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BIOLOGY

Comparison of two artificial diets to rear *Elaphria agrotina*

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ABSTRACT: *Given the increasing importance of Elaphria agrotina (Guenée) (Lepidoptera: Noctuidae) in corn crops, especially in the Brazilian Savannah biome, the present research aimed to study its development and survival on Greene's and Poitout & Bues's artificial diets (25±1°C, 70±10% relative humidity [RH] and 12h photophase). Poitout & Bues's diet was more suitable than Greene's diet, providing higher survival percent (77.51% vs. 5.57%), lower development time (49.81 days vs. 55.24 days) and higher fecundity (167.65 vs. 84.9 eggs), respectively. All the caterpillars reared on Poitout & Bues's diet passed through six instars, while almost half of the larvae reared on Greene's diet went through one less instar stage. Regarding the main reproductive parameters, higher average time of generation (T) and lower values of net rates of reproduction (Ro), and increased (rm) intrinsic and finite reason of increase (λ), were observed for larvae reared on Greene's diet. Results presented in this study indicated that Poitout & Bues's diet is more suitable for maintenance of colonies of E. agrotina as compared to Greene's diet.*
Key words: *row crops, adequacy, biology, lepidopteran-pests, maintenance of colonies.*

Comparação de duas dietas artificiais para criação de *Elaphria agrotina*

RESUMO: *Com a crescente importância de Elaphria agrotina (Guenée) (Lepidoptera: Noctuidae) em cultivos de milho, especialmente no Bioma Cerrado, o presente trabalho objetivou comparar o seu desenvolvimento e sobrevivência em dieta artificial de Greene e Poitout & Bues (25±1°C, 70±10% umidade relativa [UR] e fotofase de 12h). A dieta de Poitout & Bues foi mais adequada que a dieta de Grenne, proporcionando maior sobrevivência (77.51% e 5.57%), menor tempo de desenvolvimento (49.81 dias e 55.24 dias) e maior fecundidade (167.65 ovos e 84.9 ovos), respectivamente. Todas as lagartas alimentadas com dieta de Poitout & Bues passaram por seis instares, enquanto que praticamente metade das provenientes da dieta de Greene passou por um instar a menos. Lagartas alimentadas com dieta de Greene, apresentaram maior valor do tempo médio de cada geração (T) e menores valores das taxas líquida de reprodução (Ro), intrínseca de aumento (rm) e razão finita diária de aumento (λ). Os resultados deste estudo indicam a recomendação da dieta de Poitout & Bues para manutenção de colônias e criação massal de E. agrotina quando comparada com a dieta de Greene.*
Palavras-chave: *culturas anuais, adequação, biologia, lepidópteros-praga, manutenção de colônias de insetos.*

INTRODUCTION

Elaphria agrotina (Guenée, 1852) (Lepidoptera: Noctuidae) is a small moth whose larvae have a feeding preference for dry plant tissues. This feeding behavior causes damage to several crops, with the biggest damage observed in grain crops, such corn. Larvae of this species feed on the cob base causing toppling. In soybean, the larvae feed on pods and dry seeds (SPECHT et al., 2014), while in pineapple, they feed on the base of the bracts (GALLO et al., 2002).

This species is distributed across all American continents, especially among the latitudes 30º North and South (e.g. TARRAGÓ et al., 1975, SPECHT et al., 2004; 2014; HEPPNER, 2007). Below parallel 30° South, the entomologist Dr. C.M. Biezanko classified this species as "common" and in 1951 reported an outbreak in Pelotas municipality, Rio Grande do Sul, Brazil (SPECHT et al., 2004). In the last crop seasons, farmers reported outbreaks of this species across several sites of the Brazilian Savannah. Samples collected mainly from corn and soybean fields were identified as *E. agrotina* (Laboratory of Entomology of Embrapa Cerrados). Samples are from the West of Bahia and MATOPIBA region (Maranhão, Tocantins, Piauí and Bahia States, Brazil).

Considering the increasing economic importance of *E. agrotina*, especially in Brazilian Savannah biome, the present research intended to evaluate the *E. agrotina* development under two

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artificial diets of broad use for Noctuidae rearing, aiming to establish colony for bioassays to study the ecology of this species.

