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# Selection of an Artificial Diet for Laboratory Rearing of *Opogona sacchari* (Lepidoptera: Tineidae) (Bojer, 1856)

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**Abstract**
The banana moth *Opogona sacchari* (Bojer) (Lepidoptera: Tineidae) is a polyphagous pest that can cause serious damage, in particular to banana crops in southern Brazil. The insect is a quarantine pest in several countries, including Argentina, the main consumer market for bananas from southern Brazil. Little information is available about the biology and ecology of this moth, such as a suitable diet for laboratory rearing. In order to provide support for integrated pest management of the pest, this study furnished data for selecting two diets suitable for continuous laboratory rearing of *O. sacchari*, one based on dried beans, wheat germ, soy bran, brewer's yeast, and casein and another diet with wheat germ and casein as protein sources. With both diets, the viability of the egg-adult period exceeded 68%, with fertility over 338 eggs per female. A corrected biotic potential analysis gave similar values for the two diets.

## Introduction

In several countries, the banana moth *Opogona sacchari* (Bojer, 1856) (Lepidoptera: Tineidae) causes serious damage to agricultural and ornamental crops including banana, sugarcane, gladiolus, dahlias, yam, bamboo, and potato (Cintra 1975, (EPAGRI) Empresa de Pesquisa Agropecuária e Extensão Rural de Santa Catarina 2016, Raga 2005). Most articles dealing with *O. sacchari* have merely reported the first records in different countries and its host plants (Cintra 1975, Heppner *et al* 1987). Many countries have designated this insect as a quarantine pest (Milanez *et al* 2011, (EPPO) European and Mediterranean Plant Protection Organization 2015, (USDA) U.S. Department of Agriculture/APHIS Animal and Plant Health Inspection Service 2016), including Argentina, a major importer of bananas from Brazil ((SENASA) Servicio Nacional de Sanidad y Calidad Agroalimentaria 2016)). In the 1980s, *O. sacchari* caused economically significant losses in banana plantations in the state of São Paulo; in 2006, producers' shipments to other countries from the state of Santa Catarina were infested by this pest and rejected by agricultural inspectors (Cintra 1975, Milanez *et al* 2011).

Despite the importance of *O. sacchari*, few studies deal with aspects of its biology and ecology, information that is required for integrated pest management (IPM) programs. *Opogona sacchari* outbreaks can occur in periods without rain (low relative humidity) during the winter at the southern hemisphere (Gallo *et al* 2002). Gianotti *et al* (1977) studied the insect's larval period on an artificial diet based on bananas, and Bergmann *et al* (1995) described the larval and pupal periods of *O. sacchari* reared on an artificial diet based on beans, under controlled laboratory conditions. These authors did not investigate the suitability of the diets compared to other diets, for all developmental stages of *O. sacchari*.

The paucity of information is related to the lack of a proven suitable diet for continuous rearing of this insect in the laboratory. Insect laboratory rearing fed on an artificial diet can help to understand the pest biology and ecology and provide a continuous supply of insects for study, which can aid in developing control programs (Parra 2000).

Aiming to improve knowledge of the biological, ecological, and behavioral characteristics of *O. sacchari*, this study evaluated three artificial diets for rearing larvae of this species.

## Material and Methods

### *Biological assessment*

Larvae and pupae were collected in several banana plantations in the municipality of Luiz Alves, Santa Catarina, southern Brazil (S-26.713457, W-48.912200). After their emergence, adults were placed in cages made of polyvinyl chloride (PVC) pipe (20 cm height × 10 cm diameter) lined with bond paper as an oviposition substrate. Translucent plastic Petri dishes were used to close the ends of the pipe. A piece of bond paper folded in a fan was placed in each cage, to serve as an additional oviposition substrate. The adult moths were fed with a solution of 10% honey in water (Bergmann *et al* 1995), placed in a glass tube (4.5 cm height × 2 cm diameter), and covered with dental cotton to supply the solution by capillarity. The newly hatched larvae were "inoculated" in one of the three diets tested: Diet 1, based on the diet developed by Greene *et al* (1976); Diet 2, based on the diet of Hensley and Hammond (1968); and Diet 3, based on that of Bowling (1967) (see the modification in Table 1). These three diets were chosen because they all support a wide range of insects (Singh 1977). Each larva was placed in an individual glass vial (2.3 × 8 cm) containing approximately 20 mL of the diet and closed with hydrophobic cotton; 150 tubes were set up per diet. The tubes were kept sloping in wooden holders in an air-conditioned room at a temperature of 25 ± 2°C, RH 60 ± 10%, and photophase of 14 h. The tubes with larvae were inspected daily, in order to determine the length of the larval and prepupal-pupal periods. The viability of larvae was measured by dividing the initial number of larvae by the number of individuals that reached the prepupal stage. Similarly, the prepupal-pupal viability was measured by dividing the number of individuals that formed a cocoon by the number of adults that emerged. The prepupal and pupal periods were measured together because the larvae produce a silk cocoon that obscures the beginning of the pupal stage.

