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CROP PROTECTION

Effects of dietary palm oil on nutritional indices of *Diatraea saccharalis* (Lepidoptera: Crambidae) for *Cotesia flavipes* (Hymenoptera: Braconidae) production

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ABSTRACT. *Cotesia flavipes* parasitizes *Diatraea saccharalis*, a pest that causes major losses to sugarcane production. The mass production of *C. flavipes* and its subsequent release onto sugarcane is one form of biological control of *D. saccharalis*. An essential factor for successfully fighting this pest is the development of host diets that can increase the efficiency of *C. flavipes* production. Palm oil contains saturated fatty acids, unsaturated fatty acids, and polyunsaturated linoleic acid. However, little is known about the effects of palm oil on *C. flavipes* production in the laboratory. Different concentrations of palm oil were added to the *D. saccharalis* diet and its nutritional indices were analyzed. Subsequently, the production and sex ratio of *C. flavipes* were determined. Our results indicated that the addition of higher palm oil concentrations to an artificial diet generated a high relative consumption rate (RCR) in *D. saccharalis*, and consequently a high relative metabolic rate (RMR), which resulted in a lower production of the parasitoid. In contrast, the lowest palm oil concentration in the artificial diet generated a low RMR and resulted in high-efficiency conversion of ingested food (ECI), enabling *D. saccharalis* to yield more *C. flavipes*. This suggests that palm oil influences the quantity of *D. saccharalis* ingested, which determines the number of *C. flavipes* that will be generated. In this study, supplementation of a *D. saccharalis* diet with 0.25% palm oil increased the production of *C. flavipes*. Therefore, this level of palm oil supplementation can aid in the production of *C. flavipes* on a laboratory-scale. Further research, including increasing the number of parasitoids, must be performed to mass-produce the augmentative releases of *C. flavipes* to manage *D. saccharalis*.

Keywords: biological control; sugarcane borer; parasitoid; lipids; relative metabolic rate (RMR); efficiency of conversion of ingested food (ECI).

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Introduction

The environmental impacts of widespread insecticide use have led to practices aimed at reducing dependence on chemical pest control (Biondi et al., 2015). One of these practices involves the release of biological control agents (Parra & Junior, 2019). In this context, *Cotesia flavipes* (Cameron, 1891) (Hymenoptera: Braconidae) is a gregarious koinobiont wasp that deposits its eggs inside the larval hemocoel and is used to combat the sugarcane borer *Diatraea saccharalis* (Fabricius, 1794) (Lepidoptera: Crambidae) (Molnár, López, Gámez, & Garay, 2016). *Diatraea saccharalis* is a pest of great economic importance for sugarcane production because it can cause serious damage that contributes to significant reductions in agricultural and industrial productivity. Sugarcane borers causes damage at all the stages of plant development. The larvae penetrate plant tissues, creating galleries within the stems owing to feeding (Mello et al., 2020). This damage serves as an entrance to secondary phytopathogenic fungi, leading to greater losses in yield (Tiwari, Shukla, Jaiswal, & Tiwari, 2020). These damages include interference with the quality of sucrose content, soluble solids, purity, acidity of juice, fiber, and reduced sugar production and quality (Ferreira et al., 2018).

Diatraea saccharalis larvae are the only food source necessary for the production of the proovigenic endo-parasitoid *C. flavipes*. Therefore, for the production of *C. flavipes*, *D. saccharalis* larvae are reared in the laboratory on an artificial diet and subsequently serve as hosts for the parasitoid (Smaniotto et al., 2019). The development of artificial diets is an important scientific advancement in biological control. The use of artificial diets for herbivores has progressed research, focusing on qualitative and quantitative nutritional requirements for natural enemies (Cohen, 2018). In the production of *C. flavipes*, there is large variation in the composition of the diets used for rearing the host *D. saccharalis* (Filho et al., 2018). Most of the costs of mass rearing insects in the laboratory come from artificial diets; therefore, the development of a low-cost and effective diet is of great importance (Cohen, 2018).

