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A potential rearing method to optimize survival and reproduction of *Neoleucinodes elegantalis* (Guen., 1854) (Lepidoptera: Crambidae)

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Abstract. The aim of this study was to develop a simple and efficient laboratory rearing method for *Neoleucinodes elegantalis* (Guen., 1854) (Lepidoptera: Pyralidae). To this end, seven bioassays were performed to evaluate the survival and reproductive characteristics of *N. elegantalis* for rearing populations in the laboratory with artificial and natural fruits. *Neoleucinodes elegantalis* was affected by the size and color of artificial fruits and the number of couples per cage. It was found that larvae fed with natural fruits of *Solanum gilo* had a greater survival rate and larval and pupal duration. Pupal survival rate did not differ between treatments. However, pupae of larvae fed *S. gilo* and *S. melongena* had the highest weights in both males and females. The highest percentage of fully formed adults was observed in the *S. gilo* treatment. The sex ratio did not differ between treatments, and the increase in larvae per fruit decreased larval survival, which was not observed in pupae. The results obtained in this study are significant because they reveal biological, ecological, and behavioral aspects of *N. elengantalis* and contribute to the expansion of techniques for mass rearing of this insect pest in the laboratory, which is essential for several other entomological studies.

Keywords: Rearing method, insect biology, small tomato borer, Solanaceae, tomatoes.

In rearing systems, huge quantities of insects are produced for many purposes, such as sterile insects, biological control, research, and others (Cohen 2015). In these systems, rearing conditions must provide all the factors the insect needs to complete its cycle in continuous generations. These factors include the insects themselves with all their genetic traits, suitable food, environmental conditions, and generally some type of containment that allows the insects to be kept in an environment that is suitable for the insect and at the same time comfortable for the rearing personnel (Cohen 2018).

The small tomato borer *Neoleucinodes elegantalis* (Guenée, 1854) (Lepidoptera: Crambidae) is one of the main pests of Solanaceae species, such as *Solanum lycopersicum* (tomato), *Solanum gilo* (scarlet eggplant), and *Solanum melongena* (eggplant) (Zucchi et al. 1993; Díaz et al. 2011). The duration of the biological cycle of *N. elegantalis* varies depending on the host plant and is approximately 30 days in tomato fruit at a temperature of 25 °C, with a mean longevity of five days (Marcano 1992). *Neoleucinodes elegantalis* has been reported from South American countries (Munõz et al. 1991; Jordão & Nakano 2002); however, its presence in African, Asian, European, and Oceanic countries is considered a threat because nightshade crops are imported from South American regions where this pest causes losses (EPPO 2015; Silva et al. 2016). Infestation with *N. elegantalis* can cause production losses of 40 to 90% (Picanço et al. 1998; Revelo et al. 2010).

Although *N. elegantalis* is an important and critical pest in some solanaceous crops, its rearing methodology has not been described in detail. There is little published research on *N. elegantalis*, possibly related to the difficulty of obtaining insects, which in most cases are collected directly from the field. The nocturnal activity of the adults, the small size of the eggs, and the development of the larvae inside the fruit are the main difficulties in collecting of this insect. Therefore, it is impossible to obtain enough insects to conduct the bioassays, which are limited to the year when the pest occurs naturally. One way to facilitate the procurement of *N. elegantalis* is to grow populations in the laboratory. By rearing in the laboratory, it is possible to obtain insects at various ages and developmental stages and in sufficient numbers to perform bioassays. Another advantage is that research can be conducted continuously, regardless of whether the insect is hatching (Sing & Moore 1985; Parra 1996). Because of the importance of rearing methodology, the objective of this study was to propose a methodology and evaluate the biological characteristics of *N. elegantalis* for maintaining populations in the laboratory.

Tomato fruits with *N. elegantalis* larvae were collected from commercial crops in Coimbra, Minas Gerais State, Brazil (20°51'24'' S 42°48'10'' W). These fruits were taken to the Integrated Pest Management Laboratory of the Federal University of Viçosa, where they were packed in plastic trays (38 x 24 x 7 cm) with a layer of sand (3 cm). The sand served as a place for pupae formation after the larvae of *N. elegantalis* had left the fruit. The formed pupae were transferred to organza cages with wooden structure (45 x 45 x 45 cm), and the hatched adults were fed with the honey solution (10%) with cotton attached to the top of the cage.

