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Feeding in the Immature Phase Affects the Reproductive Performance of *Diaphaniahyalinata* L. (Lepidoptera: Crambidae)

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***Abstract*—-*Diphaniahyalinata L. (Lepidoptera: Crambidae) is an insect of agricultural importance, considered pest-key family Cucurbitaceae. Knowledge of reproductive parameters of this insect can enable a better understanding of its biology, assisting in integrated pest management programs pests. Thus, this study aimed to evaluate the influence of different food substrates consumed in the immature stage of D. hyalinata on reproductive parameters of their respective adult. Four foods were tested: pumpkin cultivar "Jacaré", cucumber cultivar "Japanese", zucchini cultivar "Caserta" and artificial diet. After the emergence of adults were evaluated reproductive parameters of insects and made fertility life tables. The highest average production of eggs per female was observed for adults from the food substrates cucumber (175.7 eggs/female) and artificial diet (173.0 eggs/female). The zucchini substrate showed the lower values of population growth and greater range of values between generations and doubling time. Thus, it is concluded that food substrate zucchini was unfavorable to the reproductive performance of D. hylinata.*

***Keywords*—Alternative hosts, Antibiosis, Egg production, Fertility.**

I. INTRODUCTION

Cucurbit borers, *Diaphania* spp. (Lepidoptera: Crambidae), are polyphagous insects, however, they prefer species from the Cucurbitaceae family [1]. These lepidoptera are pests of economic importance, occurring from the southwestern United States, Central and South America to the Caribbean islands [2]–[4]. In Brazil, insects of this genus are reported in all regions producing cucurbits [5]. *Diaphaniahyalinata* L. caterpillars are voracious broachers, attacking all vegetative and reproductive parts of the plant, including fruits, which in turn are unsuitable for commercialization and human consumption [6], [7].

Currently, there are few studies related to the investigation of biological parameters of *D. hyalinata*. These studies are fundamental for understanding the behavior and biology of the insect, thus offering support for programs related to Integrated Pest Management (IPM)[8]–[10]. One of the bases of the MIP is the sampling of pests and knowledge of the biology of the insect in association with the host culture (food), providing subsidies that enable a more careful definition of control tactics [11].

Significant results on the biology of *D. hyalinata* were obtained by Pratissoli et al. [7]. However, information on the performance and behavior of the adult phase, considered as the first indication of the presence of the pest in the crop [11], is still scarce. In addition, knowledge of the oviposition behavior and the biotic potential of the

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insect pest, when associated with a given host plant, may allow predicting the distribution and the possible number of descendants as a function of time. For this purpose, the use of the fertility life table allows the understanding of the population dynamics of the species, showing to be an excellent method for inter and intraspecific biological studies [12], [13].

Thus, the present study aimed to evaluate the reproductive performance of *D. hyalinata* from different food substrates, under laboratory conditions.

II. MATERIAL AND METHODS

Rearing of *D.hyalinata*. *Diaphaniahyalinata* caterpillars were obtained from the creation of a stock in the entomology sector of the Nucleus for Scientific and Technological Development in Phytosanitary Management of Pests and Diseases (NUDEMAFI) at the Center for Agricultural Sciences and Engineering at the Federal University of Espírito Santo (CCAE-UFES) and created according to the methodology proposed by Pratissoli et al. [7].

Bioassay. Caterpillars of 1st instar were collected from rearing stock and divided into groups being created on different food substrates for two generations, to avoid pre-imarginal conditioning. The substrates used as larval food were: pumpkin cultivar "Jacaré", cucumber cultivar “Japanese”, zucchini cultivar “Caserta” and an artificial diet recommended for the rearing of *Diatraeasaccharalis* (Fabr.) (Lepidoptera: Crambidae) proposed by Hensley and Hammond [14]. For each substrate, 200 *D. hyalinata* caterpillars were used. On natural substrates, the caterpillars were packed in pairs in gerbox-type plates (6 x 2 cm) with the substrate and a filter paper disc at the bottom. Cubes (2 cm edge) of pumpkin and zucchini and slices (1 cm thick) of cucumber were offered. The gerboxes were cleaned and the food replaced every two days. In the artificial diet, approximately 10 mL were deposited in test tubes (2.4 x 8.5 cm) and two *D. hyalinata* caterpillars were placed per tube. After larval development, pupae obtained from different substrates were placed in plastic boxes (25 x 15 x 10 cm) for 24 h, when they were sexed with the aid of a stereoscopic microscope.