The adults were sexed using morphological characters on the end of their abdomen (Bergmann *et al* 1995). After the adults emerged, 20 couples were formed and placed in individual 10-cm white PVC cages, kept in an incubator programmed to maintain a constant 25 ± 1°C, RH 60 ± 10%, and photophase of 14 h to assess the adult longevity and fertility. The female weight was assessed from 20 individuals, from each diet, collected from the tubes and not used to form the couples.

At 3-day intervals, the bond paper lining the cage and the paper fan, now with eggs, were replaced, and the bond paper with eggs was stored in a freezer for subsequent counting. An exception was the eggs laid on the second day, which were immediately used to assess egg parameters. The viability and duration of eggs laid were assessed by dividing the eggs from the second laying day into batches of ten and placing them in Petri dishes (9.5 cm diameter) containing filter paper. Ten Petri dishes were prepared for each diet. The dishes were kept at a temperature of 25 ± 1°C, RH 60 ± 10%, and photophase of 14 h. Eggs that remained unhatched 6 days after the first eggs laid on the same day started to hatch were considered non-viable. Eggs from which the larvae hatched were considered viable, and the period between egg laying and larval hatching was considered the egg period.

Dr. Vitor Osmar Becker, a specialist on Lepidoptera, confirmed the taxonomic identity of the specimens.

### *Corrected biotic potential*

To compare the diets, the corrected biotic potential (CBP) was used as modified by Vendramim & Parra (1986), based on the biotic potential proposed by Chapman (1928). The CBP can be used as an alternative to life-table analyses; a higher CBP indicates a higher insect fitness. The CBP was calculated using the following equation:

$$CBP = Rp - Er$$

Where:
CBP = Corrected biotic potential;
Rp = Reproductive potential;
Er = Environmental resistance; which includes the abiotic factors and also the biotic factors of the environment (Chapman 1928).

Since the study was done in the laboratory, the environmental resistance Er was set equal to 0, because the laboratory conditions were considered optimal for this species; therefore, CBP = Rp:
with

$$Rp = (sr \times \Omega)^g$$

Where,

$$sr = \text{sex ratio}, \left(\frac{\text{number of females}}{\text{number of females} + \text{number of males}}\right)$$

$\Omega$ = number of offspring; in this case, the number of eggs (fertility) multiplied by the viability of *O. sacchari* from egg to

Table 1 Composition of the test diets: Diet 1, based on Greene *et al* (1976); Diet 2, based on Hensley and Hammond (1968); and Diet 3, based on Bowling (1967).

| | Diet 1 | | Diet 2 | | Diet 3 | |
|---|---|---|---|---|---|---|
| Components | Quantity | Proportion (%)[a] | Quantity | Proportion (%) | Quantity | Proportion (%) |
| Beans (*Carioca* variety) | 250 g | 5.08 | – | – | 100 | 11.35 |
| Wheat germ | 200 g | 4.07 | 108 | 2.93 | – | – |
| Soy bran | 100 g | 2.03[b] | – | – | – | – |
| Granulated sugar | – | – | 180 | 4.88[b] | – | – |
| Brewer's yeast | 125 g | 2.44b | – | – | 15 | 3.47 |
| Casein | 74 g | 2.03 | 108 | 2.93 | – | – |
| Vitamin solution[bc] | 20 mL | 0.61 | 36 | 0.98 | – | – |
| Choline chloride | – | – | 3.6 | 0.10 | – | – |
| Wesson's salt | – | – | 36 | 0.98 | – | – |
| Ascorbic acid | 12 g | 0.24 | 14.4 | 0.39 | 1.5 | 0.35 |
| Sorbic acid | 6 g | 0.12 | – | – | 0.5 | 0.11 |
| Methyl parahydroxybenzoate (nipagin) | 10 g | 0.20 | 5.4 | 0.15 | 1.0 | 0.22 |
| Tetracycline | 0.25 mg | 0.007 | 1 | 0.03 | – | – |
| Formaldehyde (37%)[b] | 12 mL | 0.24 | 1.8 | 0.05 | 1.0 | 0.86 |
| Carrageenan[b] (agar) | 46 g | 1.56 | 72 | 1.95 | 9.0 | 1.41 |
| Distilled water | 3400 mL | 81.35 | 3116 | 84.64 | 3750 | 82.22 |

[a] All concentrations were modified from the original diet.

