Artificial diet for laboratory rearing of *Condylorrhiza vestigialis* (Guenée, 1854) (Lep.: Crambidae)

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ABSTRACT

The Brazilian Poplar Moth, *Condylorrhiza vestigialis* (Guenée), compromises the wood productivity of poplar trees (*Populus* sp.), mainly affecting the matchstick industry in southern Brazil. Considering the lack of information on rearing techniques for this insect, the objective of this study was to develop an artificial diet to rear *C. vestigialis* with biological characteristics similar to the wild insects. A properly diet will enable bio-ecological studies and biological control programs using the baculovirus *Condylorrhiza vestigialis* multiple nucleopolyhedrovirus (CvMNPV). To attain this objective, first, three artificial diets were tested. Only the diet based on corn, wheat germ and yeast as a protein source (Diet 3) was able to supply the nutritional requirements of the moth and support completion of its life cycle. In the second experiment, Diet 3 was compared to the natural diet of *C. vestigialis*. The artificial diet supported a viability of 81% of the eggs, while only 40% developed on the natural diet. Life-table data showed the same pattern: the net reproductive rate (*Ro*) of *C. vestigialis* reared on the artificial diet was 401.70, and on the natural diet was 151.22. The artificial diet is adequate for mass rearing of *C. vestigialis,* to support biological control programs using the baculovirus.

Key words: IPM, biological control, forest entomology, baculovirus.

INTRODUCTION

Poplar trees, *Populus* spp. (Salicales: Salicaceae), are widely distributed in the Northern Hemisphere, from the tropics to 68° N latitude. These trees are generally short-lived due to the incidence of insects and diseases, although their rapid growth often enables them to reach large sizes (Dickmann 2001). The wood is used in a large number of products (Balatinecz and Kretschmann 2001). In Brazil, it is widely grown in the Iguaçu River Basin (southern Paraná state), and provides raw material for the matchstick industry (Machado 2006).

The largest losses of productivity in poplars are caused mainly by the attack of the defoliating larva *Condylorrhiza vestigialis* (Guenée, 1854) (Lep.: Crambidae), known as the Brazilian Poplar Moth, which compromises productivity (Diodato 1999). Among the few available methods to control *C. vestigialis* is the baculovirus *Condylorrhiza vestigialis* multiple nucleopolyhedrovirus (CvMNPV), a highly specific and effective control for this pest (Castro et al. 2003).

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For large-scale production of the viral extract, large numbers of *C. vestigialis* larvae are needed. To be viable, this mass production must be conducted on an artificial diet (Castro et al. 2003, Corrêa 2006). Few studies have described standard specific diets for rearing *C. vestigialis*, which has hampered the adoption of biological control for the pest in large areas. Improved techniques to rear *C. vestigialis* larvae, using an artificial diet, will make it possible to move forward in bio-ecological studies of this pest, since it can be continuously reared in laboratory conditions, without relying on natural populations (Parra 2009), for this purpose the laboratory reared insect, should be similar to the wild, to avoid errors. This rearing method also will aid in large-scale production of the virus for biological-control programs.

Considering the sparse information about a specific artificial diet for rearing *C. vestigialis*, the present study aimed to develop an artificial diet for rearing a population with biological characteristics similar to the wild insects, compared to the natural food of the larvae.

MATERIALS AND METHODS

COMPARISON OF ARTIFICIAL DIETS FOR
Condylorrhiza vestigialis (GUENÉE, 1854)

Three artificial diets with different sources of protein were assessed (Table I), termed Diet 1 (casein-based), Diet 2 (casein and wheat germ-based) prepared with modifications of the diet proposed by Singh (1983), since this diet is used for different orders of insects; and Diet 3 (corn, wheat germ and yeast-based) proposed by Parra and Mihsfeldt (1992) for *Diatraea saccharalis* Fabricius (Lepidoptera: Crambidae). Each diet was prepared and transferred to 150 glass tubes (replication) plugged with hydrophilic cotton, previously sterilized in an oven at 160°C for 1 h. The tubes with the diet were allowed to dry for about 24 h to remove excess water. A newly

hatched larva was placed in each tube, using a soft brush. The tubes were kept in a climate-controlled room with a temperature of $25 \pm 2°C$, RH $60 \pm 10\%$ and photophase of 14 h.