With the emergence of adults, couples were formed, totaling 40 couples of each food substrate. These were individualized in cages made with PVC tubes (10 x 10 cm), paper towels, styrofoam, honey solution (food) 10% (w / v), each repetition consisting of a cage. The eggs were collected daily, removing the paper disc that was offered as an oviposition substrate. The number of eggs was counted on the paper disks and then stored in plastic boxes (25 x 15 x 10 cm) until the larvae completely hatched.

The observed parameters were: daily and total fecundity, female survival and longevity, incubation period of eggs and percentage of egg emergence. For the evaluation of the incubation period and percentage of emergence, a daily aliquot of eggs was removed from each couple/substrate that represented the respective repetitions.

Based on the daily fertility, survival and longevity values of the females, the life tables were calculated based on Silveira Neto et al. [15] and Townsend, Begon and Harper [16]. Using the values of age intervals (x), specific fertility (m_x), survival probability (l_x) of the life fertility tables, the fertility life table was made based on the Jackknife estimate [17], [18], the following parameters being determined: Net reproduction rate (R_o) (Eq.1); time interval between each generation (IMG) (Eq.2); innate capacity to increase in number (r_m) (Eq.3); finite rate of increase (λ) (Eq.4); and the time required for the population to double in number of individuals (Td) (Eq.5).

$$R_o = \sum (m_x \cdot l_x) \quad \text{(Eq. 1)}$$

$$IMG = \sum (m_x \cdot l_x \cdot x) / \sum (m_x \cdot l_x) \quad \text{(Eq. 2)}$$

$$r_m = \ln(R_o) / IMG \quad \text{(Eq. 3)}$$

$$\lambda = e^{r_m} \quad \text{(Eq. 4)}$$

$$Td = \ln(2) / r_m \quad \text{(Eq. 5)}$$

Data analysis. The daily fertility data obtained were subjected to non-linear regression analysis, while for the parameters total fertility, net reproduction rate, time interval between each generation, innate capacity to increase in number, reason finite increase and time required for the population to double in number of individuals the data were subjected to analysis of variance (ANOVA), and the means compared by the Tukey test at 5% probability when there is significance. All analyzes were processed in the computational environment R [19].

III. RESULTS

For all food substrates tested, the daily fecundities of *D. hyalinata* adjusted to the logistic model, with high levels of significance and coefficients of determination ($P < 0.0001$; $R^2 > 85.00\%$) (Figure 1). Regardless of the type of food substrate, daily fertilities showed similar behavior. The peak of daily oviposition occurred on the second day for all evaluated food substrates. The highest estimated daily fertility was observed for the cucumber substrate between the first and second days of oviposition. The zucchini substrate showed the lowest daily fertility value (Figure 1).