Researchers have reported the positive effects of using palm oil in insect diets (Beaulieu & Palmquist, 1995; Costa, Queiroga, & Pereira, 2009; Silva et al., 2014; Neumann et al., 2015). Palmitic, oleic, and linoleic acids (fatty acids) are predominantly responsible for molting, wing training, and adult emergence, and these compounds are not synthesized by insects themselves (Mattos, 2006). Therefore, this study quantified *D. saccharalis* consumption and the use of an artificial diet supplemented with palm oil, as well as evaluated how this diet influenced the production and sex ratio of the parasitoid *C. flavipes*.

Material and methods

Diatraea saccharalis production

An artificial diet based on a previous study was used (Nardin, 2004). According to Table 1, concentrations of 0.25, 0.50, and 1.00% palm oil (Campestre Ind. E Com. Vegetable oils Ltd.) (Tables 2 and 3) were added to treatment groups I, II, and III, respectively. *Diatraea saccharalis* larvae were kept in Falcon tubes (15 mL), which were sterilized beforehand and contained an artificial diet. These tubes were plugged with cotton wool and kept in a climate chamber (B.O.D. model 347) at a temperature of 27 ± 2°C and relative humidity of 70 ± 10%, with a photo phase of 14h. Diet preparation, as well as the overall method for producing sterile *D. saccharalis,* was based on techniques described in a previous study (Parra, 2007).

Table 1. Artificial control diet composition used as *Diatraea saccharalis* larvae feed (amount of substrate per 100 larvae).

Components	Control
Soybean meal	26.25 g
Wheat germ	25.00 g
Sucrose	33.75 g
Yeast sugar	37.50 g
Choline chloride	0.62 g
Ascorbic acid	1.25 g
Wesson salts	2.50 g
Nipagin	2.50 g
Vitamin solution	6.25 mL
Vita Gold	2.00 mL
Terramycin	0.60 mL
Agar	7.50 g
Palm oil	0.00 g
Water qs	500.00 mL

Table 2. Fatty acid composition of palm oil.

Components	Chemical structure	Reference value (%)
Lauric Acid	C12:0	⩽ 0.4
Myristic Acid	C14:0	0.5 – 2.0
Palmitic Acid	C16:0	40.0 – 48.0
Palmitoleic Acid	C16:1	⩽ 0.6
Stearic acid	C18:0	3.5 – 6.5
Oleic Acid (Omega 9)	C18:1	36.0 – 44.0
Linoleic Acid (Omega 6)	C18:2	6.5 – 12.0
Linolenic Acid (Omega 3)	C18:3	⩽ 0.5
Araquic Acid	C20:0	⩽ 1.0
Eicoseneic Acid	C20:1	⩽ 0.2
Behenic Acid	C22:0	⩽ 0.1
Lignoceric Acid	C24:0	⩽ 0.2

Data provided by the supplier.

Table 3. Physico-chemical characteristics of palm oil.

Indexes	Unit	Reference value
Specific Weight (50°C/20°C)	$g\ cm^{-3}$	0.891 – 0.899
Iodine	$g\ I_2\ 100\ g^{-1}$	49 – 55
Saponification	$mg\ KOH\ g^{-1}$	190 – 209
Unsaponifiable Matter	%	< 1.2
Fusion point	°C	33 – 40
Acidity, crude oil	% (oleic acid)	< 5.0
Peroxide Index	$mEq\ kg^{-1}$	< 10.0

Data provided by the supplier.

Nutritional indices

To quantify substrate consumption and use, each of *D. saccharalis* (n = 50) larva aged 24h old were introduced separately into test tubes containing 5 g of artificial diet. They were subsequently maintained under the controlled conditions mentioned above. The larvae were removed 20 d later and weighed separately. They were then sacrificed by freezing (–30°C) and dried at 55-60°C to a constant weight, together with the remainder of their diet and the feces from their test tubes. Using this procedure, the dry weight of the consumed substrate and weight gain of the larvae were determined. Both of these factors are essential for determining the rates of food consumption and utilization. In addition, an aliquot of test tubes with and without the substrate was measured, as described above, to determine the initial dry weight of the diet (Parra, 1991).

