BIOLOGICAL CONTROL

Food Consumption of *Rhammatocerus schistocercoides* Rehn (Orthoptera: Acrididae) Infected by the Fungus *Metarhizium flavoviride* Gams & Rozsypal

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Consumo Alimentar de *Rhammatocerus schistocercoides* Rehn (Orthoptera: Acrididae) Infectado pelo Fungo *Metarhizium flavoviride* Gams & Rozsypal

RESUMO - Foi examinada a capacidade de consumo foliar de ninfas e adultos do gafanhoto *Rhammatocerus schistocercoides* Rehn após infecção pelo fungo *Metarhizium flavoviride* Gams & Rozsypal. Ninfas de sexto e oitavo estádios e fêmeas adultas consumiram diariamente, em média, 6,9 ± 0,36 cm^2 (60,7 ± 2,92 mg de matéria seca), 11,0 ± 1,35 cm^2 (74,9 ± 9,24 mg m.s.), e 16,0 ± 0,50 cm^2 (126,5 ± 6,35 mg m.s.) de folhas de cana-de-açúcar, equivalente a 85,4, 35,9 e 41,9% do peso vivo dos insetos, respectivamente. Ninfas de oitavo estádio e fêmeas adultas inoculadas com o fungo *M. flavoviride* (5.000 conídios/ gafanhoto) ingeriram significativamente menos alimento a partir do terceiro dia após a inoculação do patógeno e apresentaram no décimo dia um consumo médio 74,5 e 45,6% inferior ao do controle, respectivamente. Fêmeas adultas tratadas topicamente com dose subletal (3 µl de uma solução 8 ppm/ inseto) do inseticida químico diflubenzuron apresentaram consumo semelhante àquele da testemunha (P=0,065), e a combinação do químico com o fungo não apresentou resultados diferentes daqueles obtidos com o fungo isoladamente (P=0,405). Observou-se forte correlação (r^2=0,998) entre a produção de pellets fecais por *R. schistocercoides* e o consumo foliar. Os resultados obtidos confirmaram a grande voracidade do gafanhoto *R. schistocercoides* e demonstraram marcante efeito adverso do fungo na ingestão de alimentos por adultos e ninfas da espécie.

PALAVRAS-CHAVE: Insecta, redução alimentar, consumo foliar, fungo entomopatogênico.

ABSTRACT - Foliage consumption by nymphs and adults of the grasshopper *Rhammatocerus schistocercoides* Rehn infected with the fungus *Metarhizium flavoviride* Gams & Rozsypal was evaluated. Sixth and eighth instar nymphs and female adults consumed on average (±SEM) 6.9 ± 0.36 cm^2 (60.7 ± 2.92 mg of dry matter), 11.0 ± 1.35 cm^2 (74.9 ± 9.24 mg d.m.), and 16.0 ± 0.50 cm^2 (126.5 ± 6.35 mg d.m.) of sugarcane leaves per day, equivalent to 85.4, 35.9 and 41.9% of the insect body weights, respectively. Eighth instar nymphs and female adults inoculated with *M. flavoviride* (5,000 conidia / insect) ingested less food from day three onward and showed at day 10 an average consumption 74.5

and 45.6% lower than control insects, respectively. Adult females topically treated with a sub-lethal dosage (3 µl of a 8 ppm suspension / insect) of the chemical insecticide diflubenzuron showed consumption rates comparable to untreated insects (P=0.065). The combination fungus + chemical did not show results different from those obtained with the fungus alone (P=0.405). A strong correlation (r^2=0.998) between production of fecal pellets by *R. schistocercoides* and food consumption was observed. These results confirmed the high voracity of *R. schistocercoides* and showed a remarkable adverse effect of the fungus on food intake by infected nymphs and adults.

KEY WORDS: Insecta, feeding reduction, foliage consumption, entomopathogenic fungus.

