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Scientific Note

Biological parameters of tobacco budworm (Lepidoptera: Noctuidae) reared on corn cobs at different temperatures[1]

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ABSTRACT

The tobacco budworm *Chloridea* (*Heliothis*) *virescen*s (F.) (Lepidoptera: Noctuidae) has been recently reported in corn crops. However, there is no information regarding the biology of this species feeding on corn cobs. Additionally, the relationship between temperature and tobacco budworm feeding habits is important to project the adaptation of the insect in corn crops at different times and regions. This study aimed to investigate the development of *C. virescens* feeding on corn cobs, under controlled laboratory conditions, at three temperatures (25 ± 1 ºC, 28 ± 1 ºC and 31 ± 1 ºC). Although adult budworms were observed at all temperatures, the larval development period was reduced at 31 ± 1 ºC, resulting in a lower grain consumption. On the other hand, biological parameters such as larval and pupal viability, pupal weight and sex ratio did not vary with temperature. During the adult stage, a similar fecundity was recorded at all temperatures; however, the egg viability was zero. The optimal temperature for the *C. virescens* development was between 25 ± 1 ºC and 28 ± 1 ºC. The temperature of 31 ± 1 ºC affects the eggs development, preventing the next generation of tobacco budworms.

KEYWORDS: *Chloridea* (*Heliothis*) *virescens* (F.), Heliothinae, corn pest, global warming.

RESUMO

Parâmetros biológicos da lagarta-do-fumo (Lepidoptera: Noctuidae) alimentada com espigas de milho em diferentes temperaturas

A lagarta *Chloridea* (*Heliothis*) *virescens* (F.) (Lepidoptera: Noctuidae) tem sido registrada recentemente em lavouras de milho. Apesar disso, não existem informações sobre a biologia dessa espécie alimentando-se de espigas de milho. Adicionalmente, a relação entre os hábitos alimentares da lagarta-do-fumo e a temperatura é importante para a projeção da adaptação da praga em lavouras de milho, em diferentes épocas e regiões. Objetivou-se investigar o desenvolvimento de *C. virescens* em espigas de milho, em condições controladas de laboratório, sob três temperaturas (25 ± 1 ºC, 28 ± 1 ºC e 31 ± 1 ºC). Embora os insetos tenham atingido a fase adulta em todas as temperaturas, a fase larval foi reduzida a 31 ± 1 ºC, resultando em menor consumo de grãos. Por outro lado, parâmetros biológicos como viabilidade larval e pupal, peso de pupa e razão sexual não foram influenciados pela temperatura. Durante a fase adulta, a fecundidade foi similar para todas as temperaturas; no entanto, a viabilidade de ovos foi nula. A temperatura adequada para o desenvolvimento de *C.virescens* foi entre 25 ± 1 ºC e 28 ± 1 ºC. A temperatura de 31 ± 1 ºC afeta o desenvolvimento dos ovos, não permitindo uma nova geração da praga.

PALAVRAS-CHAVE: *Chloridea* (*Heliothis*) *virescens* (F.), Heliothinae, praga do milho, aquecimento global.

The tobacco budworm [*Chloridea* (= *Heliothis*) *virescens* (Fabricius, 1777) (Lepidoptera: Noctuidae)] infests cotton crops worldwide (Fleming et al. 2018, Allen et al. 2019, Krempl et al. 2021); however, it has also been reported to infest other plant species, including white clover, upland cotton, garbanzo bean, velvetleaf (Blanco et al. 2007, Blanco et al. 2008), grapevine (Ventura et al. 2015) and soybean (Eduardo et al. 2020, Baldwin et al. 2021, Zilnik & Burrack 2021). A previous study suggests that leguminous plants and members of the Poaceae family are also susceptible to this pest (Sánchez-Vega et al. 2019). In addition to its polyphagous habit, the *C. virescens* management is hampered in diversified landscapes, because "green belts" maintain pest populations at local or regional scale.

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In Brazil, there has been an increase in the number of corn fields (Conab 2021), as the crop is cultivated at least for two seasons, depending on the region. However, the increased corn cultivation in the same region poses serious issues, such as increased use of pesticides and consequently secondary pest outbreaks (Dutcher 2007). In this sense, *C. virescens* may increase its importance in corn fields.

