PEST MANAGEMENT

Do Different Casein Concentrations Increase the Adverse Effect of Rutin on the Biology of *Anticarsia gemmatalis* Hübner (Lepidoptera: Noctuidae)?

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ABSTRACT - The flavonoid rutin is recognized as playing an important role in the protection of plants against lepidopterans. Bioassays with this compound are generally carried out using artificial diets. Proteins of high energy value, such as casein, are important ingredients of insect artificial diets as a source of essential amino acids. However, such proteins can generally increase the allelochemical activity. Our objective was to evaluate the effects of rutin on larvae of the velvetbean caterpillar *Anticarsia gemmatalis* Hübner by incorporating this allelochemical into diets with different concentrations of casein. Three casein concentrations (0, 7 g, or 14 g) combined with none, 0.65%, or 1.30% of rutin were added to the rearing diet and offered to the larvae from hatching to pupation. Rutin negatively affected larval development, the amount of food consumed, and pupal weight of *A. gemmatalis*. These negative effects were clearly seen in insects fed on diets with 7 g of casein to which any concentration of rutin was added. The effects of rutin when added to the diets without casein were stronger than in diets containing a suitable amount of casein (14 g). The greater negative effects of rutin in diets containing suboptimal concentrations of casein indicate that casein can increase the effects of rutin only when the diets are nutritionally unsuitable for insect development.

KEY WORDS: Flavonoid, velvetbean caterpillar, biological parameter

Flavonoids are chemical compounds widely distributed in plants (Simmonds 2001). They play an important role in plant defense against microorganisms and insects (Dixon & Steele 1999) and can be attractive, deterrent, or toxic to pests (Harborne & Grayer 1993). The flavonoid rutin (quercitin 3-*O*-rhamnosylglucoside) was detected in the soybean genotype PI 227687 (Hoffmann-Campo *et al* 2001, Piubelli *et al* 2005), which has been widely used in breeding programs as a source of resistance against defoliating insects. Rutin has shown antibiotic and/or antinutritional effects on many chewing insects (Stamp & Skrobola 1993), including the velvetbean caterpillar, *Anticarsia gemmatalis* Hübner (Hoffmann-Campo *et al* 2006), a major defoliating pest of soybeans in Brazil.

For insect optimal growth in artificial diets, proteins or amino acids are required in high concentrations (Panizzi & Parra 1991). Among proteins, casein has been widely used in artificial rearing diets because it contains all the essential amino acids, is soluble in water, and does not coagulate or precipitate after heating (Parra 1979). Casein also contains traces of important substances such as fatty acids, cholesterol, sugars, vitamins, and minerals (Vanderzant 1966).

The effect of plant-defense chemical compounds on the biology and behavior of insects is rarely studied independently. According to Duffey & Stout (1996), the ingredients used in artificial diets for insect rearing can increase the impact of chemical compounds related to plant defense. They also argued that high-energy proteins such as casein, which is one of the ingredients of the artificial diet of the velvetbean caterpillar, when associated with chlorogenic acid, increases its negative influence on larval growth in Lepidoptera. Chlorogenic acid and rutin are phenolic compounds, and information on the possibility of interaction between casein and rutin is essential to confirm the reliability of results obtained on the effects of flavonoids on insects when tested on artificial diets. Therefore, our main objective was to evaluate if casein can increase the effects of rutin on the biological development of *A. gemmatalis*.

Material and Methods

Anticarsia gemmatalis was maintained on an artificial diet based on beans, soybean protein, wheat germ, casein, and agar, according to Greene *et al* (1976) and as modified by Hoffmann-Campo *et al* (1985). The rutin used in the experiments was purchased from Sigma, and the amount used was based on the percentage of flavonoid observed by Hoffmann-Campo *et al* (2001) in insect-resistant soybean genotypes, which was 0.641% (dry weight) of rutin in the polar fraction of the foliar extract of PI227687.