From the 150 larvae in each treatment, 30 were randomly selected for measuring the width of the cephalic capsules, to determine the instars. These measurements were made daily, always at the same time, using a Wild® (Heerbrugg- Switzerland) digital meter linked to a stereomicroscope. On the 10th day, the phagostimulant effect of the artificial diets was evaluated, based on the start of feeding. The duration and viability of the embryonic, larval and pupal periods were also evaluated. The sex ratio was determined by the formula

$$sr = \frac{no.\ of\ females}{no.\ of\ females + no.\ of\ males}$$ based on sexual

dimorphism in the pupa, as described by Butt and Cantu (1962). Pupae 48 h old were weighed on an analytical balance, and were then placed in individual tubes until the adults emerged.

When the first adults emerged, 25 couples were combined and placed in individual PVC cages measuring 10 cm Ø (diameter) by 22 cm h and lined with paper sheets, and kept in a room with a temperature of $25 \pm 2°C$, RH $60 \pm 10\%$ and photophase of 14 h, feed with a 10% honey solution (in water), offered to the moths through cotton dental roll inserted into a plastic tube. The pre-oviposition period, daily egg laying capacity, egg viability, and lifespan of adults were evaluated. All experimental design was completely randomized.

COMPARISON OF NATURAL AND ARTIFICIAL
DIETS AS QUALITY-CONTROL CRITERION FOR *C. vestigialis*

The development of *C. vestigialis* was observed and compared on a natural diet (poplar sapling leaves) and Diet 3 (corn, wheat germ and yeast-based), which was determined to be appropriate in the first experiment (see results, Figs. 1 and 2). For the natural diet, young poplar leaves were washed

TABLE I
Composition of diets evaluated for the development of *Condylorrhiza vestigialis* in the laboratory.

Components	Diet 1		Diet 2		Diet 3	
	Amount	%	Amount	%	Amount	%
Casein	28 g	3.9	28 g	3.5	-	-
Wheat germ	-	-	24 g	3	60 g	5.7
Cornmeal	-	-	-	-	80 g	7.6
Yeast	-	-	-	-	30 g	2.9
Wheat germ oil	0.3 ml	0.04	-	-	-	-
Sunflower oil	-	-	-	-	4 g	0.4
Cellulose	10 g	1.4	80 g	9.9	-	-
Sucrose	24 g	3.4	24 g	3	26 g	2.5
Glucose	4 g	0.6	4 g	0.5	-	-
Vitamin solution*	16 ml	2.2	16 ml	2	16 ml	1.5
Wesson salts	8 g	1.1	8 g	1	7 g	0.7
Sorbic acid	0.2 g	0.03	0.2 g	0.02	-	-
Ascorbic acid	-	-	-	-	3 g	0.3
Linoleic acid	-	-	0.2 g	0.02	-	-
Benzoic acid	-	-	-	-	2 g	0.2
Cholesterin	-	-	0.4 g	0.05	-	-
Methyl parahydroxy-benzoate (Nipagin)	1.5 g	0.2	1.5 g	0.2	2 g	0.2
Tetracycline	0.1 g	0.01	0.1 g	0.01	-	-
Formaldehyde 37%	-	-	-	-	2 ml	0.2
Carrageenan	20 g	2.8	20 g	2.5	15 g	1.4
Distilled water	600 ml	84.3	600 ml	74.4	800 ml	76.4

*Vitamin solution extract from Parra (2007): Dry part (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); Liquid part (vitamin B12 (1000 mg / ml) 2.00 ml). Mix the dry and liquid parts in 1000 ml distilled water to prepare the vitamin solution.

with distilled water and transferred to 150 plastic Petri dishes (replication) with 5 cm Ø, containing moistened filter paper. A newly hatched larva was placed in each Petri dish, using a soft brush. Thirty of these larvae were randomly selected to measure the width of the cephalic capsules, which was done every day at the same time, using a Wild® digital meter linked to a stereomicroscope. The leaves were changed every two days until the larvae completed the 3rd instar, after which the leaves were changed daily, due to the high food consumption in the last instars.

Larvae of *C. vestigialis* were reared on Diet 3 according to the procedures described before. The biological parameters of duration and viability of eggs, larvae, pre-pupae and pupae, sex ratio, pupal weight, duration of the pre-oviposition period, fertility, and lifespan of adults were also analyzed as above. In addition, we determined the number of individuals with morphological deformations; and the number of mating's, assessed by counting the spermatophores inserted in the female copulation bursa (Milano et al. 2008). Based on egg-adult duration, viability, sex ratio and fertility, a life table

was constructed (Silveira Neto et al. 1976) and used to compare the two diets (natural and artificial). The entire experiment was conducted under controlled laboratory conditions (25 ± 2°C, RH 60 ± 10% and photophase of 14 h). All experimental design was completely randomized.