Recently, *C. virescens* has also been reported in Mexican corn fields (Sánchez-Vega et al. 2019). Despite the potential threat to plant reproductive structures by the tobacco budworm (Bortolotto et al. 2014, Ventura et al. 2015, Baldwin et al. 2021), little information is available on the *C. virescens* development in corn cobs, what makes developing pest management strategies difficult.

At present, there has been considerable discussion on climate change and its impact on crop pests (Skendžic et al. 2021). Therefore, it is important to examine the possible effects of temperature on insect biology, in addition to host quality, while developing pest management strategies (Clissold & Simpson 2015). Although some studies have focused on the *C. virescens* development at different temperatures, they have not evaluated the relationship between temperature and consumption of natural diets in this species (Fye & McAda 1972, Butler Junior & Hamilton 1976, Eger et al. 1982, Gulzar et al. 2012, Ghazanfar et al. 2020).

Understanding the relationship between temperature and consumption of fresh corn cobs on the *C. virescens* biology becomes necessary, as the insect development (Abarca & Spahn 2021) and the potential damage caused by the pest are strongly dependent on both temperature and diet. Additionally, this knowledge will contribute to understand the pest development on the reproductive structures of corn and its possible implications for pest management in different regions and seasons. Therefore, this study aimed to evaluate the biological parameters of *C. virescens* reared on fresh corn cobs at different temperatures.

The study was carried out under laboratory conditions at the Embrapa Soja, in Londrina, Paraná State, Brazil, where the insects were reared in a BOD-type climate chamber set [temperature: 25 ± 2 ºC; relative humidity: 70 ± 10 %; photoperiod: 14/10 h (light/dark)] and on an artificial diet (Greene et al. 1976). After the emergence of adults, a 5 % honey solution was added to the diet to promote reproduction, and the obtained eggs were used for assessing biological parameters.

Seeds of the 2B688 corn cultivar (expected to be a strain that could be cultivated during the second harvest in the Brazilian southern region) were sown in pots (5 seeds pot^{-1}) containing 8 L of sterilized soil and were then transferred to a greenhouse. A total of 75 plants were cultivated for this study. After the corn had sprouted, the plants were thinned out, keeping only one plant per pot. The seeds were sown over a period of three weeks (25 pots each week) to ensure the availability of corn cobs for different developmental stages of the pest.

Substrate irrigation was performed before and immediately after sowing to ensure uniformity in the soil moisture content in all pots. After the seedling emergence (Magalhães & Durães 2006), irrigation was performed daily. Chemical fertilizers, including nitrogen [$(NH_4)_2SO_4$], phosphorus (P_2O_5) and potassium (K_2O), were applied (Fancelli & Dourado-Neto 2000). Herbicides, fungicides and insecticides were not applied throughout the crop cycle, to avoid their interference with the results.

Corn cobs were collected at the R3 phenological stage (milky grain) (Magalhães & Durães 2006) and had their bracts removed. Thereafter, the corn cobs were cut into pieces and fed *ad libitum* to newly hatched or 24 h-old larvae placed in paraffin cups. The experimental design was completely randomized, with 10 replicates (n = 7 larvae/replicate). The paraffin cups with larvae feeding on corn cobs were incubated at three temperatures (treatments) (25 ± 1 ºC, 28 ± 1 ºC and 31 ± 1 ºC), under controlled conditions [relative humidity: 70 ± 10 %; photoperiod: 14/10 h (light/dark)], in a BOD-type climate chamber set. Fifth-instar larvae were used to determine the grain consumption, according to a protocol modified from Bortolotto et al. (2015). The corn cob pieces were weighed using an analytical scale (Dalmaq Balanças, Shymazdu AY 220, Uberaba - MG), before offering them to the insects, and replaced daily. The corn cob pieces were collected daily and kept under the same experimental conditions to naturally restore the moisture lost during the assessment. For this, the corn cob pieces in the absence of larvae (control) were also weighed as aforementioned. The average moisture loss was approximately 5 %, and the data were corrected prior to statistical analyses. During each larvae assessment, the insects were touched with a brush tip, and those that failed to move were identified as dead. The following parameters were determined: larval viability (%) and larval development period (days).