Biological activity of rutin-supplemented casein-based artificial diets on *A. gemmatalis*. Three experiments were carried out to evaluate the effects of different concentrations of casein and rutin on the biology of *A. gemmatalis*. In experiment 1, treatments consisted on a standard diet (14 g of casein) without rutin (here termed 14gC) or with 0.65% of rutin (14gC+ 0.65%R); a diet with half of the usual casein quantity (7gC), without or with 0.65% rutin (7gC+0.65%R); and a diet with no casein (0gC) and without or with 0.65% rutin (0gC+0.65%R). Experiment 2 was similar to experiment 1, but the rutin concentration was increased to 1.30%. Finally, in the third experiment, standard diets with 14 g of casein (14gC) and with or without two concentrations of rutin, 0.65% (14gC+0.65%R) and 1.30% (14gC+1.30%R), were tested. These were compared to diets without casein (0gC), without casein plus 0.65% rutin (0gC+0.65%R), and without casein plus 1.30% rutin (0gC+1.30%R).

Extraction of flavonoids from soybean proteins. The rutin from soybean proteins used in preparing the diets was extracted by using 80% methanol (MeOH) in a proportion of 10:1 (v:w) under continuous agitation (100 rpm) for approximately 18 h. The extract obtained was filtered in a Buchner funnel with a Framex quantitative fast filtration paper, and the extraction process was conducted three times to guarantee complete extraction of the flavonoids. Before the diets were prepared, all the solvent (MeOH) was completely evaporated in an air chamber.

Experiment setup. The artificial diet was prepared as described by Hoffmann-Campo *et al* (1985). Rutin was dissolved in distilled water and mixed with the artificial diet at a temperature below 50ºC. Late second larval instars kept on their respective treatment-diets since hatching, were placed individually in small acrylic cups with sterilized cardboard lids, and maintained under controlled conditions (25 ± 2ºC; 70 ± 10% UR; 12-h photoperiod). Larvae were inspected daily, and the mortality and the development time of each stage were recorded.

To estimate the dry weight of the diets, five cups were taken from each treatment, weighed, dried in an oven until constant weight (60ºC, for 72h), and re-weighed. The correction factor for fresh/dry weight of each diet was calculated, and the values obtained were multiplied by the fresh weight of food contained in each cup. To validate the correction factor for fresh/dry weight, analyses of linear regression and correlation were carried out. For the three sets of experiments, the equations were: $\hat{Y}_1 = 205.0285 + 0.1489.X_1$ ($R^2 = 0.71$) (experiment 1), $\hat{Y}_2 = 7.5566 + 0.1769.X_2$ ($R^2 = 0.94$) (experiment 2), and $\hat{Y}_3 = 142.4118 + 0.1600.\ X_3$ ($R^2 = 0.90$) (experiment 3).

The food remaining in the cups after larval consumption was separated from the feces and both were oven-dried, as described above, and weighed. To obtain the amount of food consumed by the larvae, the dry weight of the non-consumed food was subtracted from the initial dry weight of the food. At 48h after pupation, pupae were weighed, killed, and dried until constant weight to determine their dry weights.

Statistical analyses. A fully randomized block design was used for all experiments. The first and third experiments had 65 replicates each, and the second had 60 replicates. Larval mortality during each instar, total larval mortality, pupae weight (dry, in mg), total feeding time (days), food consumed (mg), and feces weight (mg) were evaluated.

The mortality was compared by using the chi-square test (χ^2), according to Banzatto & Kronka (1992), at a 5% probability level. The remaining variables were analyzed in three steps. In the first step, statistical tests were used to confirm if the data showed all the fundamentals for the analysis of variance (ANOVA), followed by Tukey and Kramer tests for comparison of treatment means, at a 5% probability level. In the last step, the analysis of covariance (ANCOVA) was carried out to estimate growth, food consumption, and the efficiency of assimilation and conversion of ingested and digested food into biomass, as proposed by Raubenheimer & Simpson (1992).

Insect growth and food consumption in the different treatments were obtained by adjusting, respectively, pupal weight and food consumption for the covariate feeding time. The efficiency in the conversion of ingested and digested food into biomass was calculated by the adjustment of pupal weight, respectively, for the food consumed and digested (the amount of food consumed minus the amount of feces produced by the larvae). The food assimilation was obtained after adjusting the amount of feces produced for the covariate amount of food consumed.