Reports on the extraordinary destructive capability of grasshoppers and locusts in the African and Asian continents in modern age, and the economic losses associated to their attacks, are well known (Steedman 1990, Showler 1995). In Brazil, reports on acridid outbreaks are relatively recent, and only a few species have been mentioned as agricultural pests, among them *Rhammatocerus schistocercoides* Rehn and *Schistocerca pallens* Thunberg (Lecoq 1991).

Rhammatocerus schistocercoides, a gregarious and univoltine species, frequently occurs in Rondônia and the western area of Mato Grosso, and there are reports of occasional occurrence in other states, such as Mato Grosso do Sul (I. Pierozzi Jr., personal communication), Goiás (W.D. Guerra, personal communication), São Paulo and Minas Gerais (G.W. Cosenza, personal communication), as well as in other South American countries (Léon 1996, Miranda *et al.* 1996, Lecoq & Assis-Pujol 1998). *R. schistocercoides* is a polyphagous insect, showing preference for native grasses, rice and sugarcane crops. Despite its voracity, information regarding the economic impact of this pest is difficult to assess due to the large number of host plants, lack of notifications to official phytossanitary authorities when infested areas are treated, and inprecise estimates of the actual losses in attacked crops and pastures. To overcome the problem in infested areas of Mato Grosso, from 1984 to 1988, the Brazilian government spent more than US$ 5 million, mainly in purchase of chemical insecticides (approx. 60.000 liters of fenitrothion and malathion) and aerial spraying costs (Guerra & Manfio 1996).

A research program focusing on the biological control of *R. schistocercoides* was started in 1993, and resulted in the selection of a fungal pathogen, *Metarhizium flavoviride* Gams & Rozsypal. Field trials carried out in Mato Grosso, have demonstrated that mortality of nymphs treated with this fungus is initiated seven days after spraying, and peaks around the tenth day (B. Magalhães, M. Faria, S. Xavier-Santos & S. Mendez, unpublished). Unlike chemical insecticides which have knockdown effect, *M. flavoviride* has a slow action, although infected insects show reduction in the locomotory and feeding capacities. Sub-lethal dosages of chemical insecticides can disturb the locomotory, reproductive and alimentary functions of insects (Haynes 1988). In preliminary studies on the compatibility of chemical insecticides and *M. flavoviride,* we have observed that, in contrast with the insecticides used to control acridids in Brazil (malathion and fenitrothion), diflubenzuron shows notably less adverse effects on the conidial germination and growth of *M. flavoviride* (S. Xavier-Santos, B. Magalhães & M. Faria, unpublished).

The purpose of this work was to estimate the food intake of nymphs and adults of *R. schistocercoides* treated with *M. flavoviride*, and investigate the relationship between food ingestion and production of fecal pellets.

Material and Methods

Insects.Third instar nymphs of *R. schistocercoides* were collected from the field and kept in laboratory until used in bioassays. Due to the short duration of initial instars, the food consumption experiments were performed with sixth and eighth instars and adult females (approx. 15 days following the final molt). Sex determination was based on morphological traits of the external genitalia (Lecoq & Pierozzi Jr. 1994).

Assessment of Leaf Consumption. In a preliminary experiment, leaf consumption by sixth instar nymphs was assessed over eight days. Fifteen females and 15 males were individually kept in transparent 300-ml plastic cups with perforated lid. Insects had been moved to cups 24 h before initiation of experiment and were fed fresh sugarcane. The initial weight of each insect was determined with an analytical balance. Pieces of fresh sugarcane leaf (2,7 x 6,0cm), one end wrapped in wet cotton and aluminum foil, were offered daily. The wrapping was shown to maintain the turgidity of leaf pieces for at least 48 h. Insects were maintained at 27ºC, 57% RH and 12 h photophase. Leaf pieces were removed daily and their area determined with the use of an area meter (CI-203 Area Meter, Cid Inc., Vancouver, WA, USA). Insects that underwent molt or were damaged during the experiment were discarded. Nymphs showing a loss in body weight were assumed to be strongly stressed, and were not considered. Pieces of fresh sugarcane leaves were weighted before and after drying at 70ºC for 48 h, to determine wet and dry matter ingested per nymph. Treatment comparisons were performed using the t-test (α=0.05).