DATA ANALYSIS

The number of instars was defined according to Dyar's rule, using a graph of frequency distribution of the cephalic capsule widths and their respective linear regressions; an ANOVA of the linear regression was performed at the 5% significance level (Parra and Haddad 1989).

The data from laboratory rearing method were analyzed using generalized linear models (GLM) (Nelder and Wedderburn 1972); the number of adults with deformations, sex ratio, larval and pupal viability through a binomial distribution; and the data for fertility analysis, copulation number and pupal weight by quasi-Poisson distribution. The quasi-binomial distribution was used for analysis of the egg viability. The "F" value was calculated by ANOVA of the models. The goodness of fit was determined using a half-normal graph of probabilities with a simulation envelope (Demétrio and Hinde 1997, Hinde and Demétrio 1998). When there was a significant difference between treatments, multiple comparisons (Tukey test, $P < 0.05$) were done with the glht function from the multcomp package (Hothorn et al. 2008) with "p" adjusted by the single-step method. The data for durations of egg, larva, pre-pupa, pupa, pre-oviposition stage and longevity were analyzed using the survival curve, where the means and standard errors were calculated with the Kaplan-Meier estimator with the corresponding survival function; and the means compared by Log-rank test ($P < 0.05$). All analyses were performed using the R statistical program (The R Foundation for Statistical Computing; http://www.R-project.org). The parameters estimated from the life table were compared using a Jackknife analysis; each parameter was compared using the 95% confidence interval.

RESULTS AND DISCUSSION

COMPARISON OF ARTIFICIAL DIETS FOR
Condylorrhiza vestigialis (GUENÉE, 1854)

For Diet 1 (casein-based), the mortality recorded on the 10th day was 73.5%, and on the 15th day all larvae had died before reaching the 3rd instar, with a prolonged development period of up to 8 days in the 2nd instar. For Diet 2 (casein and wheat germ-based), mortality was 33.1% on the 10th day, and all the larvae had died by the 15th day, before reaching the 4th instar. Diet 3 (corn, wheat germ and yeast-based) was the only one where the larvae completed their development; on the 10th day the mortality rate was 2%; on the 13th day, pre-pupae were observed; and after 22 days the first adults emerged. Therefore, Diet 3 has a good phagostimulant action, with 98% of the larvae starting to feed, unlike Diets 1 and 2 where only 26.5% and 66.9% of the larvae started feeding, respectively.

On Diet 3, the larval stage had 5 instars (Fig. 1), with mean durations of 3, 2, 1.6, 2, and 3.5 days, respectively, for instars 1-5 (Fig. 1 and 2). The pre-pupal stage lasted 24 hours; the pupal stage averaged 7.8 days (Fig. 2). In this diet, the viability of the larval and pupal stages was 97.3% and 95.1% respectively. The percentage of adults emerged without deformation was 96.3%, with a sex ratio of 0.5 (Table II). The lifespans of males and females emerged from Diet 3 were 17.56 and 13.60 days, respectively, with a pre-oviposition period of 3.08 days. The female egg production capacity was 964.4 eggs, on average, and the viability of these eggs was 92.1%.