After the ANCOVA, whenever the interaction between the covariate and the treatment was significant, the effect of the treatments adjusted for the covariate was included. However, when the interaction (covariate x treatment) was not significant, the parallel-lines model was used, considering only the effect of the treatments. All statistical analyses were performed using the SAS - Statistical Analysis System statistical package (SAS Institute 1996).

Results

Experiment 1. Mortality, feeding time, pupal weight, food consumption, and feces weight of *A. gemmatalis*. Most of the treatments caused some mortality. The highest mortality ($\chi^2 = 51.86$, $P > 0.05$) was observed for larvae fed a diet containing 7gC + 0.65%R (38.29%), followed by those fed 0gC + 0.65%R (26.15%), 14gC + 0.65%R (10.77%), and 14gC and 0gC (1.54%). No mortality was observed on insects fed the 7gC diet.

A significant effect of the treatments on pupae weight, food consumption, feces weight, and feeding time was observed (Table 1). The weight of the pupae whose larvae ate the 0gC diet did not differ from pupae from larvae fed the 14gC and 7gC diets. The lowest pupal weights and longest feeding times were observed when larvae were fed on diets with rutin (0gC+0.65%R, 7gC+0.65%R, and 14gC+0.65%R). The highest consumption was observed in

Table 1 Means (±SE) of dry weight of pupae, consumed diet, feces and feeding time of *Anticarsia gemmatalis* fed on artificial diet containing different concentrations of casein (C) plus 0,65% rutin (R).

Diet	Weight (mg)			Feeding time (days)
	Pupae	Consumed diet	Feces	
14gC	75.37 ± 3.33 a	216.04 ± 7.83 ab	128.75 ± 4.43 ab	9.89 ± 0.22 c
14gC+R	42.36 ± 3.33 b	177.07 ± 7.83 c	109.22 ± 4.43 c	12.71 ± 0.22 b
7gC	75.24 ± 3.22 a	221.69 ± 7.56 a	123.98 ± 4.28 abc	9.58 ± 0.21 c
7gC+R	39.05 ± 4.21 b	167.68 ± 9.90 c	111.82 ± 5.60 bc	14.03 ± 0.28 a
0gC	67.21 ± 3.19 a	187.85 ± 7.50 bc	133.42 ± 4.24 a	9.84 ± 0.21 c
0gC+R	42.00 ± 3.84 b	154.96 ± 9.04 c	111.33 ± 5.11 bc	14.02 ± 0.25 a
F Values	24.64***	10.30***	5.09***	82.31***

[1]Means followed by the same letter on the column are not significantly different by Tukey and Kramer test, at 5% probability; *** P < 0.001.

larvae fed on rutin-free casein-based diets (7gC and 14gC), when compared to the other treatments. Larvae fed the 0gC diet produced the most feces.

Growth, food consumption, conversion efficiency of ingested and digested food into biomass, and assimilation of food of *A. gemmatalis* fed artificial diets with different concentrations of casein and 0.65% rutin. The ANCOVA indicated a significant and interactive effect between treatments and the covariates feeding time (Table 2a), food consumption (Table 2c), and food digested (Table 2d), regarding pupal weight. There was also an interaction between treatments and the covariate food consumption, regarding feces weight (Table 2c). There was no significant interactive effect between treatments and feeding time, regarding food consumption (Table 2a); consequently, this relationship can be represented by the parallel-lines model (Figs 1b).

The extension in larval feeding time (covariate) did not result in an increase in pupal weight, considering the negative or stable trends of the curves that represent the data obtained (Fig 2a). A negative relationship between food consumption

Table 2 Analysis of covariance (ANCOVA) adjusting *Anticarsia gemmatalis* pupae weight and consumed diet by covariate feeding time (a, b); pupae weight and feces weight by covariate consumed diet (c); and pupae weight by covariate digested food (d).