Nutritional indices were based on previous assessments (Waldbauer, 1968), following recommendations of previous studies (Parra, 1991; Panizzi & Parra, 2009). The following calculations were performed.

Relative consumption rate (RCR, $g\ g^{-1}\ day^{-1}$) = $I/Bm \times T$,

Metabolic rate (RMR, $g\ g^{-1}\ day^{-1}$) = $M/B_m \times T$,

Relative growth rate (RGR, $g\ g^{-1} day^{-1}$) = $B/B_m \times T$

Efficiency of conversion of ingested food (ECI, %) = $(B/I) \times 100$

Efficiency of conversion of digested food (ECD, %) = $(B/I\text{-}F) \times 100$

Approximate digestibility (AD, %) = $(I\text{-}F/I) \times 100$,

where T = feeding period in days, I = substrate intake (in g) during T, B = larval weight gain (in g) during T, F = undigested food + excreted product (in g), $M = (I - F) - B$ = food metabolized during T (in g), $I - F$ = food assimilated during T (in g), and B_m = mean larval weight during T (in g).

Production and sex ratio of the parasitoid *Cotesia flavipes*

The parasitoid *C. flavipes* was produced and provided by the laboratory of Usina Santa Terezinha, located in Iguatemi District, Paraná State, Brazil. The parasitoids had no access to any carbohydrate source before or during the parasitism experiments, and they were +24h old. *Diatraea saccharalis* larvae were used to evaluate the effects of palm oil levels on *C. flavipes* production and their sex ratios. The larvae were reared under the same conditions as described above. After 18 days, 50 larvae from each treatment (n = 50) were individually subjected to parasitoids, following a previously described technique (Viel, 2009). Parasitoid females oviposited on the dorsal region of the integument of *D. saccharalis* larvae, which were kept separately in Petri dishes (6 cm diameter) containing a meal substrate until parasitoid emergence and cocoons were present on the integument of the host larvae. These masses were placed in paper cups and returned to the climate chamber until adult parasitoids emerged, and subsequently died. Finally, dead *C. flavipes* individuals were separated by sex, based on the sexual dimorphism of the antennae. The antennae in males are longer than those in females, although both have the same number of antennomeres (Wilkinson, 1928). The final number of adult *C. flavipes* parasitoids per *D. saccharalis* individual was estimated by adding the numbers of male and female individuals. The number of unviable cocoons was determined by counting the cocoons without adult emergence.

Data analysis

The distribution of the nutritional indices (RCR, RMR, RGR, ECI, ECD, and AD), sex ratio, and parasitoid production were analyzed using the Shapiro–Wilk test ($p < 0.05$), as well as by analyzing Quantile–Quantile plots (QQ-Plots) using the qqnorm package in the R software (R Core Team, 2013). The Shapiro–Wilk test indicated that the data were not normally distributed ($p < 0.0001$). The data were subsequently analyzed using the Kruskal–Wallis test ($p < 0.05$) to identify whether there were differences among the dietary treatments in nutritional indices, sex ratio, or in the production of parasitoids.

When significant differences were found, a post-hoc Dunn's test was performed to specify which treatments were significantly different from each other. Spearman's rank correlation was used to analyze the relationship between ECI and parasitoid production. Once the correlation between the variables was determined, they were evaluated qualitatively for their intensity using the criteria proposed previously (Callegari-Jaqcques, 2003). For all tests, we used R software. Scatter plots were generated using the CAR package (Fox & Weisberg, 2011).