The egg viability on Diet 3 was 82.2%, higher than the 75% considered the minimum threshold required for an artificial diet to be considered adequate for insect laboratory rearing (Singh 1977).
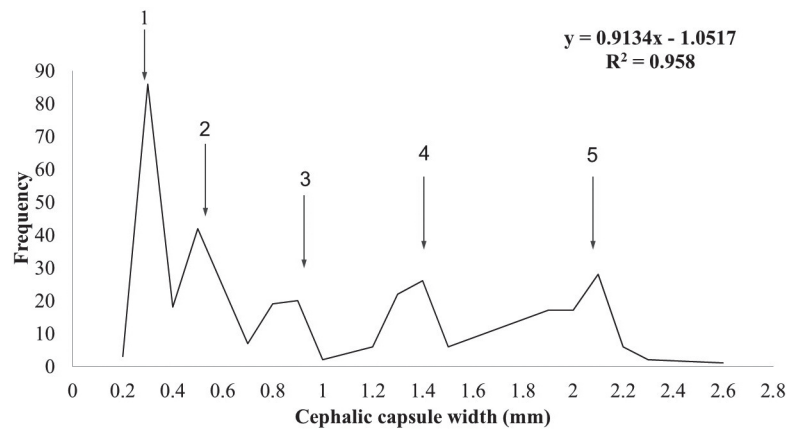
Figure 1 - Larval instar number of *Condylorrhiza vestigialis* and the cephalic-capsule widths on the corn, wheat germ and yeast-based Diet 3. Temperature 25 ± 2°C, RH 60 ± 10% and photophase of 14 h. Arrows indicate the instars.
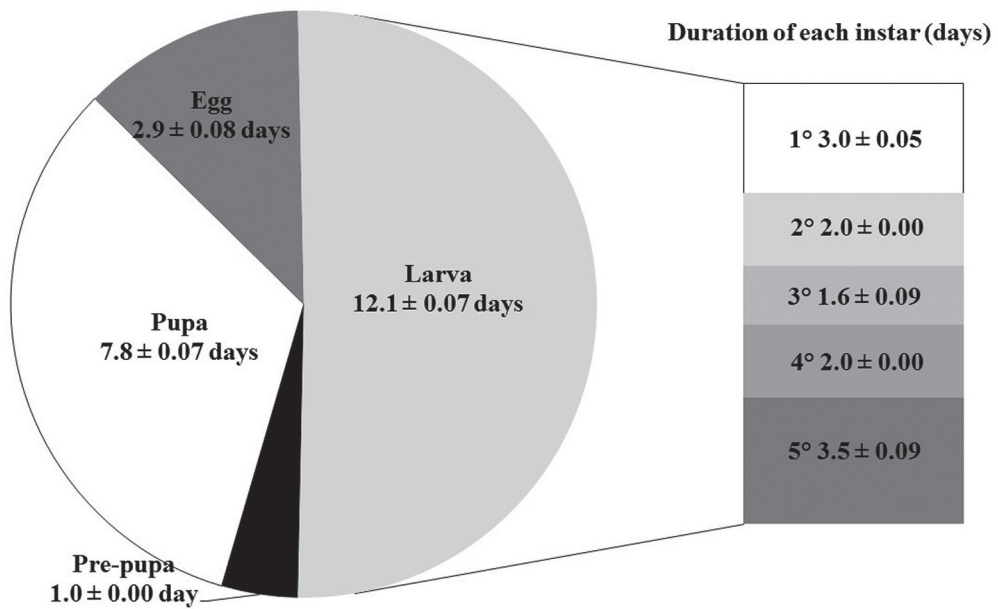

Figure 2 - Biological cycle for *Condylorrhiza vestigialis* (egg to adult, N=30) with the duration of each stage and the standard error of the mean, on Diet 3- corn, wheat germ and yeast-based. Temperature 25 ± 2°C, RH 60 ± 10% and photophase of 14 hours.

TABLE II

Condylorrhiza vestigialis pupal weight, pre-oviposition period, sex ratio, lifespan of females and males, fertility, and mean egg viability observed on Diet 3 (corn, wheat germ and yeast-based).

Pupal weight (mg)		Pre-oviposition period (days)	Sex ratio	Lifespan (days)		Fertility	Egg viability (%)
♀	♂			♀	♂		
126.8 ± 1.4 (N=72)	124.5 ± 1.3 (N=71)	3.08 ± 0.31 (N=30)	0.5 (N=143)	13.6 ± 0.4 (N=25)	17.6 ± 0.5 (N=25)	964.48 ± 83.06 (N=25)	92.05 ± 0.04 (N=25)