Variation	GL	F value		
		Pupae weight	Consumed diet	Feces weight
(a) Feeding time (covariate)	1	17.84***	8.43**	-
Treatment	5	3.54**	0.61 ^{NS}	-
Feeding time x treatment	5	2.26*	0.71 ^{NS}	-
Residual	298	-	-	-
(b) Treatment	5	-	3.12**	-
Feeding time	1	-	8.47**	-
Residual	303	-	-	-
(c) Consumed diet (covariate)	1	91.04***	-	701.48***
Treatment	5	1.45 ^{NS}	-	1.35 ^{NS}
Consumed diet x treatment	5	4.75**	-	4.32**
Residual	298	-	-	-
(d) Digested diet (covariate)	1	0.71 ^{NS}	-	-
Treatment	5	40.77***	-	-
Digested diet x treatment	5	4.32**	-	-
Residual	298	-	-	-

^{NS}Non significant; *P < 0.05; **P < 0.01; *** P < 0.001

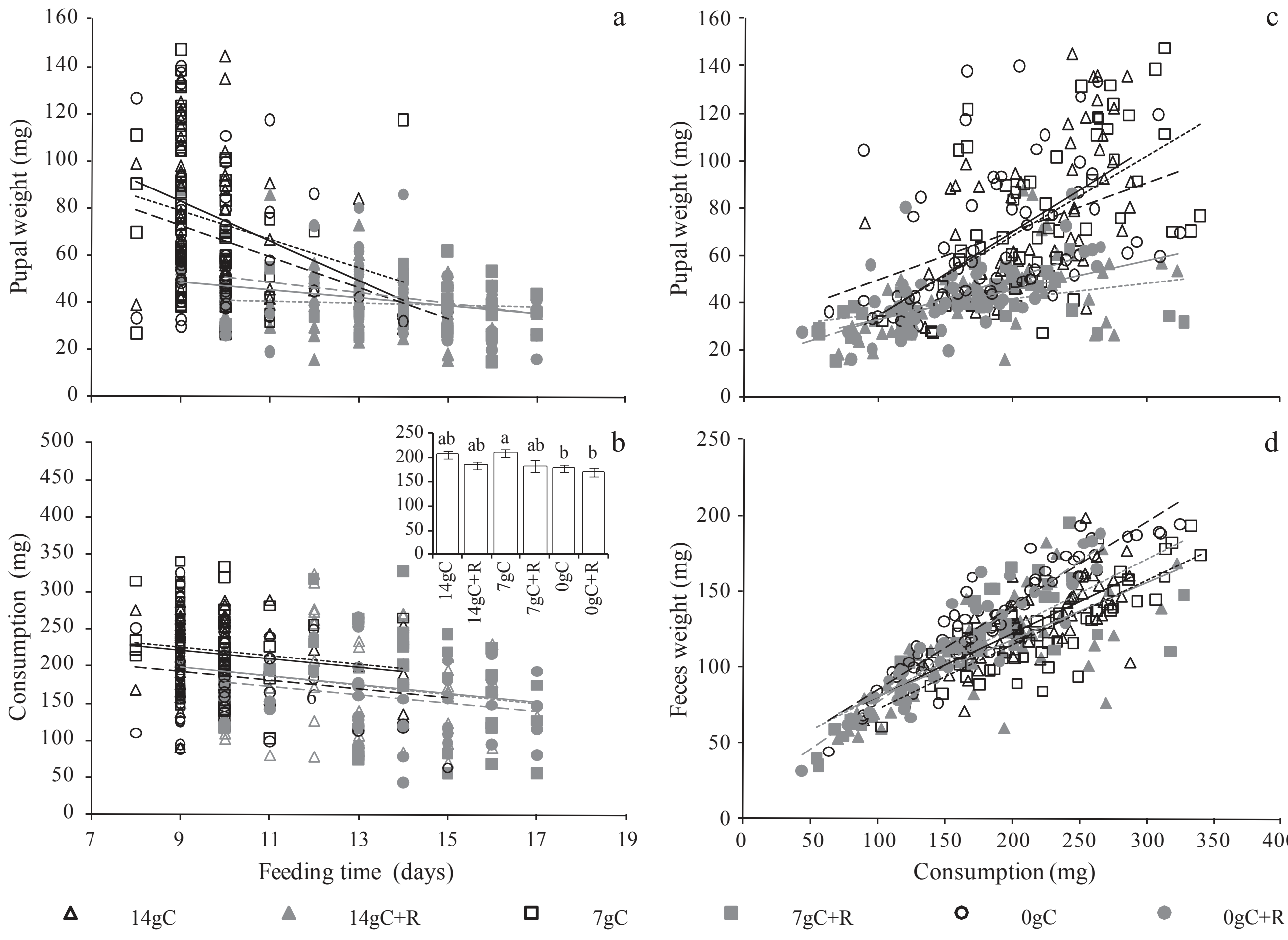

Fig 1 Relationship between feeding time (days) and pupal weight (mg) (a) or consumption (mg) (b), and relationship between consumption (mg) and pupal weight (mg) (c) or feces weight (mg) (d) of *Anticarsia gemmatalis* fed on artificial diet containing different concentrations of casein plus 0.65% rutin. Inserted graph shows ANCOVA means. (For statistical analysis see Table 2).

and insect feeding time (Fig 2b) was also observed; larvae that extended their feeding time did not consume more food. In Fig 2b, it is possible to observe the occurrence of a quantitative factor (Horton & Redak 1996) affecting larvae fed on the diet with rutin. The least-squares mean (graph insert, Fig 2b) indicates that the larvae fed the 0gC diet (without casein and without casein plus rutin) consumed less food than those fed on the 7gC diet without rutin.