Results

Palm oil modifies the relative consumption and metabolic rate of *D. saccharalis*

The relative consumption rates for *D. saccharalis* differed between treatments (χ^2 = 30.494, df = 3, p < 0.0001). Significant differences were found when treatments II (p = 0.0037) and III (p = 0.0009) were compared to the control (Figure 1A). This indicated that, when subjected to treatment III (Figure 1A), *D. saccharalis* had the largest relative consumption. The metabolic rate of *D. saccharalis* also changed after being subjected to the different treatments (χ^2 = 33.2202; df = 3, p < 0.0001). Higher metabolic rates were generated in treatment II (p = 0.0018) and III (p = 0.001) than for the control (Figure 1B). In contrast, different palm oil addition levels did not influence the relative growth rate (χ^2 = 0.3137, df = 3, p = 0.9574) (Figure 1C).

The different palm oil concentrations also resulted in changes in the conversion of the ingested substrate (χ^2 = 11.6696, df = 3, p = 0.0086). The largest ingested substrate conversion ratio was observed in treatment I (p = 0.0028) (Figure 1D), whereas treatments II (p = 0.0264) and III (p = 0.0226) had the lowest conversion ratios of ingested food (Figure 1D).

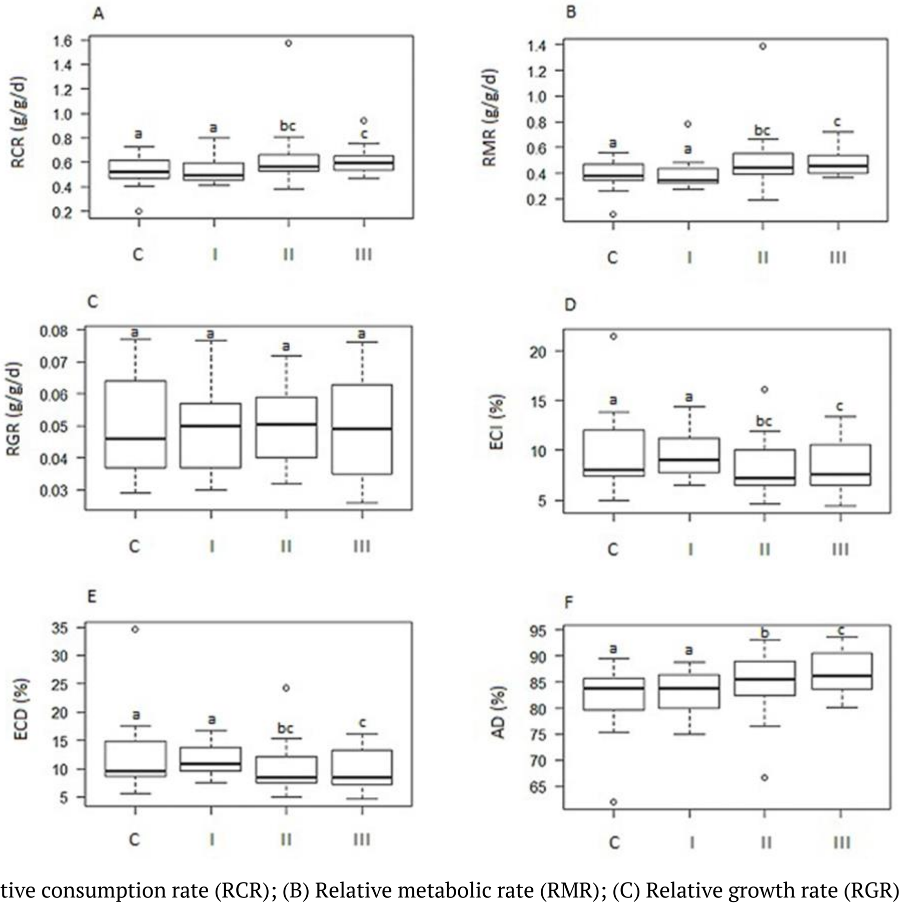

Figure 1. (A) Relative consumption rate (RCR); (B) Relative metabolic rate (RMR); (C) Relative growth rate (RGR); (D) Efficiency of conversion of ingested food (ECI); (E) Efficiency of conversion of digested food (ECD); (F) Approximate digestibility (AD) of sugarcane borer larvae fed with treatment (C) control, (I) 0.25%, (II) 0.50%, and (III) 1.00% palm oil. Different letters among the treatments indicate statistical differences of p < 0.05.