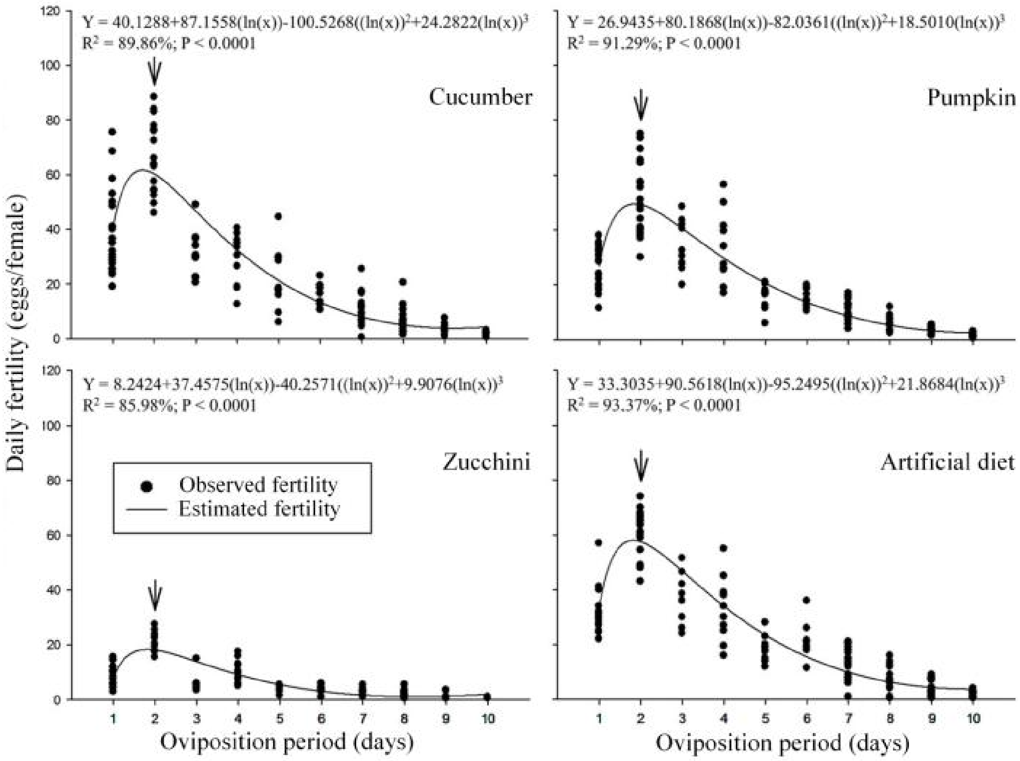

Fig. 1. Daily fecundity of adult female Diaphaniahyalinata from caterpillars reared on different food substrates (Temperature 25 ± 1 ° C, relative humidity 70 ± 10% and photophase 14 hours). The arrows indicate maximum daily fecundity.

The total fecundity of *D. hyalinata* varied significantly between the tested food substrates (F = 124.60; p <0.001) (Table 1). The highest average egg production per female was observed for adults from cucumber (175.7 eggs / female) and artificial diet (173.0 eggs/female) substrates. Again, zucchini food substrate showed the lowest egg production (86.4 eggs/female).

Table. 1: Total fecundity, incubation period and viability of Diaphaniahyalinata eggs from adults from caterpillars reared on different food substrates (Temperature 25 ± 1ºC, relative humidity 70 ± 10% and photophase 14 hours)

Food Substrates	**Total fecundity (eggs/female)**	**Incubation period (days)**	**Egg viability (%)**
Cucumber	175.7 a	4.0 a	89.7 a
Pumpkin	148.3 b	3.5 b	91.9 a
Zucchini	86.4 c	4.0 a	68.0 b
A. diet	173.0 a	4.0 a	90.7 a
CV (%) [1]	16.12	4.36	4.65
F	124.6	3.33	333.27
DFR [2]	156	156	156
p	<0.001	0.02	<0.001

Means followed by the same letter in the column do not differ by Tukey test (*p* = *.05*);

[1] Coefficient of variation;

[2] Degrees of freedom of the residue.

The incubation period for *D. hyalinata* eggs was influenced by food substrates (F = 3.33; p = 0.02) (Table 1). Despite being statistically different, the variation was small (3.5 to 4.0 days). The pumpkin substrate had the shortest incubation period, differing from the other food substrates.

The viability of *D. hyalinata* eggs were affected by the food substrates (F = 333.27; p <0.001), varying between 91.9 and 68.0% (Table 1). There was no significant

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difference between the cucumber, pumpkin and artificial diet substrates, with only a difference between these food substrates and the zucchini substrate (89.7; 91.9; 68.0 and 90.7%, respectively).

The net growth rate (R_o) suffered a significant variation (F = 277.61; p <0.001) (Table 2), ranging from 22.73 to 80.26 females/female. The food substrates cucumber, pumpkin and artificial diet differed only from the substrate zucchini.

Table. 2: Fertility life table parameters of Diaphaniahyalinata adults from caterpillars reared on different food substrates (Temperature 25 ± 1°C, relative humidity 70 ± 10% and photophase 14 hours)

Food Substrates	R_o	r_m	λ	*IMG*	*Td*
Cucumber	79.28 a	0.156 a	1.169 a	28.00 b	4.44 c
Pumpkin	74.28 a	0.143 b	1.154 b	30.14 a	4.85 b
Zucchini	22.73 b	0.104 c	1.110 c	29.97 a	6.65 a
A. diet	80.26 a	0.145 b	1.157 b	30.15 a	4.77 b
CV (%)	16.41	3.75	0.52	1.19	4.13
F	277.60	780.33	762.42	356.21	872.28
DFR	156	156	156	156	156
p	<0.001	<0.001	<0.001	<0.001	<0.001

Means followed by the same letter in the column do not differ by Tukey test ($p \leq .05$);

R_o = net rate of reproduction;

r_m = innate ability to increase in number;

λ = finite rate of increase;

IMG = time interval between each generation;

Td = time required for population to double in number of individuals.

The innate capacity to increase in number (r_m) was affected by food substrates (F = 780.33; p <0.001) (Table 2), with a variation between 0.10 and 0.15. Likewise, the finite rate of increase (λ) was also affected by the food substrates (F = 762.42; p <0.001) (Table 2). Regardless of the difference observed for the parameters r_m and λ, the values were positive, demonstrating that the food substrates provided an increase in the populations of *D. hyalinata*. The cucumber food substrate obtained the highest values of r_m (0.15) and λ (1.17 females/female/day).