Seven bioassays were performed to evaluate factors affecting egg laying and development of *N. elegantalis* in the laboratory. All bioassays were conducted under controlled conditions at 25 ± 1 ºC, relative humidity of 75 ± 5%, and photophase of 12h. Artificial fruits consisted of Styrofoam balls with a piece of string (3 cm) glued to one end and then dipped in dyed paraffin were used as substrate for oviposition. Insects up to 24 hours old were used for adult bioassays. Larvae from oviposition of these adults were used for developmental bioassays.

The first bioassay performed was to evaluate the effects of cage

size on egg laying by *N. elegantalis*. The experimental design for this bioassay was completely randomized (n = 5). Treatments were 30 dm³ (31 x 31 x 30 cm), 90 dm³ (45 x 45 x 45 cm), and 480 dm³ (90 x 101 x 52 cm) wooden cages covered with organza. The experimental plot consisted of a cage with three artificial fruits with a diameter of 3 cm covered with green paraffin and 25 pairs of *N. elegantalis*. The artificial fruits were replaced daily and the number of eggs in the fruits was counted.

Three additional bioassays were performed to evaluate the effects of cage size, colors, and the number of artificial fruits per cage. Bioassays for artificial fruit size were performed under choice conditions (n = 6). Each replicate consisted of an organza cage with a wooden structure (45 x 45 x 45 cm) containing 10 couples of *N. elegantalis*. Adults were randomly selected 24 hours after hatching. Artificial green fruits of 2.0, 3.0, 3.8, 5.2, and 7.7 cm in diameter were tested. These sizes were chosen because they correspond to the fruits in which *N. elegantalis* is observed ovipositing in the field (Blackmer et al. 2001). The bioassay with fruit colors was performed in a similar manner, testing artificial fruits (3.8 cm Φ) in green, red, white, and yellow colors.

In the bioassay for the density of artificial fruit per cage, the densities of 3, 6, 9, and 12 artificial fruits (3.8 cm Φ green) per cage were tested. Each density was evaluated four times. The same number of replicates was performed for the fruit size and color bioassays. For the three bioassays, egg laying by *N. elegantalis* was examined for five days.

Wooden-structure organza cages (45 x 45 x 45 cm) with 10, 25, and 50 couples of *N. elegantalis* per cage were used to evaluate the effects of density of couples per cage. Each density of couples was tested three times. Three artificial fruits (3.8 cm Φ green) were also placed in each cage. The oviposition of *N. elegantalis* was evaluated for five days.

A sixth bioassay was also performed to evaluate larval development on different Solanaceae host plants. In this bioassay, three different fruits of solanaceous plants (Solanaceae) were used: Tomato (*Solanum lycopersicum*), Scarlet Eggplant (*S. gilo*) and Eggplant (*Solanum melongena*). The bioassay was performed in a completely randomized design (n = 5). Each replicate consisted of a plastic tray (28 x 20 x 6 cm) containing 400 g of whole fruit. Newly hatched larvae of *N. elegantalis* were transferred to these fruits at a ratio of three larvae per 50 g of fruit, for a total of 24 larvae per replicate. Five days after the larval transfer, a paper towel was placed over the fruit to serve as a pupation site. Survival and duration of larval and pupal periods, pupal weight, percentage of adults, and sex ratio were evaluated.

In the last bioassay, the number of larvae per fruit was evaluated. The bioassay was performed in a completely randomized design (n = 4). Each replicate consisted of 10 *S. gilo* fruits (mean 50 grams per fruit) packed in a plastic tray (28 x 20 x 6 cm). The newly hatched larvae of *N. elegantalis* were transferred to the fruits at densities of 2, 4, 6, 8, and 12 larvae per fruit. After five days, the fruits were covered with paper towels. Survival larvae and pupae was evaluated.

Data were subjected to ANOVA, and treatment means were compared using the Tukey test ($P < 0.05$) (PROC GLM, SAS Institute 2008). Sex ratio was analyzed using the chi-square test ($P < 0.05$). Bioassay data number of fruits per cage and larvae per fruit were subjected to linear regression analysis (P <0.05) (TableCurve 2D, SPSS 2000). Fruit size, fruit colour, and pupal survival data were transformed into log (x + 1), √x, and arcsin √x/100 to meet assumptions of normality and homoscedasticity.