Larvae fed on diets with rutin, 7gC+0.65%R, 14gC+0.65%R, and 0gC+0.65%R, were less efficient in converting the ingested food into biomass (pupal weight adjusted for the covariate consumption) (Fig 1c). The increase in consumption did not result in an increase in pupal weight. In the evaluation of food assimilation (Fig 1d), feces production was dependent on the different treatments, as well as on the quantity of ingested food. The data indicate a positive relationship between the response variable (weight of feces) and the covariate (consumption); larvae that consumed more food also produced more feces.

Larvae fed on diets with rutin, independently of casein concentration, were less efficient in converting the food digested [pupae weight adjusted by the covariate food digested (consumption – feces)] into biomass (Fig 2a), as suggested by the lower inflections observed in the curves that represent these treatments.

Experiment 2: Mortality, feeding time, pupa weight, food consumption, and feces weight of *A*. *gemmatalis*. Similarly to the results of the previous experiment, the highest mortality rate (χ^2 = 134,02, P > 05) occurred for the larvae fed the 7gC+1.30% R (64.98%) diet, followed by those fed the 0gC+ 1.30%R (53.32%), 14gC+ 1.30%R (49.98%), and 7gC (1.66%) diets. All the larvae fed the 14gC and 0gC diets survived.

The feeding time of the larvae was shorter for insects fed the 14gC diet without rutin, and longer for those fed the diets with rutin, 7gC+1.30%R, 0gC+1.30%R, and 14gC+1.30%R (Table 3). Larvae fed the 14gC+1.30% and 0gC+1.30%R diets had lower pupal weights when compared to those fed rutin-free casein-based diets (7gC and 14gC). The highest consumption was observed for insects fed the 14gC diet and the lowest on the 0gC diet; no statistically significant differences were observed among the other treatments (14gC+1.30%R, 7gC, 7gC+1.30%R, 0gC+1.30%R). The lowest amount of feces was observed in the 0gC treatment, differing from diets containing casein but without rutin (14gC and 7gC).