MATERIALS AND METHODS

Elaphria agrotina adults (n=10) were collected from the Embrapa Cerrados Experimental Station (15° 36′ 7.1″ S; 47° 42′ 46.67″ W, 1000m, a.s.l.) using light trap. They were kept in metallic cages covered with 30cm^2 catching nets and an adult diet (105 honey in water solution). Corn leaves' bouquets were available to stimulate oviposition. A total of 1,348 eggs were collected from the cages with female adults and two colonies were established to test the artificial diets of Greene (GREENE et al., 1976) and Poitout & Bues (POITOUT & BUES 1974) (Table 1). Sixty hundred eighty larvae were used to establish each colony. Because this species does not present cannibalistic behavior, 10 larvae each were kept in 30mL container.

The comparative developmental study on the two different diets was performed with neonates from the second generation of each colony reared on Greene's and Poitout and Bues's artificial diets. The experiment was conducted under controlled conditions (25±1°C, 70±10% relative humidity [RH] and 12h of photophase), being 200 eggs for each diet, individualized on the first day after oviposition by cutting part of the substrate and placing it on damp filter papers, which were in turn placed in petri dishes until larval eclosion.

After larval eclosion, the neonates were individualized in plastic containers with transparent lids (30mL) containing 5mL of diet. The containers were observed daily to identify changes in the larval instars. After feeding was ceased and size reduction initiated (which is the characteristic of the pre-pupal period), larvae were transferred to other containers with autoclaved vermiculite to stimulate the larvae to build the pupal chamber.

Pupae were kept in the same container with damp vermiculite. On the second day after metamorphosis, gender identification was performed based on BUTT & CANTU (1962) and each pupa was weighted on semi analytical scale (precision of one-hundredth of a gram).

Adults from the same colony and those that emerged on the same day were coupled and kept in PVC cages (dimension: 16cm diameter x 21cm height). A total of 20 couples were formed with individuals from the Poitout & Bues's diet and ten couples were formed with individuals from the Greene's diet. Interior of each cage was lined with craft paper, which was replaced every 24h for egg counting.

The duration of each developmental stage (including the pre-pupal period) and the pupal weight of individuals that had undergone through five and

Table 1 - Percentage of components for the preparation of Greene et al. (1976) and Poitout & Bues (1974)'s artificial diets.

Component	Commercial brand	Greene et al. (1976)	Poitout & Bues (1974)
Distilled water (mL)	-	79.87	77.95
Bacteriological agar (g)	Vetec™	1.08	1.83
Bean - dry (g)	Kicaldo® - Branco, classe 1	5.87	-
Toasted wheat germ (g)	Frutos da Terra®	4.70	3.21
Soybean protein (g)	PTS Miúda Clara - Frutos da Terra®	2.35	-
Casein (g)	P.A. - Dinâmica®	1.75	-
Yeast extract (g)	Kasvi®	2.93	3.43
Ascorbic acid (g)	P.A. - Dinâmica®	0.28	0.45
Vitamin mixture (Vanderzant)	Manipulate by Farmacotécnica (*)	0.50	-
Tetracycline Hydrochloride	Medquímica®	0.01	-
Formalin (mL) – diluted on distilled water	P.A. Proquimios®	0.28	0.05
Methyl P-hydroxybenzoate (g)	P.A. - Dinâmica®	0.24	0.11
Sorbic acid (g)	P.A. - Dinâmica®	0.14	-
Cornflour (g)	Variety Embrapa BRS 4103**	-	12.84
Benzoic acid (g)	P.A. Vetec™	-	0.13

*each gram contends: Niacinamide (2.000mg), calcium pantothenate (2.000mg), Thiamine HCl (0.500mg), Riboflavin (1.000mg), Pyridoxine HCl (0.500mg), Folic acid (0.500mg), Biotin (0.040mg), Vitamin B12 (0.004mg), plus inert.
**tamanho máximo dos grãos 1.18mm.

six instars were compared with the Wilcoxon's non-parametrical test at 5% probability. The following reproductive parameters from adults were considered: fecundity (number of eggs per female), fertility (number of larvae per female), and pre- and post- oviposition periods (days). From the immature and adult data, fertility life tables were elaborated to represent graphically the probability of survival values (survival rate - lx) and the number of eggs oviposited per female (specific fertility - mx) by week. The average generation time (T), liquid reproduction rate (Ro), intrinsic increase rate (Rm), and finite increase ratio (λ) were estimated (SILVEIRA NETO et al., 1976).