[b] Ingredient modified from the original diet.

[c] Vitamin solution: dry ingredients (niacinamide 1.00 g; calcium pantothenate 1.00 g; riboflavin 0.50 g; thiamine 0.25 g; pyridoxine 0.25 g; folic acid 0.10 g; biotin 0.02 mg); wet ingredient: vitamin $B_{12}$ (1000 mg/mL) 2.00 mL. The dry and wet ingredients were mixed in 1 L distilled water.

adulthood, i.e., the number of eggs that generated adults. This differs from the proposal by Chapman (1928), who did not include the viability of the different life stages; *g* = number of generations during a period, in this case 1 year.

*Data analyses*

For the duration of the development times of eggs, larvae, and prepupae-pupae, as well as adult longevity, Kaplan-

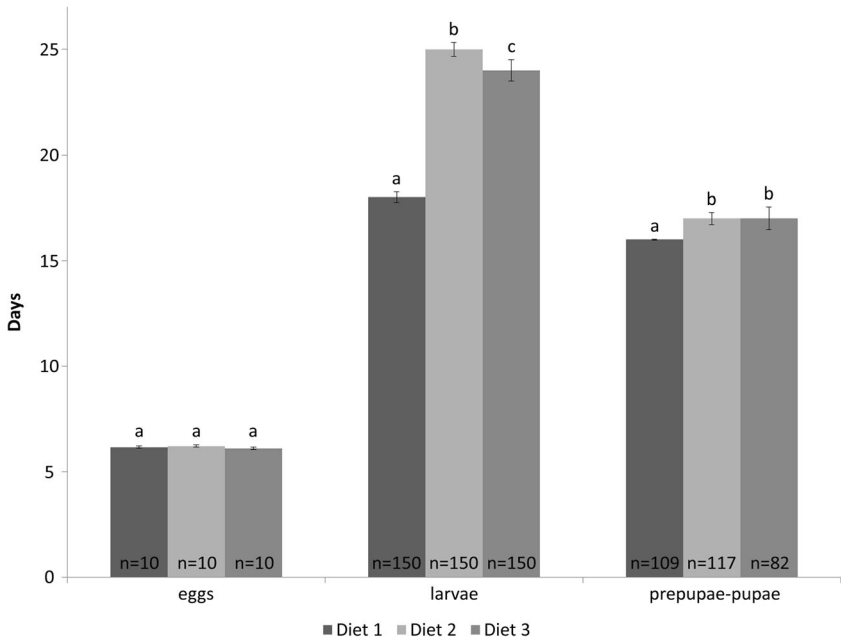


Fig 1 Duration of egg ± SE, larval ± SE, and prepupal/pupal ± SE periods of *Opogona sacchari* reared on three different diets. The eggs assessed came from insects reared on the three different diets. Temperature 25°C; RH 60%; photophase 14 h. Means followed by the same *letter* do not differ in the log-rank test ($p < 0.05$). Statistics: *egg* $\chi^2 = 0$; df = 2; $p = 0.98$; *larvae* $\chi^2 = 222$; df = 2; $p = 0$; *prepupae-pupae* $\chi^2 = 19.5$; df = 2; $p < 0.01$. Protein source: *Diet 1* bean, wheat germ, soy bran, brewer's yeast, casein; *Diet 2* wheat germ, casein; *Diet 3* bean, brewer's yeast.

