0.05$). All plots were made with the ggplot2 package (Wickham 2016).

RESULTS AND DISCUSSION

The feeding source interfered with the *L. luminosa* larval development period ($p < 0.001$). A shorter period was observed in larvae fed with AGD and a more extended period in grape berries. Only in the control treatment, the females lived longer than the males, while no difference was detected in the artificial diets. For the pupal stage, no difference was observed between treatments ($p = 0.40$). Considering the larval-adult period, the feeding source showed an effect ($p < 0.001$). The pattern followed the same as the larval period, with a shorter AGD period followed by HZD and grape berries. Also, only differences between sexes were observed for the control treatment, in which the females showed a higher larval-pupal period (Fig. 2, Dias et al. 2025).



*Significant difference at 5% between sexes.

Figure 2. *Lasiothyris luminosa* development periods in two artificial diets (*Anticarsia gemmatalis* diet–AGD; *Helicoverpa zea* diet–HZD) and grape berries, at 25 ± 1°C, 65 ± 10% relative air humidity and 12-h photophase. Boxplots followed by the same letter do not vary statistically from each others (lower case: females; upper case: males) in the same insect stage using log-rank test ($p < 0.05$). No difference was observed for the pupal stage.

It is reported for lepidopterans that high-quality foods result in higher larval growth rates and, thus, a shorter development period (Suits et al. 2017, Borzoui et al. 2018, Truzi et al. 2021). Proteins (P) and carbohydrates (C) are the most essential dietary macronutrients for insects (Behmer 2009). These elements' proportion is also important. Caterpillars generally require intermediate or higher P:C ratios (Lee et al. 2004, 2006, Thompson and Redak 2005). The AGD treatment shows a higher P:C compared to HZD. The protein quality can also interfere with HZD, whose primary protein source are beans, which offer low concentrations of some amino acids, such as methionine and cysteine (Panizzi and Parra 2012). Some protein sources, such as soybean and casein, were present only in AGD. Besides that, the AGD presents a vitamin-B complex that is not present in HZD. These micronutrients function as coenzymes, and their absence can extend the insect development time (Douglas 2017). *L. luminosa* most extended larval and larva-pupal period was in the grape berries. The fruit presents a very low P:C ratio, which means the insect needs to increase its food intake to meet its minimum requirements to achieve the pupal stage (Behmer 2009). Also, the study method used detached grapes, which naturally will start to decompose and will alter the grape composition. A study with *L. botrana* also noticed a longer development time for the larvae reared in grape berries than an artificial diet, mainly because these insects showed a higher proportion needing an extra larval instar (Pavan et al. 2013).

The larval developmental time for *L. luminosa* observed (25°C) in both artificial diets was close to what was detected for *L. botrana* in the artificial diet at 24°C (Preto et al. 2019). At the same time, this period in grape berries was similar to that already observed in a previous study with *L. luminosa* in the same host (Costa-Lima et al. 2021). No difference between treatments was observed in the pupal stage duration for *L. luminosa*. Similar results were found for other lepidopterans that compared artificial diet and natural host (Silva et al. 2021, Wang et al. 2021). These similar *L. luminosa* pupal durations reflect that the shorter larval-pupal period resulted from the larval stage.

The larval viability was similar between treatments ($p = 0.21$), ranging from 64.41 (grape berries) to 73.66% (AGD). However, the diet interfered with the *L. luminosa* pupal viability ($p < 0.05$), higher in AGD (92.34%) compared to HZD (82.01%), and none differed from the grape berries (86.66%) (Fig. 3, Dias et al. 2025). These values are superior when compared to the combined viability larvae to adult for *L. botrana* developed in artificial diets (Tzanakakis & Savopoulou 1973) and similar to when fed with 'Cabernet sauvignon' grape berries (Thiéry and Moreau 2005). However, the larval viability is lower when compared to other tortricids reared in artificial diets, such as *A. sphaleropa*. (Manfredi-Coimbra et al. 2005) and *Grapholita molesta* (Busck) (Su et al. 2020). Adjustments can probably still be made in the *L. luminosa* diet, aiming at variations that can interfere with larval survival, such as nutrition composition and balance (Behmer 2009, Panizzi and Parra 2012). No difference was observed in the sex ratio between treatments, which ranged from 0.50 (HZD) to 0.55 (AGD) ($p = 0.73$).