RESULTS

The population larvae of *E. agrotina* reared on the Greene's diet declined and has died out completely by the third generation of the colony. Larvae from the colony reared on Poitout & Bues's diet continued for five generations, with increasing insect number, and no decrease in vitality.

The total survival of *E. agrotina* colony was higher under Poitout & Bues's diet. On Greene's diet, the survival was very low percent was note, especially in the larval and egg stages (Table 2).

Comparisons among insects reared on Greene's diet presented with significant differences between the female and male adults that went through the fifth and sixth instars. All larvae reared on Poitout & Bues's diet went through six instars. Nearly half of those reared on Greene's diets presented one less instar stage (Table 3).

Comparisons among caterpillars of the same sex that underwent six instars and were reared on Greene's and Poitout & Bues's presented with significant differences in all instars. Duration of egg and larval stages of *E. agrotina* was significantly higher for larvae reared on Greene's diet (Table 2), for individuals with both five and six instars (Table 3). There were significant differences in the same gender pupae duration on individuals who went through the same number of instars. Regarding pupal weight, significant differences were observed when comparing diets (Greene's - 0.09±0.05mm; Poitout & Bues's - 0.10±0.01mm) ($F=0.13$; $P<0.05$) (Table 3).

Longevity of adults from the colony reared on Poitout & Bues's diet was significantly higher than of those reared on Greene's diet (Table 2), which can be explained by the longer longevity of male adults (Table 4). Even though there was no significant difference on female adult' longevity, significant longer oviposition and pre-oviposition periods of female adults the colony reared on Poitout & Bues's diet. Fecundity and fertility followed the same pattern (Table 4). Insects reared on Greene's diet presented higher mean generation time (T) and lower reproduction rates (Ro), intrinsic increase rate (Rm) and finite increase ratio (λ) than did the insects from colony reared on Poitout & Bues's diet.

Figure 1 presents the differences between individuals from both colonies, considering their survival on immature stages and specific fertility. Overall, the oviposition peak occurred around the seventh day after eclosion.

DISCUSSION

Results of this study indicated the benefits of the Poitout & Bues's diet when establishing an *E. agrotina* colony in laboratory. Insects under Poitout & Bues's diet developed better and could be maintained through five generations. Results of

Table 2 - Viability (%) and duration (days) of the *Elaphria agrotina* stages, with larvae reared on Grenee's and Poitout & Bues's artificial diets (25±1ºC, 70±10% RH and 12 hour photophase).

Diet	------------------------------Grenee	------------------------------	----------------------------Poitout & Bues	--------------------------
Stage	Viability	Duration (X±SD)	Viability	Duration (X±SD)
Egg	30.98	3.79±0.46*	80.32	3.17±0.37
Larval	24.00	22.79±4.36*	97.00	19.00±2.16
Prepupal	100.00	4.18±2.01*	99.48	2.75±0.78
Pupal	75.00	9.83±2.75	100.00	8.97±1.86
Adult	-	14.65±2.27*	-	15.92±1.55
Overall	5.57	55.24	77.51	49.81

*Significance by the Wilcoxon test at the 5% probability level.

Table 3 - Duration (days) and pupal weight (mg) of larvae of *Elaphria agrotina* fed with Greene's and Poitout & Bues's artificial diets, considering the sex and number of larval instars (25 ± 1 ° C, 70 ± 10% RH and 12h photophase).

Diet	Greene's diet				Poitout & Bues's diet	
	Five instars		Six instars		Six instars	
Instar	Female (10)	Male (13)	Female (14)	Male (11)	Female (101)	Male (93)
Duration (Days)						
I	3.00±0.95	3.08±0.95	3.64±0.63^{+}	3.72±0.78^{+}	2.28±0.68^{+}	2.22±0.63^{+}
II	4.95±2.24	5.31±2.52	4.5±2.21^{+}	5.63±1.02^{+}	3.02±0.45^{+}	3.18±0.74^{+}
III	3.86±1.05	3.77±0.83	3.71±1.32^{+}	4.54±1.36^{+}	3.17±0.95^{+}	3.14±0.89^{+}
IV	3.43±1.44	3.77±1.58	4.07±1.59*	2.90±0.94^{*+}	3.88±1.22	3.75±1.11^{+}
V	4.86±3.12	5.08±3.96	4.64±1.39^{+}	4.00±1.34	3.55±1.11^{+}	3.41±0.84^{+}
VI	-	-	4.21±2.04*	5.45±1.57*	3.22±0.99^{+}	3.13±0.84^{+}
Total	19.00±3.97	21.00±5.01	24.78±2.08^{+}	25.81±2.13^{+}	19.15±2.33^{+}	18.84±1.94^{+}
Pre-pupae	2.90±1.59	3.76±2.12	5.57±1.98^{+}	4.09±1.30^{+}	2.66±0.38^{+}	2.83±0.77^{+}
Total	21.90±5.96	24.76±6.61	30.35±2.43^{+}	29.90±2.54^{+}	21.81±2.32^{+}	21.67±2.04^{+}
Pupae	8.5±3.25	8.90±4.15	10.16±0.57^{*+}	11.63±1.59^{*+}	8.84±1.66^{+}	9.15±2.04^{+}
Weight (mg)						
Pupae	0.09±0.01	0.08±0.01	0.09±0.06^{+}	0.08±0.02^{+}	0.10±0.01^{+}	0.10±0.01^{+}