COMPARISON REARING ON NATURAL AND
ARTIFICIAL DIETS AS QUALITY-CONTROL
CRITERIA FOR *C. vestigialis*

The number of instars (five) was the same on the
natural and artificial diets (Fig. 3). Considering
that the number of instars is one parameter that
indicates nutritional adequacy (Parra et al. 2009),
Diet 3 demonstrated its nutritional quality for *C.
vestigialis*. The results for instar determination on
both diets had an R² coefficient higher than 95%
(Fig. 3). No difference was found for the embryonic
period ($\chi^2 = 1.7$; df = 1; $P = 0.19$) that was around
three days. The durations of the larval, pre-pupal
and pupal stages of *C. vestigialis* were shorter on
the natural than on the artificial diet (larva- $\chi^2 =
63.1$; df = 1; $P < 0.001$/pre-pupa- $\chi^2 = 63.6$; df = 1;
$P < 0.001$/pupa- $\chi^2 = 33.2$; df = 1; $P < 0.001$) (Fig.
4). The larval stage on the natural diet was about
two days shorter than on the artificial diet. The pre-
oviposition period was about four days without
difference between natural and artificial diet ($\chi^2 =
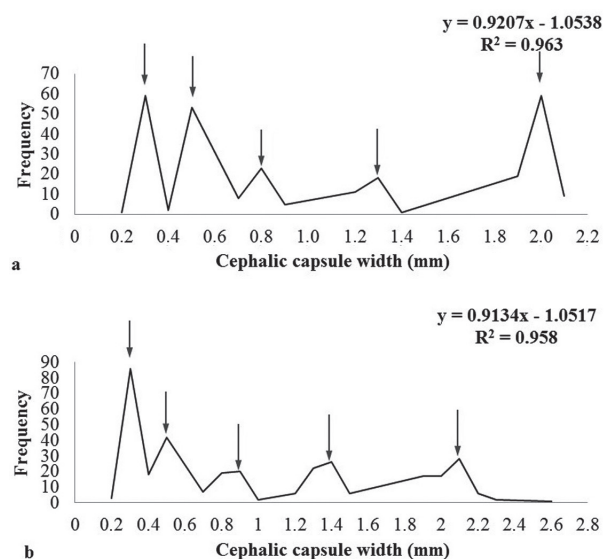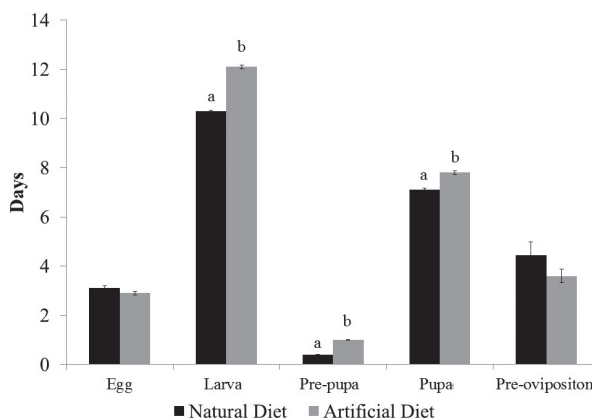1.9$; df = 1; $P = 0.16$) (Fig. 4).

Figure 4 - Duration of the development stages (egg, larva,
pre-pupa, pupa, N=30) and pre-oviposition of *Condylorrhiza
vestigialis* on natural (poplar leaves) and artificial (corn, wheat
germ and yeast-based) diets; temperature 25 ± 2 °C, RH 60
± 10% and photophase of 14 h. Means followed by the same
letter do not differ by the log-rank test ($P > 0.05$).

Adults from larvae fed the artificial diet
showed better reproductive performance. The
estimated number of mating's per female reared
on the artificial diet was higher than the females
on the natural diet, about three copulations more
per female (F = 25.49; df = 1; $P < 0.001$) (Table
III). No morphological deformities were recorded
in adults reared on natural diet while five adults
emerged with deformed (specially on wings) on
Diet 3 ($\chi^2 = 7.87$; df = 1; $P = 0.0087$). The egg
production of females emerged from larvae fed on
the artificial diet was 1.7 times higher compared to
females from the natural diet. The mean number of
eggs laid by females given the artificial diet was
965 (F = 14.59; df = 1; $P = 0.001$) (Table III). The
higher posture capacity may be a reflection of the
pupal weight, since pupae (female) from larvae
fed the artificial diet were nearly 20 mg heavier
than larvae fed the natural diet (F = 70.9; df = 1;
$P = 0.001$) (Table III). Daumal and Boinel (1994)
found a direct relationship between the weight of
insects and their fertility.

The adult lifespan was not affected by the diet
on which the insect was reared (Table III). On both
diets, males lived longer than females ($\chi^2 = 31.6$; df

Figure 3 - Larval instars of *Condylorrhiza vestigialis* and the
width of the cephalic capsule on natural diet (**a**) and artificial
diet (**b**). Temperature 25 ± 2°C, RH 60 ± 10% and photophase
of 14 h. Arrows indicate the instars.

= 1; $P < 0.01$). The shorter lifespan of females may be related to energy expenditure for reproduction. Coelho Jr and Parra (2013) observed the same pattern for *Anagasta kuehniella* (Zeller) lifespan.