Approximately 24 h after pupation, the pupae were weighed using an analytical scale. Individual pupae were observed to determine the duration of pupal period (days), sex ratio (days) and pupal viability (%). The pupal viability was quantified according to adult emergence. After emergence, 24 h-old adults of opposite sexes were paired and fed with a 5 % honey solution to improve longevity and promote reproduction. The pairs were placed in polyvinyl chloride tubes, which were covered on the inside with bond paper for oviposition. The eggs were collected daily and maintained inside a Gerbox in a BOD-type climate chamber set. After a female insect had died, the fecundity (total number of eggs) and egg viability (%) were evaluated. The viability was estimated by quantifying the hatched eggs.

Table 1. Biological parameters of the larval stage of *Chloridea virescens* fed with fresh corn grains at different temperatures (relative humidity: 70 ± 10 %; photoperiod: 14 h).

Temperature	Biological parameters		
	Duration (days)	Viability (%)[2]	Consume (g)[1]
25 ± 1 ºC	13.45 ± 0.56 a	67.14 ± 0.00	3.34 ± 0.29 a
28 ± 1 ºC	12.63 ± 0.32 a	54.29 ± 0.06	3.01 ± 0.30 ab
31 ± 1 ºC	10.47 ± 0.35 b	65.71 ± 0.05	2.03 ± 0.33 b
CV (%)	3.54	13.80	28.97
P	0.0001	0.3015	0.0191
F	15.16	1.28	4.97
DF_{model}	2	2	2
$DF_{residue}$	18	18	18

[1] Mean ± SEM followed by the same letter in the column do not differ by the Tukey test at 5 % of probability. [2] The results followed original analysis carried out with the data transformed into arcsin √(x/100).

The data were subjected to exploratory analyses, to assess the assumptions of normality of residuals (Shapiro & Wilk 1965), homogeneity of variance of treatments and additivity of the model (Burr & Foster 1972), prior to the analysis of variance. All the data met the parametric assumptions. Only the percentage data was transformed to arcsin $\sqrt{(x/100)}$ to enable the data analysis. Means were compared using the Tukey test ($p \leq 0.05$) (SAS Institute 2001).

The *Chloridea virescens* completed its life cycle on fresh corn cobs at all temperatures. However, the larval phase was reduced at 31 ± 1 ºC, when compared to 25 ± 1 ºC and 28 ± 1 ºC. Grain consumption was also reduced at the highest temperature (31 ± 1 ºC) (Table 1). Regarding the larval viability, this parameter was similar for the three temperatures ($p > 0.05$) (Table 1).

Regarding the pupal parameters, the pupal weight, pupal viability and sexual ratio were not affected by the tested temperatures ($p > 0.05$) (Table 2). On the other hand, the pupa-adult period was shorter at 31 ± 1 ºC than at 25 ± 1 ºC and 28 ± 1 ºC (Table 2). In general, the female fecundity did not vary with temperature (Table 3). However, egg viability was reported only at 25 ± 1 ºC and 28 ± 1 ºC, and was 0 % at 31 ± 1 ºC (Table 3).

To the best of our knowledge, this is the first study to report the *C. virescens* biology on corn cobs. Our findings suggest that the potential damage caused by the pest may be affected by higher temperatures. This observation supports a recent report that identified *C. virescens* damaging the reproductive structures of corn plants in Mexico (Sánchez-Vega et al. 2019). However, it is important to consider that the ears are protected by corn husks in the field,

Table 2. Biological parameters of *Chloridea virescens* pupal stage fed with fresh corn grains at different temperatures (relative humidity: 70 ± 10 %; photoperiod: 14 h).

Temperature	Pupal weight (g)[1]	Pupa-adult period (days)[1]	Viability (%)[1;2]	Sex ratio[1]
25 ± 1 ºC	0.21 ± 0.00	15.33 ± 0.16 a	75.71 ± 0.07	0.38 ± 0.11
28 ± 1 ºC	0.20 ± 0.01	14.19 ± 0.26 b	78.00 ± 0.08	0.50 ± 0.11
31 ± 1 ºC	0.23 ± 0.01	7.45 ± 0.21 c	89.29 ± 0.05	0.41 ± 0.08
CV (%)	8.97	4.56	11.58	63.41
P	0.0722	< 0.0001	0.3382	0.6879
F	3.05	402.01	1.15	0.38
DF_{model}	2	2	2	2
$DF_{residue}$	18	18	18	18

[1] Mean ± SEM followed by the same letter in the column do not differ by the Tukey test at 5 % of probability. [2] The results followed original analysis carried out with the data transformed into arcsin √(x/100).