Leaf Consumption by Eighth Instar Nymphs Treated with *M. flavoviride* (CG 423). Following an adaptation period of 24 h, 20 nymphs were topically inoculated with 3 µl of soybean oil containing conidia of *M. flavoviride* (5,000 conidia / nymph). Control nymphs (n=15) were inoculated with 3 µl of pure soybean oil. Leaf consumption was assessed for 10 days as described above. Fungus-treated nymphs that died during the experiment were held in wet chambers, and only those killed by the fungus were considered. Treatment comparisons were performed using the t-test (α=0.05).

Leaf Consumption by Adult Females Treated with *M. flavoviride* and Diflubenzuron. Food consumption was determined for healthy (oil-inoculated), fungus-inoculated (5,000 conidia / female), diflubenzuron-treated (Dimilin®, Basf) (3 µl of a 8 ppm suspension), and fungus + diflubenzuron-treated adult females (using previous dosages). Previous experiments had shown that, at the doses used in this experiment, diflubenzuron alone did not kill treated *R. schistocercoides*. For both oil and diflubenzuron-treated insects, only those showing a positive gain in weight during the experiment were considered. For treatments with *M. flavoviride*, only data referring to insects confirmly killed by the fungus were considered in the statistical analyses (t-test).

Food Consumption and Production of Fecal Pellets. Adult females were individually kept in plastic cups and allowed a 24 h period on fast. Pieces of fresh sugarcane leaves with known area were then offered to insects once a day. For each 24 h period, both the consumed area and the fecal pellets production were determined. Fecal pellets were dried at 70°C for 24 h, and then weighted. For each day the correlation coefficient (r^2) was calculated through linear regression considering all insects (n=10), with the exception of the fourth and fifth days, which were grouped before the analysis. Since the objective of this experiment was to determine if there was a correlation between food intake and production of fecal pellets by adult females, all insects, independently of presenting or not a positive weight gain, were considered in calculations.

Results e Discussion

Significant differences in daily consumption of fresh sugarcane leaf between male and female sixth instar nymphs were observed (P=0.041; t-test) (Table 1). However, no statistical differences between gender were observed when food consumption per mg of body weight was used (P=0.193). The initial average weight of female nymphs (8.3% higher than for males) seems to explain the greater food consumption. Male and female sixth instar nymphs were quite voracious, ingesting the equivalent to 81.3 and 85.4% of their body weight per day, respectively, in terms of wet matter. The average food consumption of less than 7 cm^2 is lower than the approximately 10 cm^2 reported to fifth instar nymphs of the locust *Schistocerca gregaria* Forskål (Mathews 1992). According to Steedman (1990), one of the main reasons for the extraordinary damage potential of many locust species is the amount of food ingested daily by the insects, in some cases equivalent to their body weight. Although less voracious than several locust species, the grasshopper *R. schistocercoides* is highly destructive due to a high populational density, up to 10,000 nymphs / m^2 (Miranda *et al*. 1996).