It was found that the oviposition of *N. elegantalis* was greater in the 90 dm³ cages than in the 30 and 480 dm³ cages. This fact was due to greater competition between females in the smallest cage (30 dm³) and the difficulty of females in the larger cage (480 dm³) to find artificial fruit.

We found differences in egg laying by *N. elegantalis* as a function of size (F = 10.13; df = 4,20; $P < 0.001$) and color (F = 10.44; df = 3,15; $P < 0.001$) of artificial fruits. The greatest number of eggs was observed in fruits with a diameter of 3.8 cm (Fig. 1A) and green color (Fig. 1B). The greater oviposition in green fruits with a diameter of 3.8 cm may be because they have the natural size and color of the fruits in which *N. elegantalis* most frequently lay eggs in the field (Blackmer et al. 2001).

The number of artificial fruits per cage (3 to 12 artificial fruits) did not affect the total number eggs laid by *N. elegantalis*. However, the number of eggs per fruit decreased with increasing density of fruits per cage (Fig. 1C). It is possible that females use this mechanism of distributing eggs among different fruits to reduce intraspecific competition among larvae entering the fruit (Damman 1991; Kagata & Ohgushi 2001). The use of many fruits per cage increased the transfer of newly hatched larvae to their food and required a greater number of artificial fruits. However, the use of fewer fruits per cage (three fruits) resulted in higher mortality of newly hatched larvae due to cannibalism. The ideal density is one that lies between the two extremes, i.e., six fruits per cage.

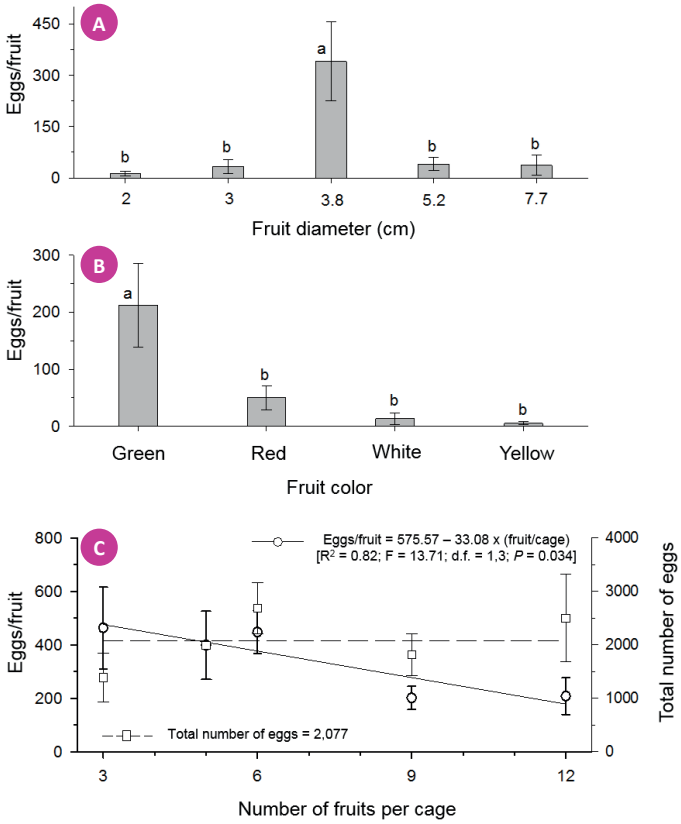

Figure 1. Number of eggs of *Neoleucinodes elegantalis* (Lepidoptera: Crambidae) as a function of (A) size, (B) color, and (C) number of artificial fruits per cage. Means (± SE) labeled with the same letter are not different by Tukey test ($P < 0.05$).

The number of couples per cage did not affect the fertility of *N. elegantalis* (F = 1.32; df = 2.4; $P = 0.363$; mean ± SE = 3.7 ± 1.1) but did, as expected, affect the total number of eggs (F = 6.61; df = 2.4; $P = 0.030$). Higher egg laying per cage occurred at densities of 25 (687.0 ± 103.9) and 50 (687.7 ± 164.4) couples of *N. elegantalis* per cage, but no significant difference was found between these two densities. The lowest egg production was observed at 10 couples (138.7 ± 87.9), which was due to the low density of insects per cage. The fact that egg laying did not increase when 50 couples were used must be due to greater competition between females when laying eggs in artificial fruit at the highest insect density.