Table 3. Biological parameters of adult *Chloridea virescens* fed with fresh corn grains at different temperatures (relative humidity: 70 ± 10 %; photoperiod: 14 h).

Temperature	Fecundity[1]	Viability[1;2]
25 ± 1 ºC	261.11 ± 16.43	47.33 ± 0.08 a
28 ± 1 ºC	250.44 ± 21.21	28.78 ± 0.03 a
31 ± 1 ºC	298.89 ± 20.18	0.00 ± 0.00 b
CV (%)	68.69	59.68
P	0.1022	< 0.0001
F	2.51	20.03
DF_{model}	2	2
$DF_{residue}$	24	24

[1] Mean ± SEM followed by the same letter in the column do not differ by the Tukey test at 5 % of probability. [2] The results followed original analysis carried out with the data transformed into arcsin √(x/100).

unlike in the present study. This fact is relevant to justify the low occurrence of the species on corn cobs in corn fields.

Although there have been no studies evaluating the *C. virescens* biology on Poaceae members, some studies have demonstrated the damage caused by *C. virescens* to plant species from other families. For example, Bortolotto et al. (2014) evaluated the development of *C. virescens* on soybean leaves and pods and recorded a larval phase of approximately 17 days and larval viability of 37 %. These results are higher to the *C. virescens* development on cotton bolls (larval phase: 16 days; larval viability: 20-30 %) (Tollefson 1979). However, the present study reported a shorter life cycle of approximately 13 days, with viability approximately twice and three times as that previously reported for soybean and cotton bolls, respectively. This suggests that fresh corn cobs are nutritionally more suitable for larval development than cotton bolls and soybean (leaves and pods), indicating the potential threat to corn fields.

Although no studies have estimated the damage caused by *C. virescens* in corn fields, other Heliothinae species, such as *Helicoverpa armigera*, have been reported to reduce the weight of corn cobs by up to 14 % (Keszthelyi et al. 2011). Therefore, to avoid the underestimation of its importance in corn cultivation, it is necessary to study the *C. virescens* biology because of its morphological similarity to other Heliothinae species that attack corn cobs, including *H. zea* (Matrangolo et al. 1998) and *H. armigera* (Fefelova & Frolov 2008). Despite the lack of scientific investigation, the grain consumption by *C. virescens* (3.4 g) was twice for corn cobs than for soybean pods (Bortolotto et al. 2014), thereby suggesting its potential for corn damage. In addition to direct damage, which affects yield, it is important to consider the mode of infection of phytopathogens (Pruter et al. 2019) that decrease the corn production.

Similarly to the findings of a previous study (Souza et al. 2001), a shorter larval development period and a reduction in grain consumption at 31 ºC were observed. This observation may be justified by the hypothesis that a higher temperature favors the development of the insect in the larval phase; however, it affects the viability of moth eggs. Although the present study did not determine the nutritional quality of corn cobs, previous studies have demonstrated that high temperatures may affect food quality. For example, high temperatures increase the hardness and indigestibility of food, especially for chewing insects (Clissold & Simpson 2015). However, as the larval viability was not affected by the highest temperature (31 ± 1 ºC) in the present study, this hypothesis is inconsistent to our results.

The fecundity was not affected by the temperatures, despite the 0 % for egg viability at 31 ºC. This observation may be explained by the egg desiccation. A previous study (Gulzar et al. 2012) demonstrated a lower fecundity (33-59 %) in *C. virescens* reared on cotton bolls at 30 ºC. This evidence indicates that it is not only the direct effects of temperature on *C. virescens*, but also the effects of temperature on food quality that affect the pest biology. This is reinforced by the fact that the nutritional balance between carbohydrates and proteins is affected by temperature (Clissold & Simpson 2015).

Therefore, the present study helps in predicting the probability of *C. virescens* occurrence, which will assist in monitoring *C. virescens* populations as well as in the development of control management strategies aimed at this species.

The present study showed that 25 ± 1 ºC and 28 ± 1 ºC are optimal temperatures for the survival and development of *C. virescens*. At the highest temperature (31 ºC), a reduction was observed for the larval phase, grain consumption and pupa-adult period. On the other hand, the temperature affects the egg viability and prevents the development of another generation. Further investigation is necessary to assess the pest performance on other corn cultivars.

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