The time interval between each generation (*IMG*) differed statistically between the tested substrates (*F = 356.21, p < .001*), but the variation was not significant (28.0 to 30.1 days) (Table 2). The time required for the population to double in number of individuals (*Td*) was significantly affected (*F = 872.28, p < .001*). For both *IMG* and *Td*, caterpillars fed with the cucumber substrate had the lowest values (28.0 and 4.4 days, respectively) (Table 2).

IV. DISCUSSION

Egg production by *D. hyalinata* was affected by the food. The daily fertility curves of the food substrates cucumber, pumpkin and artificial diet showed the highest angular and linear coefficients, which implies greater egg production when *D. hyalinata* is raised on these substrates. The reproductive performance of this insect may be directly influenced by the nutritional constitution of the food. Raw cucumber is, among the natural food substrates evaluated, the one with the lowest amounts of calories, proteins, lipids, carbohydrates, minerals and vitamins and the one with the highest percentage of moisture [20]. Thus, it can be assumed that due to the fact that this food does not have high nutritional values, as a measure of survival, *D. hyalinata* intensified its egg production in order to guarantee the survival of its descendants. Such behavior of presenting an expressive increase in order to prevail the species in unfavorable circumstances is considered by some authors as hormone [1], [21], [22]. Hormesis is based on providing favorable biological responses to organisms when exposed to stressors in small amounts or underdeveloped conditions, such as stimulatory effects. However, confirmation of this event requires detailed studies about chemical analysis for biological organisms with greater complexity, such as insects.

Another hypothesis to be considered is the presence of secondary metabolic agents that may be directly or indirectly affecting the acceptance and / or consumption of these natural substrates, such as phage stimulants and deterrents [23]–[26]. Plants have secondary metabolism which can promote a type of resistance in the plant that directly influences the insect's food and development, and in some cases can lead to death [24]–[28]. On the other hand, the fact that *D. hyalinata* occurs naturally in cucurbit cultures, makes it possible to assume that this insect is adapted to these secondary metabolic processes of these plants [7]. However, the occurrence / absence and, or difference in the concentration of these compounds can affect the behavior of the insect, the same eating habit [28].

Some reproductive and biological parameters, such as egg production and larval survival, can be influenced by physical or chemical differences in the food, or by the amount ingested in the larval phase [8], [27]. From this perspective, the influence of food acting on reducing the survival of caterpillars has already been reported for *D. hyalinata* when fed with the food substrate cucumber [7]. However, the planting around the zucchini has been suggested, since *Diaphania* spp. has a preference for this plant [5]. In this circumstance, this plant is used as trap plants.

The fact that the substrate zucchini had the lowest percentage of emergence implies the possibility that secondary substances are affecting the embryonic development of *D. hyalinata*. Regarding the possibility of transovarian action, some substances are easily translocated and accumulated, which implies the possibility of reducing the viability of eggs due to the death or malformation of embryos [26].

The net growth rate (R_o) was similar for the cucumber, pumpkin and artificial diet food substrates. When the immatures of *D. hyalinata* were fed with these substrates, adults could increase in number, on average, 77.94 times per generation, whereas when fed with the substrate zucchini this insect increases in number only by 22.73 times per generation. Although in the present study *D. hyalinata* was created on the respective food substrates for two generations in order to break some pre-marginal conditioning of these insects, the results for an artificial diet were expressive when compared to natural substrates. This possibly be associated with the absence of secondary substances in the artificial diet, a fact that, according to some authors, directly and indirectly influence the development and performance of insects [23], [24], [26]–[29].

The innate capacity to increase in number (r_m) the finite rate of increase (λ), although affected by the substrates, presented positive values which implies a greater speed of population growth [13], [15], [16]. For the cucumber substrate, the values of r_m and λ were higher, a fact that implies a greater population growth in *D. hyalinata* in less time. The values obtained for the time interval between each generation (*IMG*) and the time required for the population to double in number of individuals (*Td*) reinforce this result.

V. CONCLUSION

The food consumed during the immature phase of *D. hyalinata* affected the reproductive performance of adults;

The evaluated food substrates did not influence the oviposition behavior of *D. hyalinata*, but on the daily and total fecundity;

The zucchini food substrate negatively affected the reproductive performance of *D. hylinata*, while the cucumber, pumpkin and artificial dietary substrates were favorable for *D. hyalinata*, so they can be used as a food source in mass breeding of this lepidopteran.

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