It was found that insects fed *S. gilo* fruit had higher larval survival (F = 4.60; df = 2.12; $P = 0.033$) and longer larval (F = 15.02; df = 2.10; $P < 0.001$) and pupal (F = 5.34; df = 2.10; $P < 0.026$) duration. There were no significant differences in pupal survival (F = 0.65; df = 2,10; $P = 0.538$). However, pupae from larvae fed *S. gilo* and *S. melongena* had the highest weights in females (F = 4.93; df = 2,10; $P = 0.032$) and males (F = 9.83; df = 2.10; $P = 0.004$). Larval feeding also affected the number of adults (F = 5.51; df = 2.12; $P = 0.020$), with the highest percentage observed in the *S. gilo* fruit treatment. The sex ratio varied from 0.52 to 0.60, but there were no significant differences ($x^2 = 0.97$; df = 2; $P = 0.616$). *Solanum gilo* was more suitable for larval production than the other fruits because it had a longer residence time in our experiments, which allowed the larvae to obtain high quality food during their larval

development.

We observed that increasing the number of larvae per fruit resulted in decrease in larval survival. Pupal survival was not affected by the number of larvae per fruit (Fig. 2). Higher larval mortality was observed with increasing density of *N. elegantalis* larvae per fruit. There was no effect of larval density per fruit on pupal mortality. Fewer adults per fruit were observed with increasing larval density per fruit. The occurrence of intraspecific competition could explain these facts. This competition may lead to a reduction in the number of adults produced (Yoon et al. 2001; Danho et al. 2002), larval period (Yoon et al. 2001), larval survival, and adult fertility (Giga et al. 1991).

A schematic representation of the methodology for rearing *N. elegantalis* in the laboratory is shown in Fig. 3. Individuals were maintained at constant temperature (25 ± 1 °C), relative humidity (75% ± 5%), and photoperiod (12 h). The 25 adult pairs are placed in an organza cage with wooden structures (45 x 45 x 45 cm), with six green artificial fruits (3.8 cm diameter). The adults are fed with a 10% honey solution. The artificial fruits are used as substrates for egg-laying. After hatching, the larvae are transferred to scarlet eggplant fruits in groups of three individuals and remain in these fruits until they reach the pupal stage. Scarlet eggplant fruits are placed in plastic trays and covered with paper towels. The paper towel serves as a substrate for pupation. The pupae on the paper towel were placed in a plastic container until the adults hatched. The total cycle is about 33 days, i.e., the duration of the egg, larval, and pupal stages is about 6, 15, and 12 days, respectively.

In conclusion, we have found a useful method for rearing *N. elegantalis*. We believe that this method can be of fundamental importance in stimulating research interest in this species, both in terms of management of the species, and in the fields of biology, ecology, and genetics.

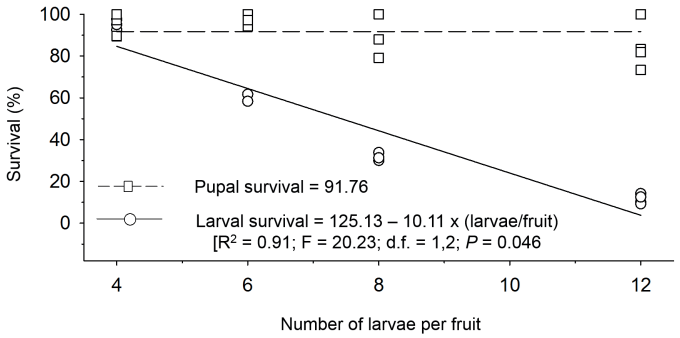

Figure 2. Survival of larvae and pupae of *Neoleucinodes elegantalis* (Lepidoptera: Crambidae) as a function of the number of larvae per fruit of *Solanum gilo*.