*Significance by the Wilcoxon test at 5% probability level for male and female adults raised on the same artificial diet. $^{+}$Significance by the Wilcoxon test at 5% probability level for sixth instar individuals of the same sex raised on the Greene's and Poitout & Bues's diets.

differences between the two tested diets reinforce the importance of evaluating different artificial diets to optimize quality and vitality of the insects, allowing populations' maintenance for several generations and for across several biology and behaviour bioassays (PARRA, 2009; SCHNEIDER, 2009). One explanation for the success of the *E. agrotina* colony with the Poitout & Bues's diet is the fact that the larva of this species has a preference for dry tissues and cereals, especially corn (SPECHT et al., 2014), which is the main component of this diet (POITOUT & BUES, 1974).

However, even on Poitout & Bues's diet, the *E. agrotina* development was relatively slow as compared to that of *E. nucicolora* (Guenée) (approximately 60 days) reared at 21.11°C with

Table 4 - Longevity, the pre-, post- and oviposition periods (days); fecundity and fertility; and reproductive parameters of *Elaphria agrotina* whose larvae were reared on Greene's and Poitout & Bues's diet (25±1 °C, 70±10% RH and 12 hours of photophase).

Larval diet		Greene	Poitout & Bues
Sex	Parameter	Mean ±SD	Mean ± SD
Female	Longevity (days)	15.80±1.93	16.5±1.76
	Pre-oviposition (days)	4.00±0.0	4.3±0.57
	Oviposition (days)	4.60±3.10	9.8±1.10*
	Post-oviposition (days)	0.80±0.42	1.95±0.60*
	Fecundity (eggs)	84.90±137.66	167.65±79.84*
	Fertility (caterpillars)	26.30±32.86	134.65±64.25*
Male	Longevity (days)	13.51±1.96	15.35±1.08*
Reproductive parameters			
T	-	54.02	44.10
Ro	-	2.44	64.63
Rm	-	0.12	0.66
λ	-	1.12	1.94

*Significance by the Wilcoxon test at 5% probability level.