The average daily consumption of healthy eighth instar female nymphs was 11.0 ± 1.35 cm^2 (74.9 ± 9.24 mg of dry matter) (Table 2), equivalent to 35.9% of their body weight, which is considerably less than that obtained for sixth instar nymphs. The food consumption of fungus-infected nymphs was significantly lower than that of healthy nymphs from day three onward (P=0.024). The average amount of food consumed daily by fungus-infected eighth instar nymphs was drastically reduced to 2.8 cm^2, which is 74.5% lower than that of healthy nymphs. The adverse effect of pathogens on food intake by acridid nymphs has been reported in a number of studies. Food reduction rates from 15.3 to 74.3% at 21 days post-treatment, were observed in fourth instar *Melanoplus sanguinipes* (Fabricius) treated with spores of the microsporidian *Nosema locustae* Canning (Johnson & Pavlikova 1986). First instar *M. sanguinipes* treated with $5x10^3$ inclusion bodies of an entomopoxvirus showed reduction in food consumption levels of 24 and 40%, at 5 days and two weeks post-inoculation, respectively (Olfert & Erlandson 1991). For sixth instar *Schistocerca americana* Drury, a significant reduction on food consumption by individuals treated with *M. flavoviride* was observed in the second day following inoculation, resulting in 36.6% reduction of food ingestion from 5 to 8 days after treatment (Sieglaff *et al*. 1997).

Table 1.Consumption of fresh sugarcane leaves, initial and final body weight of sixth instar nymphs of *R. schistocercoides.*

	Females (n=10)	Males (n=6)[1]	
Mean daily consumption (cm^2) per insect (±SEM)	6.9 ± 0.36	6.0 ± 0.31	(P=0.041)
Initial mean body weight (mg)	214.4 ± 7.88	197.9 ± 6.70	
Final mean body weight (mg)	243.3 ± 6.62	227.9 ± 10.75	

[1]The experiment was initiated with 15 nymphs per gender. Calculations were based only on nymphs that survived the eight-day experiment and showed a positive gain in body weight during this period.

Table 2. Consumption of fresh sugarcane leaves by eighth instar female nymphs of *R. schistocercoides*.

Days post-inoculation	Mean daily consumption (cm^2) per insect (±SEM)		
	Control[1]	Fungus[2]	Reduction (%)[3]
1	2.2 ± 0.55	2.5 ± 0.79 (17)	-15.7 (P=0,385)
2	5.5 ± 0.88	5.1 ± 1.09 (17)	7.1 (P=0.400)
3	9.5 ± 1.05	5.8 ± 1.23 (17)	39.1 (P=0.024)
4	11.7 ± 1.26	2.6 ± 0.91 (17)	77.7 (P<0.001)
5/6	23.1 ± 2.37	5.6 ± 2.05 (17)	75.8 (P<0.001)
7	13.9 ± 1.50	0.5 ± 0.25 (15)	96.4 (P<0.001)
8	13.6 ± 1.74	0.0 ± 0.00 (2)	100.0 (P=0.003)
9	15.2 ± 1.61	-	-
10	15.4 ± 1.16	-	-
Mean	11.0 ± 1.35	2.8 ± 0.18	74.5 (P<0.001)
Initial body weight (mg)	795.7 ± 24.54	788.0 ± 23.06	-
Final body weight (mg)	869.1 ± 30.61	ND	-

[1]Each nymph (n=10) was topically inoculated with 3 µl of soybean oil.
[2]Each nymph was topically inoculated with 3 µl of soybean oil containing conidia of *M. flavoviride* (approx. 5,000 conidia per insect). Only data referring to nymphs killed by the fungus were used. The number of nymphs considered in the figures of mean daily consumption (± SEM) are in brackets. All nymphs that succumbed to *M. flavoviride* infection died by day eight.
[3]Negative value indicates that food consumption by fungus-infected nymphs was higher than that observed to untreated nymphs. Values in brackets refer to the P-value obtained at t-test (α=0.05).

The average intake of fresh sugarcane leaves by healthy adult female *R. schistocercoides* was 16.0 ± 0.50 cm^2 (Table 3), equivalent to 126.5 ± 6.35 mg of dry matter per insect. *M. flavoviride*-treated insects ingested significantly less food than untreated insects from day three onward (P=0.006), and the average consumption during the experiment was 8.7 ± 1.50 cm^2, which is 45.6% less than control females. Adult females of *Melanoplus differentialis* (Thomas) treated as nymphs with spores of *N. locustae* ingested up to 55% less food than untreated insects (Oma & Hewitt 1984). Moore *et al*. (1992) demonstrated a correlation between the dose of *M. flavoviride* applied and the level of reduction of food consumption by infected *S. gregaria* adults. Those authors reported that dosages of 2,700 and 17,000 conidia per insect caused a clear reduction on food intake after the fifth and sixth days and the level of reduction of food consumption on day 8 was 17.9 and 33.8%, respectively. The invasion of *R. schistocercoides* hemocoel by structures of *M. flavoviride* (isolate CG 423) from three to four days following inoculation (Vicentini & Magalhães 1996), may explain the significant reduction on food intake observed in this study for both nymphs and adults.