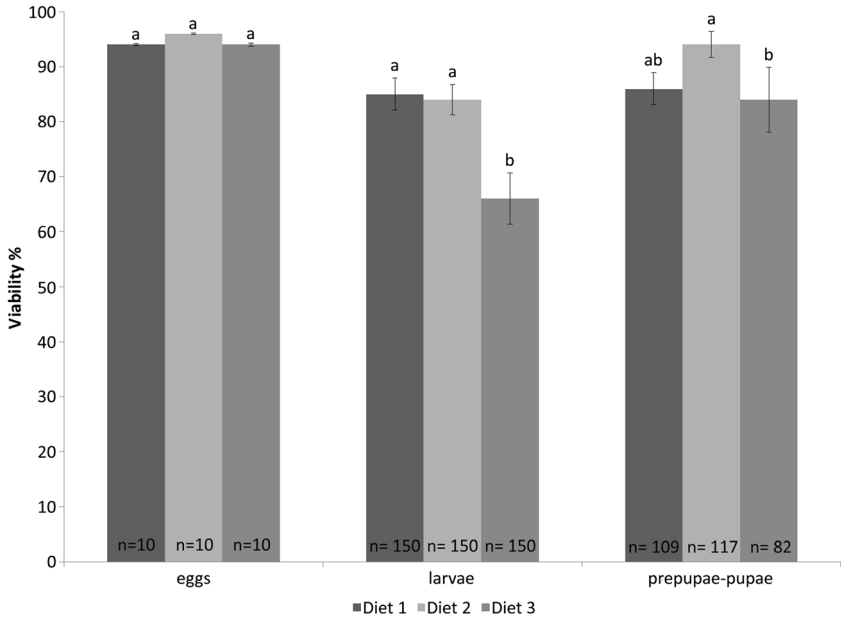


Fig 2 Viability of egg-adult ± SE period of *Opogona sacchari* reared on three different diets. Temperature 25°C; RH 60%; photophase 14 h. The eggs assessed came from insects reared on the three different diets. Means followed by the same *letter* do not differ statistically ($p < 0.05$). Statistics: *egg*, $F = 0.20$; df = 2; $p = 0.75$; *larvae* $\chi^2 = 10.6$; df = 2; $p < 0.01$; *prepupae-pupae* $\chi^2 = 3.7$; df = 2; $p < 0.01$. *Diet 1* bean, wheat germ, soybean bran, brewer's yeast, casein; *Diet 2* wheat germ, casein; *Diet 3* bean, brewer's yeast.

Meier estimators were obtained for each treatment (Therneau 2015). To test for significant differences, pairwise tests were performed using log-rank tests (Matthews & Farewell 2007). For the data on egg viability and sex ratio, quasi-binomial generalized linear models were fitted; and for the fecundity data, a quasi-Poisson generalized linear model was fitted. The effects were tested with an *F* test, because the data were overdispersed (Demétrio *et al* 2014). Binomial generalized linear models were fitted to the data for the viability during the larval and prepupal-pupal periods, and an analysis of deviance was carried out to assess the significance of the effects. An analysis of variance model was fitted to the data for female weight. When there were significant treatment effects ($p < 0.05$), multiple comparisons were performed by obtaining the 95% confidence intervals for the linear predictors for the generalized linear models; and for the analysis of variance model, a Tukey test was performed. Goodness-of-fit for all models was assessed using half-normal plots with a simulated envelope package (Moral *et al* 2016). A cluster analysis was carried out and a dendrogram was obtained to rank the diets according to total viability, fertility, sex ratio, and egg-adult period, using Euclidean distance. The corrected biotic potential was compared using 95% bootstrap confidence intervals from 10,000 simulations of the values for each treatment. Since values between $1.71^{14}$ and $1.19^{18}$ were found, they were transformed

Table 2 Total egg-adult period, viability, fecundity, and life span of *Opogona sacchari* fed on three different diets.

| Diet | Egg-adult period (days)* | Viability in egg-adult period (%)** | Fertility*** | Adult longevity (days)** | |
|---|---|---|---|---|---|
| | | | | ♂ | ♀ |
| Diet 1[a] | 40 ± 0.35 a | 68.5 ± 2.76 ab | 338.42 ± 39.83 | 21.0 ± 1.5 abA | 14.0 ± 0.6 B |
| Diet 2[b] | 48 ± 0.61 b | 75.8 ± 3.27 a | 364.00 ± 51.35 | 19.0 ± 0.6 bA | 14.0 ± 0.4 B |
| Diet 3[c] | 47 ± 0.88 b | 51.6 ± 7.30 b | 293.70 ± 50.64 | 23.5 ± 1.2 aA | 16.0 ± 0.6 B |
| Statistics | $\chi^2 = 37$; df = 2; $p < 0.01$ | F = 5.38; df = 2; $p = 0.01$ | F = 0.55; df = 2; $p = 0.57$ | $\chi^2 = 94.6$; df = 2; $p = 0$ | |

Temperature: 25 ± 1°C, RH: 60 ± 10%, photophase: 14 h.

*Means followed by the same letter do not differ in the Log-rank test ($p < 0.05$); **means followed by the same letter do not differ statistically in the Tukey test ($p < 0.05$); ***no statistical difference by quasi-Poisson GLM model.

[a] Protein source: bean, wheat germ, soy bran, brewer's yeast, casein.

[b] Protein source: wheat germ, casein.

[c] Bean, brewer's yeast.