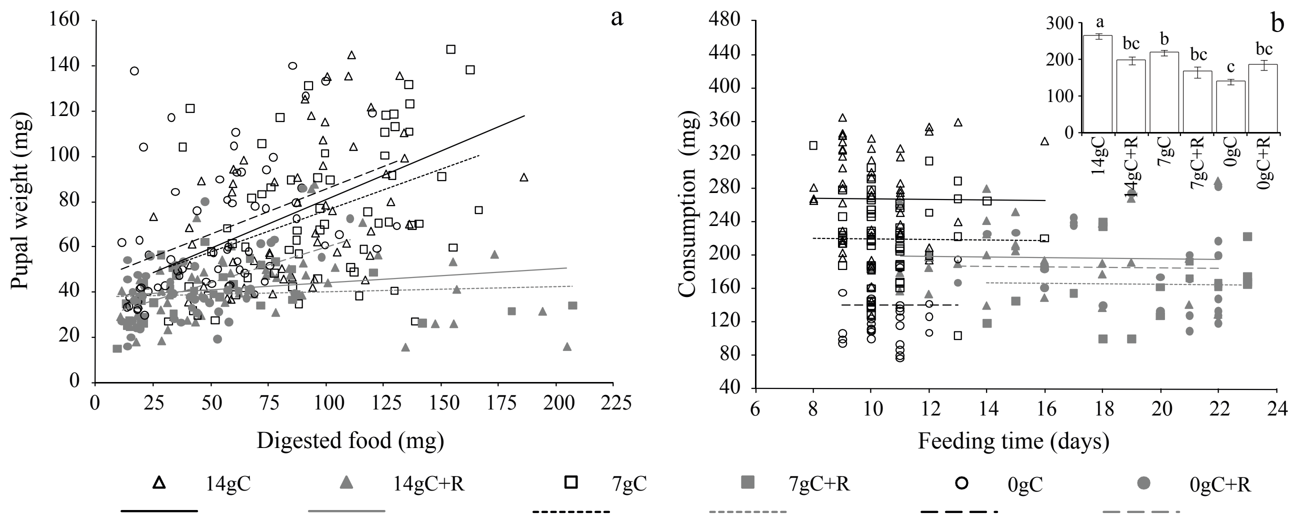

Fig 2 Relationship between weight of digested food (mg) and pupal weight and of *Anticarsia gemmatalis* fed on artificial diet containing different concentrations of casein plus 0.65% rutin (a), and relationship between feeding time (days) and consumption (mg) of *A. gemmatalis* fed on artificial diet containing different concentrations of casein plus 1.30% rutin (b). Inserted graph shows ANCOVA means. (For statistical analysis see Tables 2 and 4, respectively).

Growth, food consumption, conversion efficiency of ingested and digested food into biomass, and assimilation of food of *A. gemmatalis* fed artificial diets with different concentrations of casein and 1.30% rutin. The ANCOVA indicated that there was no interaction between treatments and the covariate feeding time, regarding either pupal weight or diet consumed (Table 4a). Also, there were no interactions between treatments and the covariates food consumption (Table 4c), or food digested (Table 4e) in their relationship to pupa weight. Therefore, the interactive effect was observed only between treatments and the covariate food consumption, regarding feces weight (Table 4c).

The insects' consumption was affected by the treatments (Table 4a), with no effect of feeding time (covariate). The analysis of the curves tendency in Fig 2b showed that the larvae fed on the diets containing rutin had an extended feeding time without increasing their consumption. Larvae fed the 14gC diet consumed the largest amount of food, followed by those fed the 7gC diet. The lowest consumption was observed for larvae fed the 0gC diet (graph insert, Fig 2b).

The conversion efficiency of the food ingested (pupa weight x food consumption covariate) showed a positive relationship (Fig 3a); the more food was consumed by the larvae, the higher was the pupae weight, with those fed the diets without casein and rutin being more efficient in the conversion of ingested food when compared to those fed standard diet with (14gC + R) or without rutin (14gC) (graph insert, Fig 3a). The insects fed the 0C or 7gC diets, but without rutin, converted more of the food ingested into biomass than those fed the 14g + R diet.

The interaction between the food consumption covariate and feces weight suggested that the production of feces depended on the treatment, but also on the quantity of food consumed (Fig 3b). The tendency curves of the data indicate a positive relationship; in other words, the

Table 3 Means (±SE) of dry weight of pupae, consumed diet, feces and feeding time of *Anticarsia gemmatalis* fed on artificial diet containing different concentrations of casein (C) plus 1.30% rutin (R).