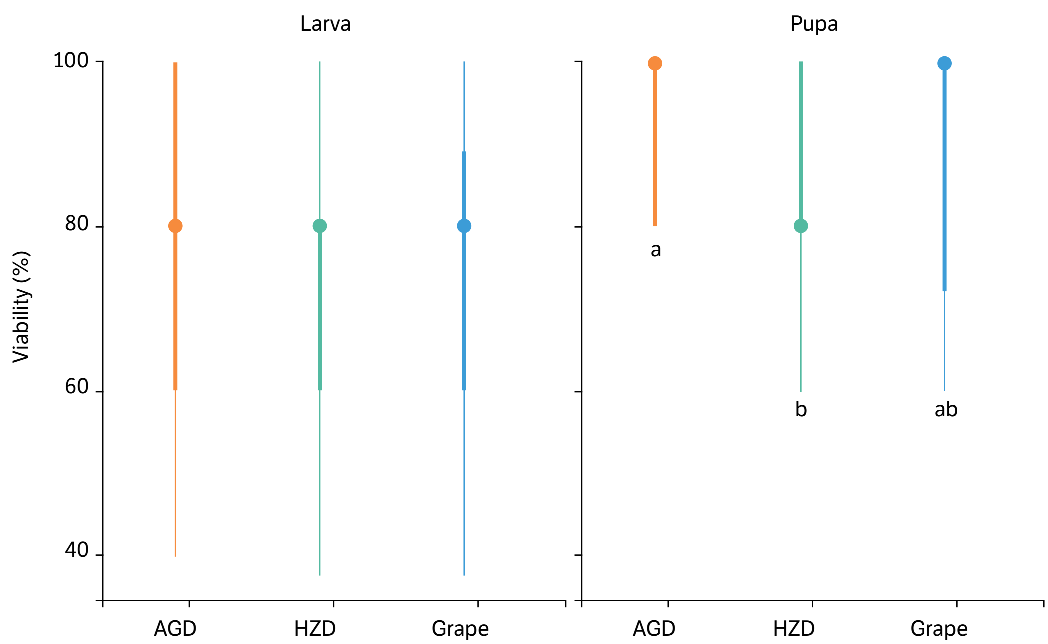
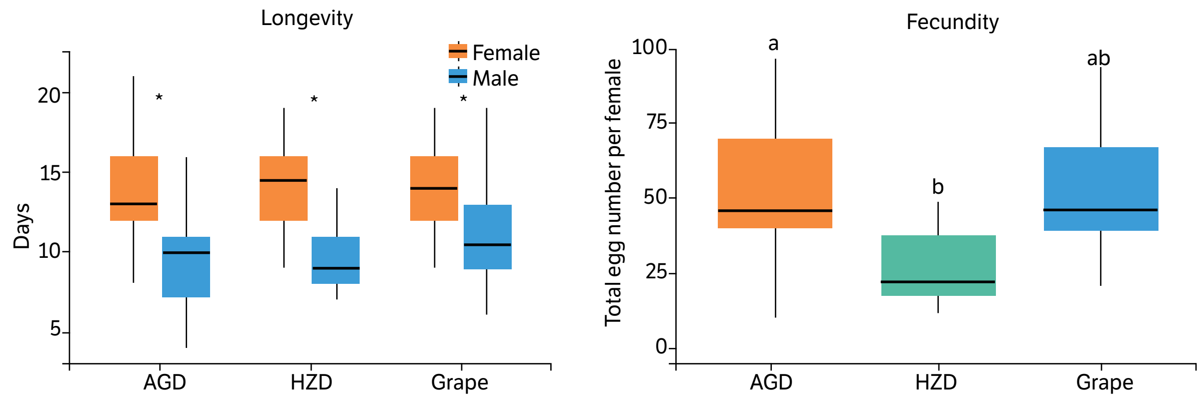


Figure 3. *Lasiothyris luminosa* larval and pupal viability in two artificial diets (*Anticarsia gemmatalis* diet–AGD; *Helicoverpa zea* diet–HZD) and grape berries, at 25 ± 1°C, 65 ± 10% relative air humidity and 12-h photophase. Means followed by the same letter do not vary statistically from each other using generalized linear model with quasi-binomial distribution followed by multiple comparisons (Tukey's test, $p < 0.05$). No difference was observed for the larval stage.