A pattern similar to the ingested food conversion ratio was generated for the digested food conversion efficiency. Treatment I (p = 0.0003) showed the most efficient digestion by larvae, whereas treatments II (p = 0.0038) and III (p = 0.0028) had the lowest ingested food conversion ratios (Figure. 1E).

Furthermore, palm oil addition modified the approximate digestibility index of *D. saccharalis* ($\chi^2 = 25.6426$, df = 3, $p < 0.0001$). The highest palm oil concentrations clearly caused a higher digestibility (Figure 1F), with treatment III showing the highest apparent digestibility index ($p < 0.0001$) followed by treatment II ($p = 0.0039$). For the RCR, RMR, ECI, ECD, and AD indices, some *D. saccharalis* individuals showed atypical consumption, and these data were therefore characterized as outliers.

Production and sex ratio of *Cotesia flavipes*

The addition of palm oil changed the number of parasitoids emerging from *D. saccharalis larvae* ($\chi^2 = 32.7623$, df = 3, $p < 0.0001$). Treatment I generated the largest number of *C. flavipes* ($p = 0.0017$; n = 3991) and the smallest number of non-viable cocoons ($p = 0.0381$; n = 50) among the treatments (Table 2). The hosts in treatment III had a lower production of parasitoids ($p = 0.0022$, n = 1519) and the highest rate of non-viable cocoons (n = 166) (Table 4). After separation by sex, all treatments, including the control group, produced higher frequencies of parasitoid females (Table 4). The *C. flavipes* sex ratio of males to female adults emerging from *D. saccharalis* larvae was 0.2 in the control group and treatment III. For treatments I and II, the sex ratio was 0.25 (Table 4).

Table 4. Production, number of unviable cocoons, and sex ratio of males (♂) and female (♀) of *Cotesia flavipes* that emerged from *Diatraea saccharalis* per treatment. The treatments consisted of: only the artificial diet (C), or the diet supplemented with palm oil at 0.25% (I), 0.50% (II), and 1.00% (III).

Treatment	Production in numbers	Unviable cocoons	Sex ratio (♂:♀)
C	2719^a	152^a	0.2^a
I	3991^b	50^b	0.25^a
II	3127^a	55^b	0.25^a
III	1519^c	166^a	0.2^a

Different letters in the same column indicate significant diferences.

Correlations between substrate conversion and production rates of *Cotesia flavipes*

There was a weak positive correlation between the ECI and treatment control output (*rho* = 0.058) and I (*rho* = 0.202), and a weak negative correlation between the ECI and production of parasitoids in treatments II (*rho* = -0.281) and III (*rho* = -0.155) (Figure 2). The degree of correlation between the two variables indicated that high ECI values were accompanied by high parasitoid production, although high ECI values were not necessary to achieve high digestibility. Because treatment I larvae had larger ECI values, they were more capable of being parasitoid hosts, thus creating a higher yield. Moreover, because high ECI values were not associated with high digestibility rates, treatment I was an economically favorable treatment compared with the control and treatment III because an increase in the amount of palm oil (> 0.25%) induced a decrease in *C. flavipes* production (Figure 2). Therefore, the potential of larvae to host the parasitoid *C. flavipes* increased as well. Moreover, treatment III had high levels of digestibility compared to the control treatment (Figure 2), which caused the metabolic rate to be high, resulting in more energy being spent during the feeding period. Consequently, less energy became available for ECI in these larvae, causing them to have a lower potential for parasitoid production.