Table 3 Corrected biotic potential (CBP) of *Opogona sacchari* on three different diets, for 365 days.

| Diet | Absolute value | Bootstrap CI* | Logarithm value | Logarithm bootstrap CI* |
|---|---|---|---|---|
| Diet 1[a] | $1.19^{18}$ | $9.9^{16}$: $1.16^{19}$ a | 18.08 | 17.00: 19.07 b |
| Diet 2[b] | $1.77^{16}$ | $1.95^{15}$: $1.11^{17}$ ab | 16.25 | 15.29: 17.05 ab |
| Diet 3[c] | $1.71^{14}$ | $7.2^{12}$: $2.48^{15}$ b | 14.25 | 12.86: 15.4 b |

CBP (absolute value) and logarithm value and the respective confidence intervals calculated by bootstrap.

* Confidence interval followed by the same letter do not differ (significancy 95% from 10,000 simulations).

[a] Protein source: dried beans, wheat germ, soy bran, brewer's yeast, casein.

[b] Protein source: wheat germ, casein.

[c] Protein source: dried beans, brewer's yeast.

to logarithm scale to better express the CBP. All analyses were carried out in R software (R Development Core Team 2015).

## Results and Discussion

None of the diets affected the duration and viability of *O. sacchari* eggs (Figs 1 and 2). Eggs laid by the females reared on the three different diets showed a similar embryonic period of around 6 days, with viability greater than 94%.

The diets affected the duration and viability of the larval and prepupal-pupal stages and consequently the total duration and survival (Figs 1 and 2, Table 2). For Diet 1, the larval period was 18 ± 0.24 days, at least 6 days shorter than the periods obtained with the other experimental diets. The percentages of larval survival were statistically similar (84 ± 3.13% and 85 ± 3.22%, respectively) for Diets 1 and 2, higher than the 66 ± 7.77% obtained with Diet 3 (Figs 1 and 2). Gianotti *et al* (1977) reported that the larval period of *O. sacchari* ranges from 42 to 70 days with a diet based on bananas, although they did not report the temperature at which the insects were reared. The larval period with a diet based on cornflakes was 58 days (Mourikis & Vassilaina-Alexopoulou *apud* Bergmann *et al* 1995). The data of Bergmann *et al* (1995) were similar to those obtained in the present study, i.e., the larval period lasted 24.19 days with a bean-based diet and a temperature of 25 ± 1°C, RH 70%, and photophase 12 h.

The prepupal-pupal period lasted at least 16 days, and again, Diet 1 provided the faster development. Diet 2 supported high viability for this phase of development, with an emergence rate higher than 94% (Figs 1 and 2). The period obtained by Bergmann *et al* (1995) was 11.24 days under similar environmental conditions. This 5-day difference in the duration of the pupal stage may be related to the initiation of observations at the prepupal stage, because of the obscuring silk cocoon (see Material and Methods).

The sex ratio did not vary ($\chi^2$ = 10.6, df = 2, $p$ = 0.76) and was consistently 0.5. The weight of females was similar for all diets ($F$ = 2.8, df = 2, $p$ = 0.07), ranging from 32 to 35 mg. Females had lower longevity than males under the rearing conditions. Fertility was also not affected by the different diets, averaging over 290 eggs per female ($\chi^2$ = 10.6, df = 2, $p$ = 0.76) (Table 2). Daumal & Boinel (1994), Chapman (1998) and Coelho & Parra (2013) mentioned that there is a direct relationship between female weight and egg production, which can explain the similar fertility obtained in this study.

Over a long rearing period (1 year), Diets 1 and 2 gave the best values of CBP (Table 3). Although Diet 2 provided an overall 75.8% viability, an acceptable value for insect laboratory rearing according to Singh (1983), the CBP value for a longer period was similar to that obtained with Diet 1. This relatively high CBP for insects reared with Diet 1, based on dried beans, wheat germ, soy bran, brewer's yeast, and casein, is explained by the shorter egg-adult period, which provides at least two more generations of *O. sacchari* per year under laboratory conditions. The dendrogram composed from all biological parameters measured shows the similarity between Diets 1 and 2, which concords with the results for the corrected biotic potential. The biological parameters of


Fig 3 Dendrogram of the biological characteristics (total viability, fertility, sex ratio, and egg-adult period) of *Opogona sacchari* reared on the different diets.

*O. sacchari* reared on Diets 1 and 2 were grouped into the same clade at a distance of 30%, separating them from Diet 3 (Fig 3).

Therefore, based on the biological results, diets based on dried beans, wheat germ, soy bran, brewer's yeast, and casein (Diet 1) and wheat germ and casein (Diet 2) can be used for rearing *O. sacchari*. These are the first artificial diets studied specifically for this target species, under controlled laboratory conditions.

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