Adult females inoculated with sub-lethal dosage of diflubenzuron showed a food ingestion equivalent to that of healthy ones (P=0.141). The combination of fungus and chemical insecticide did not show a synergistic effect either on food consumption (P=0.405) or on the mean survival time of infected in-

Table 3. Effect of the fungus *Metarhzium flavoviride* and the chemical insecticide diflubenzuron on foliage consumption by adult female *R. schistocercoides*.

Days post-inoculation	Mean daily consumption (cm^2) per insect (± SEM)						
	Control[1]	Fungus[2]	Reduction[5](%)	Insecticide[3]	Reduction[5](%)	Insecticide+Fungus[4]	Reduction[5](%)
1	16.4 ± 1.52	19.0 ± 1.46(12)	-15.9(P=0.118)	9.7 ± 1.94	41.5(P=0.005)	11.8 ± 0.74(4)	28.1(P=0.06)
2	12.0 ± 1.74	12.0 ± 1.56(12)	0.0 (P=0.500)	14.1 ± 1.70	-17.5(P=0.203)	11.9 ± 2.81(4)	0.8(P=0.486)
3	15.8 ± 1.07	10.6 ± 1.55(12)	32.9(P=0.006)	17.8 ± 1.02	-12.7(P=0.099)	9.3 ± 0.88(4)	41.4(P=0.003)
4	16.4 ± 0.90	10.2 ± 1.42(12)	37.4(P<0.001)	19.4 ± 0.63	-19.2(P=0.007)	10.9 ± 2.71(4)	33.1(P=0.012)
5	17.3 ± 1.23	6.3 ± 1.55(12)	63.6(P<0.001)	15.2 ± 1.11	12.4(P=0.114)	4.0 ± 1.10(4)	76.9(P<0.001)
6	16.0 ± 0.90	4.1 ± 0.75(12)	74.4(P<0.001)	18.3 ± 1.68	-14.4(P=0.117)	6.6 ± 1.21(4)	58.8(P<0.001)
7	15.9 ± 0.93	4.6 ± 0.97(11)	71.1(P<0.001)	13.1 ± 1.06	22.6(P=0.028)	15.6 ± 0.04(4)	1.9(P=0.433)
8	16.3 ± 0.99	3.3 ± 0.97(11)	81.6(P<0.001)	14.3 ± 1.82	12.3(P=0.167)	7.4 ± 1.37(4)	54.6(P<0.001)
9	15.5 ± 0.99	9.9 (1)	36.1	7.4 ± 1.76	52.3(P<0.001)	1.7 ± 0.76(4)	89.3(P<0.001)
10	18.1 ± 1.55	7.7 (1)	57.5	9.0 ± 1.52	50.3(P<0.001)	3.2 ± 1.26(2)	82.3(P=0.002)
Mean	16.0 ± 0.50	8.7 ± 1.50	45.6(P<0.001)	13.8 ± 1.30	13.8(P=0.141)	8.2 ± 1.41	48.8(P<0.001)
Initial body weight (mg)	981.6 ± 42.82	1024.7 ± 31.64		958.5 ± 36.79		940.3 ± 48.70	
Final body weight (mg)	1065.8 ± 33.81	970.3 ± 33.11		1079.9 ± 33.38		940.1 ± 66.70	

[1]Each adult (n=13) was topically inoculated with 3 µl of soybean oil.