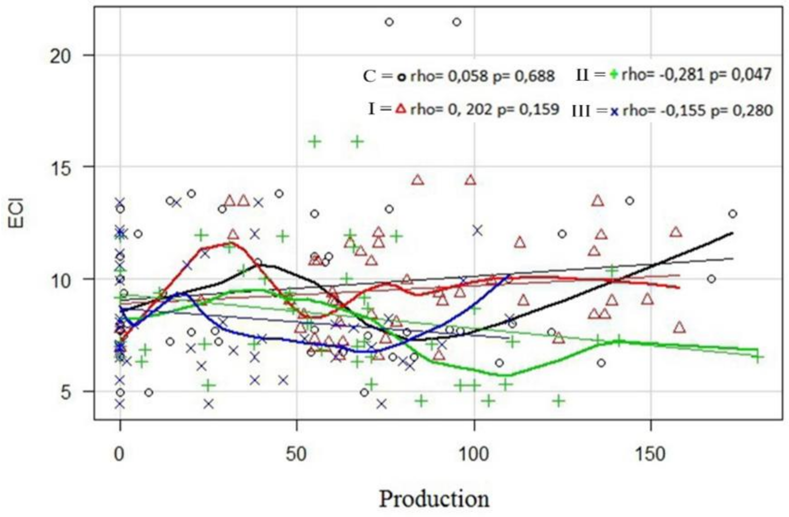

Figure 2. Scatterplot with correlations between the efficiency of conversion of ingested food (ECI) and the production of *Cotesia flavipes*.

Discussion

The indices of consumption and substrate use for insects, applied as assessment criteria, have been used in most studies as indicators of the adequacy of diets, revealing that their specific components can profoundly affect insect behavior and physiology (Crocomo & Parra, 1985). For example, Straub, Tanga, Osuga, Windisch, and Subramanian (2019) studied five different diets in crickets and locusts to assess their consumption, biomass gain, and feed conversion. These results will lead to further efforts to explore the enormous potential of insect production as a novel livestock and sustainable protein source for human food and animal feed.

Nutritional values obtained in group I (0.25% palm oil) were in the range of values reported previously (Souza, Ávila, & Parra, 2001) for *D. saccharalis* reared on an artificial diet at 25-30°C. Furthermore, comparing the nutritional indices of parasitized and non-parasitized *D. saccharalis* larvae revealed a reduction in consumption rate (RCR), growth rate (RGR), and metabolic rate (RMR) in parasitized larvae, and an increase in digested food conversion efficiency (ECD). However, ingested food conversion efficiency (ECI) and approximate digestibility (AD) were not affected by *C. flavipes* parasitism (Rossi, Salvador, & Cônsoli, 2014).

In Lepidoptera, most of the energy used for maintenance and reproduction in the adult stage is accumulated during the larval stage because these insects do not feed as adults. The fat body functions as a major site for nutrient storage, energy metabolism, innate immunity, and detoxification (Li, Yu, & Feng, 2019). This tissue plays an important role in the integration of hormonal and nutritional signals, which regulate larval growth, body size, and feeding behavior (Li et al., 2019). Compared with larvae (Pompilho, 2006), adult females of *D. saccharalis* exhibit a large reduction in hemolymph and fat bodies. However, the ovaries occupy most of the insect's abdominal cavity. During egg growth, lipids are delivered through spores from lipids stored in the fat body during the larval stage (Canavoso & Wells, 2001).

In the treatment containing 0.25% palm oil (the lowest concentration evaluated), *D. saccharalis* larvae had a lower diet intake because of their lower metabolic expenditure. This increases the conversion efficiencies of the ingested and digested food. However, by increasing palm oil concentration, consumption increased, generating high metabolic costs by reducing the efficiency of assimilating food. This consequently resulted in decreased production of *C. flavipes*.

In insects, lipids assimilated by the midgut are transported to other tissues via the spores present in the hemolymph (Canavoso, Jouni, Karnas, Pennington, & Wells, 2001). Lipids are digested in the midgut, where triacylglycerol (TAG), the main lipid component of the diet, is hydrolyzed by TAG lipase into free fatty acids and monoacylglycerol (Arrese & Soulages, 2010). Overall, fatty acids are absorbed by enterocytes and converted to diacylglycerol (DAG), TAG, and phospholipids. DAG can be converted to TAG, which serves as a fatty acid reserve. Alternatively, DAG can be secreted into hemolymph-associated spores (Canavoso & Wells, 2001). As the midgut plays an important role in food absorption, it is a target for different pest control methods. *Bacillus thuringiensis* serovar Aizawai GC-91 leads to morphological changes in the midgut epithelial cells of *D. saccharalis,* resulting in changes in nutrient absorption and cellular homeostasis, leading to insect mortality (Daquila et al., 2019).