The sex ratio of individuals from the natural diet was 0.53, and 0.49 for individuals reared on the artificial diet; these values are statistically similar ($F = 0.36$; df = 1; $P = 0.54$).

TABLE III
Female pupal weight, sex ratio, number of deformed adults, mean number of copulations, fertility, and lifespan of females and males of *Condylorrhiza vestigialis* reared on natural and artificial diets.

Treatments	Female pupal wt.[1] (mg)	Sex ratio	No. of deformed adults[1]	Mean no. of matings[1]*	Fertility[1]	Lifespan[2] (days) ♀	Lifespan[2] (days) ♂
Natural diet	107.4 ± 1.9 a (N=143)	0.53 ± 9.04 (N=136)	0 ± 0.00 a (N=138)	1.80 ± 0.34 a (1-6)** (N=25)	568.1 ± 63.2a (N=25)	13.9 ± 0.7 aA (N=25)	18.6 ± 1.3 aB (N=25)
Artificial diet 3	126.8 ± 1.4 b (N=134)	0.49 ± 0.04 (N=134)	5 ± 0.02 b (N=134)	4.76 ± 0.46 b (1-9)** (N=25)	964.5 ± 83.1b (N=25)	13.6 ± 0.4 aA (N=25)	17.6 ± 0.5 aB (N=25)

[1] Means followed by the same letter do not differ by Tukey test ($P < 0.05$).
[2] Means followed by the same lower-case letter in a column and the same capital letter on a line do not differ by log-rank test ($P < 0.05$).
* Determined by counting the number of spermatophores in the female copulation bursa. ** Min. and max. no. of spermatophores in the bursa.

The viability of the egg stage from females reared on the natural diet was less than 50%, lower than eggs laid by females fed the artificial diet (88%) ($F = 30.6$; df = 1; $P < 0.001$) (Table IV). Possibly, the high viability values obtained on artificial diet are related to the fact that artificial diets have more quantity and equilibrium of all nutrients required for the insect development. The viability of the other development stages was always higher than 90%, with no difference between the natural and artificial diets (larva- $\chi^2 = 3.53$; df = 1; $P = 0.06$/ pupa- $\chi^2 = 0.27$; df = 1; $P = 0.60$). The higher viability of the egg stage of *C. vestigialis* fed the artificial diet was one of the key factors indicating the suitability of this diet for the species, considering that the estimated total viability was 81%, higher than Singh (1977) recommendation (75%), while for the natural diet the total viability was only 40%.

These biological parameters produced a net reproductive rate (*Ro*), i.e. how much the population increases in each generation, of 151.22 on the natural diet and 401.70 on the artificial diet.

TABLE IV
Viability (%) of egg, larval, and pupal stages of *Condylorrhiza vestigialis* on natural and artificial diets.

Treatment	Egg (%)	Larvae (%)	Pupae (%)
Natural diet	46.0 ± 3.9 a[1] (N=25)	92.7 ± 2.1 (N=150)	96.4 ± 1.6 (N=145)
Artificial diet 3	88.0 ± 7.8 b (N=25)	97.3 ± 1.3 (N=150)	95.1 ± 1.8 (N= 139)

[1] Means followed by the same letter do not differ by the Tukey test ($P < 0.05$).

The finite rate of increase λ for the artificial diet was 1.25, higher than 1.21 for the natural diet; these values are statistically different (Table V). Accordingly, the artificial diet developed by Parra and Mihsfeldt (1992) for *D. saccharalis* rearing, with corn, wheat germ and yeast as the protein source, can replace the natural diet (poplar leaves) for mass production of *C. vestigialis* larvae, to support industrial production of the baculovirus to be used as a pest control in field conditions. Taking into account the higher *Ro* of the artificial diet and the viability above 75%, this contribution describes the first practical artificial diet for *C. vestigialis*.

TABLE V

Fertility life table of *Condylorrhiza vestigialis* from the parameters of moths reared on natural and artificial diets. Mean generation time (*T*), net reproductive rate (*Ro*), intrinsic rate of increase (*r_m*) and finite rate of increase (λ).

Treatments	Parameters			
	T^1	Ro^1	$r_m{}^1$	λ^1
Natural diet	26.06 a	151.22 a	0.192 a	1.21 a
Artificial diet 3	26.84 b	401.70 b	0.223 b	1.25 b

[1] Parameters followed by the same letter do not differ by the Jackknife test.

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