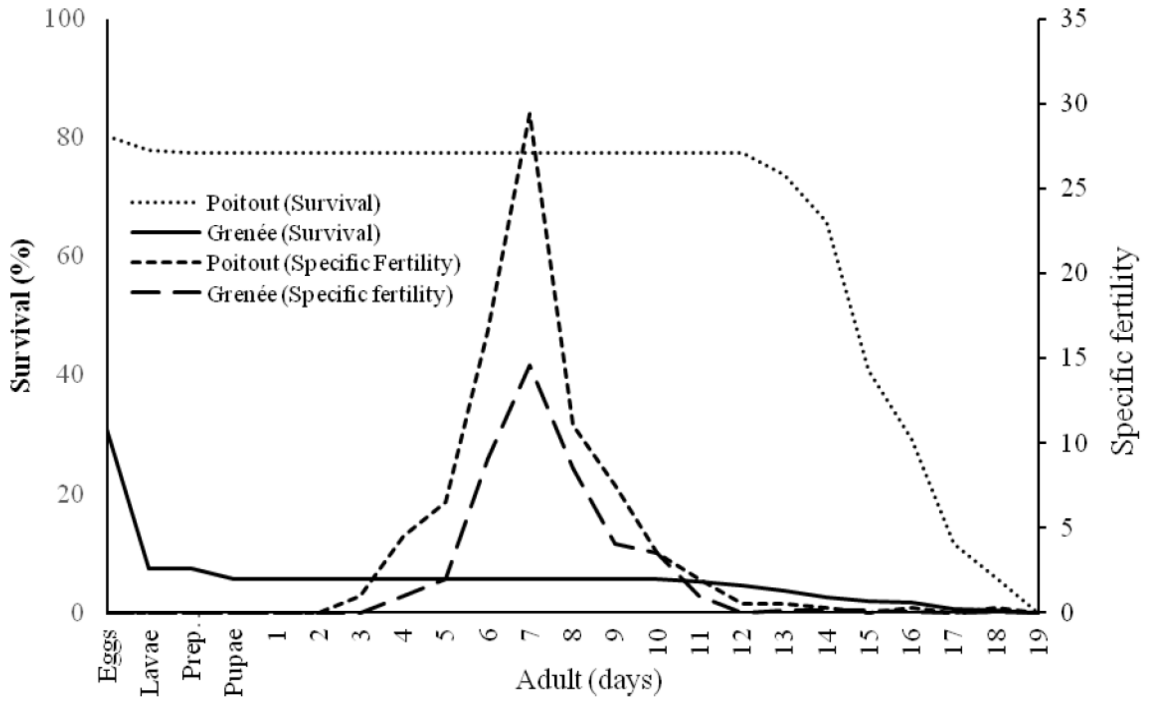

Figure 1 - Relationship between fertility (mx) and survival rate (lx) of *Elaphria agrotina* whose larvae where reared on Greene's and Poitout & Bues's artificial diets (25±1°C, 70±10% RH and 12h of photophase).

larvae reared on rape leaves (HABECK, 1965). The development time of this small species is even slower when compared to noctuids such as *Spodotera albula* (Walker), *S. eridania* (Stoll) and *S. frugiperda* (J.E. Smith) (BUSATO et al., 2006; MONTEZANO et al., 2013; 2014), which can complete their cycle in about a month and their pupae weight the double or triple when raised in similar temperature conditions.

An increase in the number of the instars is expected in lepidopterans (ESPERK et al., 2007) due to inadequacy of a diet. In the present study, *E. agrotina* colony reared on Greene's diet had only five instars. The same results were observed in some species, such as *Psacothea hilaris* (Pascoe) (Coleoptera: Cerambycidae), which presents a reduction of one instar stage under starvation condition (MUNYIRI et al., 2003). Reduction of one instar and the anticipated pupation with smaller size could benefit the species rather than extend its development in a hostile environment. *Elaphria agrotina* larvae has the same feeding behaviour as *P. hilaris*, which preferably feeds on dead tissues and survives close to dry plant tissues, such as straws in non-tillage systems, and on litter in a natural savannah (SPECHT et al., 2014).

Regarding the diets' composition, several aspects can influence the development of *E. agrotina*, including: water quantity, as the insect usually lives on less humid environments; fibers quantity, as chewing insects do not develop properly with low amount of fibers; reducing sugars, which is an important composition to the diet to provide energy; nitrogen derived from proteins, which are broken to obtain amino acids and smaller molecules that are used to build tissues and possess biological functions associated with vital activities; and lipids, which are used in cellular wall and membrane formation and production of hormones, transporters, energy and other molecules and structural components (COHEN, 2003; PARRA, 2009; SCHNEIDER, 2009).

The overall fecundity of *E. agrotina* was also reduced when compared to *E. nucicolora*, which can lay more than 900 eggs (HABECK, 1965) and other Noctuidae from the genus *Spodoptera* (e.g. BUSATO et al., 2006; MONTEZANO et al., 2013; 2014).

The colony of *E. agrotina* was kept in laboratory through five generations, without losing vitality. In addition, the larval survival was greater than 75%, which is considered adequate for the establishment of insect' colonies in laboratory (SINGH, 1983; SCHNEIDER, 2009). The conclusion is that Poitout & Bues's diet is adequate to maintain *E. agrotina* colony in laboratory.

CONCLUSION

Results presented in this study indicated that Poitout & Bues's artificial diet is more appropriate to the maintenance of *E. agrotina* in laboratory than Greene's diet, due to its suitability for mass rearing aiming to obtain insects for bioassays and for the maintenance of multiple generations in the laboratory.

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DECLARATION OF CONFLICTING INTERESTS

The authors declare no conflicting interests.

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