Different palm oil levels induced significant changes in the rates of substrate consumption by *D. saccharalis*, leading to significant changes in the production of *C. flavipes*. Specifically, we found that when hosts were fed higher palm oil concentrations, smaller numbers of parasitoids emerged, which may be related to high metabolic cost and decreased food conversion efficiency. This observation suggests that the production of *C. flavipes* was reduced by the lack of available nutrients in the *D. saccharalis* larvae.

Parasitism by *C. flavipes* prolongs the retention time of food in the host digestive tract (Rossi et al., 2014). In addition, the increase in food residence time in the digestive tract of parasitized larvae is probably due to reduced peristaltic activity, as diagnosed in *Manduca sexta* larvae parasitized by *Cotesia congregata* (Beckage & Gelman, 2004). Parasitism by *C. flavipes* significantly reduces the host's maintenance energy expenditure, although in our study, the results showed that feeding larvae higher concentrations of palm oil (1%) increased the relative metabolic cost (Rossi et al., 2014). However, the reduction in food consumption detected by the larvae in the treatment containing 0.25% palm oil was compensated for by better utilization of the digested food, which had a positive effect on the production of *C. flavipes*. The gut microbiota assists in food digestion and utilization by producing enzymes that act on food. Parasitization of *D. saccharalis* and *Spodoptera frugiperda* by *C. flavipes* altered the composition and contribution of the gut microbiota. These changes induced by the parasitoid could favor host exploitation by *C. flavipes* (Oliveira & Cônsoli, 2020).

This study showed that there is a feasible measure of palm oil that can be added to the host's diet to increase parasitoid emergence. Parasitoid production was the highest in diets with 0.25% palm oil, whereas diets with 0.50% and 1% palm oil showed a decrease in production. The number of unviable cocoons in the control (n = 152) and 1% palm oil (n = 166) were similar, as were those in 0.25% (n = 50) and 0.50% (n = 55). Successive oviposition in the same host increased both the number of viable and unviable cocoons due to food competition (Yamauchi, Gobbi, Chaud-Neto, & Campos-Farinha, 1997). However, in our study, the treatment with the largest production of cocoons was also the lowest of those unviable, indicating that the supplementation provided a good source of nutrients.

Different dietary palm oil levels did not affect the sex ratio among the treatments in our study, although they influenced the total number of females in the *C. flavipes* population. Our observed sex ratios (0.25) are lower than earlier findings of *C. flavipes* male to female sex ratios, which ranged between 0.61 and 0.82 (Campos-Farinha & Chaud-Netto, 2000; Carvalho, Vacari, De Bortoli, & Viel, 2008; Vacari, Genovez, Laurentis, & De Bortoli, 2012; Volpe et al., 2014; Bortoli et al., 2015). High frequencies of male parasitoids compared to females are unfavorable for the biological control of *D. saccharalis*, as female *C. flavipes* are responsible for parasitism. Therefore, a palm oil addition level of 0.25% is the most suitable for the production of the host, as it results in a sex ratio of 0.25, which matches most closely with the recommendations of having more females, with an average of 80 parasitoids per cocoon mass (Campos-Farinha & Chaud-Netto, 2000).

Conclusion

In conclusion, an artificial diet containing 0.25% palm oil is most suitable for the production of *D. saccharalis*. This diet resulted in increased production of the parasitoid *C. flavipes*, and this concentration provided a favorable *C. flavipes* sex ratio. Therefore, this level of supplementation with palm oil can aid in the production of *C. flavipes* on a laboratory-scale. Further studies, including different generations of parasitoids, must be performed to achieve mass production for augmentative releases of *C. flavipes* to manage *D. saccharalis* and prevent damage to sugarcane cultivation.

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