[2]Each insect was topically inoculated with 3 µl of soybean oil containing conidia of *M. flavoviride* (approx. 5,000 conidia per insect). Only data referring to adults killed by the fungus were used. The number of adults considered in the figures of mean daily consumption (± SEM) are in brackets.

[3]Each insect (n=11) was treated with 3 µl of soybean oil containing 8 ppm of diflubenzuron.

[4]Each adult was topically inoculated with 3 µl of soybean oil containing conidia of *M. flavoviride* (approx. 5,000 conidia per insect), and 8 ppm of diflubenzuron.

[5]Negative values indicate that food consumption by treated insects was higher than that observed to untreated controls. Values in brackets refer to the P-value obtained at t-test (α=0.05).

sects. In fact, the mean survival time of insects that succumbed to fungal infection was eight days and when the fungus was used in combination with diflubenzuron, 9.5 days. As an insect growth regulator (IGR), diflubenzuron is not expected to cause the death of adult insects. Data presented in this study demonstrate that this IGR, used in association with *M. flavoviride*, is neither able to increase the virulence nor the invasive capability of the fungus. In contrast, Sanyang & Van Emden (1996) observed that the association of *M. flavoviride* and the chemical insecticide cypermethrim inhibited feeding by 50% on day one onwards, and advanced onset of mortality by 48 h. For the non-migratory and gregarious *R. schistocercoides* females, it was observed a daily average of wet matter consumption equivalent to 41.9% of their own body weight. In a similar study, Davey (1954) reported that adults of *S. gregaria* eat approximately 50% of their own weight per day.

The experiment with adult female *R. schistocercoides* confirmed a strong correlation (r^2=0.998) between the food intake and the production of fecal pellets (Table 4). This evidence allows studies to assess the reduction of food intake by infected acridids without using expensive equipment to measure leaf areas. Furthermore, it is possible to collect and store feces for future weight measurements. Production of fecal pellets by grasshoppers as an indicator of food consumption rate has been recently used as an indication of food consumption (Sanyang & Van Emden 1996, R. Milner, personal communication).

Table 4. Correlation between the consumption of fresh sugarcane leaves by adult females of *R. schistocercoides* and production of fecal pellets.

Days	Mean daily consumption[1] (cm^2)	Fecal pellets[2] (mg)	$(r^2)^3$
1	9.7 ± 2.56	43.3 ± 10.91	0.934
2	12.3 ± 3.13	70.9 ± 18.31	0.959
3	15.1 ± 4.62	77.2 ± 24.44	0.982
4/5	15.8 ± 3.67	93.7 ± 20.21	0.972
6	20.3 ± 3.67	100.4 ± 18.28	0.980
7	14.0 ± 3.80	77.4 ± 20.46	0.992
8	9.5 ± 2.44	43.8 ± 14.01	0.964
9	7.9 ± 2.69	40.6 ± 15.59	0.926
10	4.9 ± 1.55	23.0 ± 7.27	0.954
Total per insect	109.0 ± 25.36	567.9 ± 135.50	0.998

[1]Based on 10 insects kept on fast for 24 h before starting the experiment. All insects, independently of presenting or not gain in body weight, were considered in calculations.
[2]Dry weight, obtained after drying fecal pellets at 70°C for 24 h.
[3]Correlation coefficient calculated through linear regression analyses (α=0.05).

Although less voracious than many locust species, nymphs and adults of *R. schistocercoides* present a high food consumption potential. In addition to ingestion of food, a considerable damage is observed when this insect cut the stalk of rice plants just below flowering seed heads. Due to high population densities that may be achieved, the reduction on food intake following application of *M. flavoviride* would not be sufficient to avoid economic losses to attacked crops and pastures. Nevertheless, the strategy based on control of young *R. schistocercoides* bands, which are usually found in native pastures and are very sensitive to the fungus, seems to be a suitable biological control approach for avoiding damage.

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