For *L. luminosa* adults, females showed longer life spans compared to males regardless the food source ($p < 0.001$); the same was also observed for *L. botrana* (Ioriatti et al. 2012). The survivorship showed no difference between treatments for the same sex (Fig. 4, Dias et al. 2025). The *L. luminosa* females lived an average of 14 days, four days more than *L. botrana* (Moreau et al. 2006). The diet consumed by the larvae affected the *L. luminosa* fecundity ($p < 0.05$). Females that the larvae fed in AGD showed higher egg mean (57.78) compared to HZD (34.68), but no difference was observed in grape berries (55.72) (Fig. 4, Dias et al. 2025). It is known that larval nutrition can affect adult traits, such as fecundity (Awmack and Leather 2002, Boggs and Freeman 2005). As commented, the AGD has a higher P:C ratio and a vitamin solution that is not present in HZD. The observed fecundity for *L. luminosa* was similar compared to a review of the European grapevine moth (Ioriatti et al. 2012) and lower to study with *L. botrana* in three *Vitis vinifera* L. cultivars (Moreau et al. 2006).



*Significant difference at 5% between sexes.

Figure 4. *Lasiothyris luminosa* longevity and fecundity from females originated from larvae developed in two artificial diets (*Anticarsia gemmatalis* diet–AGD; *Helicoverpa zea* diet–HZD) and grape berries, at 25 ± 1°C, 65 ± 10% relative air humidity, and 12-h photophase. No difference was observed between treatments for longevity using a log-rank test ($p < 0.05$). Fecundity boxplots followed by the same letter do not vary statistically from each other using generalized linear model with quasi-Poisson distribution followed by multiple comparisons (Tukey's test, $p < 0.05$).

Basic biological studies and rearing methods are crucial to developing different management strategies (Parra and Coelho Jr. 2022). The present study presented the first artificial diets that can enable the maintenance of an *L. luminosa* population in the laboratory. Considering another tortricid species, the rearing establishment of *Gymnandrosoma aurantianum* (Lima) (Garcia and Parra 1999) permitted several studies towards its management that led to the synthesis of the species' synthetic pheromone (Leal et al. 2001). A study estimated that citrus growers in 10 years avoided losses of up to 1.3 billion dollars after using the sex pheromone (Bento et al. 2016). The artificial diets evaluated for *L. luminosa* are already permitting the initiation of studies to identify the species's sex pheromone.

CONCLUSION

The HZD and AGD can complete *L. luminosa* cycle in the laboratory. However, the AGD guarantees a shorter larval and larval-adult period and a higher pupal viability and fecundity.

CONFLICT OF INTEREST

Nothing to declare.

AUTHORS' CONTRIBUTION

Conceptualization: Costa-Lima, T. C.; **Data curation:** Dias, G. S.; **Formal analysis:** Costa-Lima, T. C.; **Investigation:** Dias, G. S., Gama, F. C., Teles, E. R. and Duarte, F. R. M.; **Methodology:** Costa-Lima, T. C., Gama, F. C., Dias, G. S. and Gervásio, R. C. R. G.; **Writing – original article:** Dias, G. S., Costa-Lima, T. C. and Gervásio, R. C. R. G.; **Writing – review & editing:** Costa-Lima, T. C., Dias, G. S., Gervásio, R. C. R. G., Gama, F. C., Teles, E. R. and Duarte, F. R. M.; **Final approval:** Costa-Lima, T. C.

DATA AVAILABILITY STATEMENT

Supplementary data are available at figshare. https://doi.org/10.6084/m9.figshare.28352780.v1

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