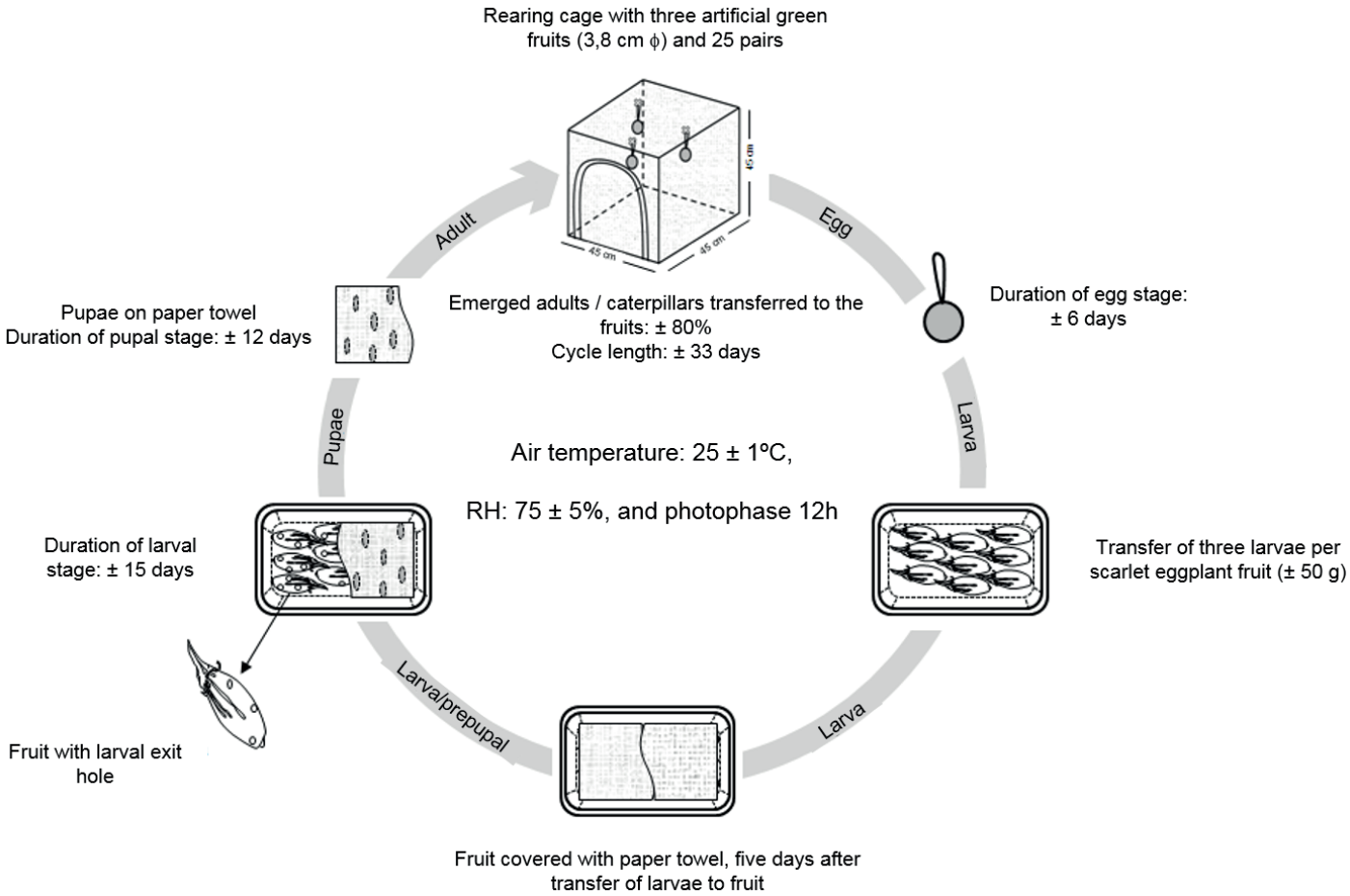

Figure 3. A schematic representation of the methodology for rearing *Neoleucinodes elegantalis* (Lepidoptera: Crambidae) in the laboratory.

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Authors' Contributions

Conceptualization and methodology: Pablo da Costa Gontijo (PCG), Marcelo Coutinho Picanço (MCP), and Júlio Cláudio Martins (JCM); Formal analysis and investigation: PCG, Ézio Marques da Silva (SEM), Hudson Vaner Ventura Tomé (HVVT), and Rodrigo Soares Ramos (RRS); Writing - original draft preparation: PCG, JCM, MCP, and Ricardo Siqueira da Silva (RSS); Writing - review and editing: all authors.

Conflict of Interest Statement

All authors declare that they have no conflict of interest.

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