Diet	Weight (mg)			Feeding time (days)
	Pupae	Consumed diet	Feces	
14gC	53.12 ± 1.33 a	266.83 ± 6.43 a	131.42 ± 3.91 a	10.18 ± 0.27 c
14gC+R	45.87 ± 1.82 b	197.25 ± 8.78 b	121.70 ± 5.35 ab	15.73 ± 0.37 b
7gC	53.35 ± 1.32 a	219.49 ± 6.37 b	131.76 ± 3.88 a	10.57 ± 0.27 c
7gC+R	47.42 ± 2.42 ab	164.71 ± 11.67 bc	111.58 ± 7.11 ab	19.82 ± 0.49 a
0gC	48.71 ± 1.31 ab	140.37 ± 6.32 c	101.68 ± 3.85 b	10.72 ± 0.26 c
0gC+R	46.04 ± 1.99 b	184.93 ± 9.62 bc	115.68 ± 5.86 ab	19.12 ± 0.40 a
F Values	4.63***	43.60***	8.48***	147.31***

Means followed by the same letter on the column are not significantly different by Tukey and Kramer, at 5% probability; *** P < 0.001.

Table 4 Analysis of covariance (ANCOVA) adjusting *Anticarsia gemmatalis* pupae weight and consumed diet by covariates feeding time (a, b); pupae weight and feces weight by covariate consumed diet (c, d); and pupae weight by covariate digested food (e, f).

Variation	GL	F value		
		Pupae weight	Consumed diet	Feces weight
(a) Feeding time (covariate)	1	0.07 [NS]	0.03 [NS]	-
Treatment	5	0.17 [NS]	1.89 [NS]	-
Feeding time x treatments	5	0.37 [NS]	0.95 [NS]	-
Residual	231	-	-	-
(b) Treatment	5	2.74 [NS]	40.23***	-
Feeding time	1	0.08 [NS]	0.03 [NS]	-
Residual	236	-	-	-
(c) Consumed diet (covariate)	1	97.27***	-	290.82***
Treatment	5	0.74 [NS]	-	8.30***
Consumed diet x treatment	5	0.64 [NS]	-	14.26***
Residual	231	-	-	-
(d) Treatment	5	6.76***	-	-
Consumed diet	1	98.01***	-	-
Residual	236	-	-	-
(e) Digested food (covariate)	1	40.81***	-	-
Treatment	5	1.09 [NS]	-	-
Digested food x treatment	5	0.98 [NS]	-	-
Residual	231	-	-	-
(f) Treatment	5	3.96**	-	-
Digested food	1	40.83***	-	-
Residual	236	-	-	-

[NS]Non significant; * P < 0.05; **P < 0.01; *** P < 0.001

higher the consumption, the more feces were produced. However, the tendency curve of the data for the insects fed the 14gC diet showed a lower inflection, suggesting higher food assimilation, considering that even with increased consumption, the larvae produced relatively less feces, probably retaining more food. The conversion of food digested into biomass (pupa weight x food digested covariate) (Fig 4a) followed the same tendency observed in the conversion of ingested food (Fig 3a), with larvae increasing the amount of food digested and producing heavier pupae. The insects fed the 7gC and 0gC diets were most efficient in converting digested food into biomass (graph insert, Fig 4a).

Experiment 3: Mortality, feeding time, pupae weight, food consumption, and feces weight of *A. gemmatalis*. According to the χ^2 test (P > 0.05), the treatments caused some mortality. Larvae fed the 0gC + 1.30%R diet showed the highest mortality rate (60%), followed by the 14gC + 1.30R (43.07%), 0gC+0.65R (27.70%), and 14gC+0.65R (18.46%) diets. Mortality rates of larvae fed the diets without rutin (14gC and 0gC) were 1.54% and 7.70%, respectively. The highest mortality percentages were observed during third instar for both treatments.

Table 5 shows that the 0gC + 1.30%R treatment yielded pupae with the lowest mean weight, differing from the other treatments; the larval weight in the standard diet (14gC) was the highest. In this treatment, higher consumption and shorter feeding time compared to the other treatments were also observed; larvae fed on diets without casein showed lower consumption and lower feces weight. The longest feeding time was observed in insects fed the 0gC + 1.30%R diet (15.0 ± 0.55 days).

Growth, food consumption, conversion efficiency of ingested and digested food into biomass, and food assimilation of *A. gemmatalis* fed an artificial diet with different concentrations of casein and rutin. An interactive effect (evaluated by ANCOVA) between treatment and food consumption covariate was observed, in relation to the amount of feces (Table 6c). However, there was no interactive effect between treatment and

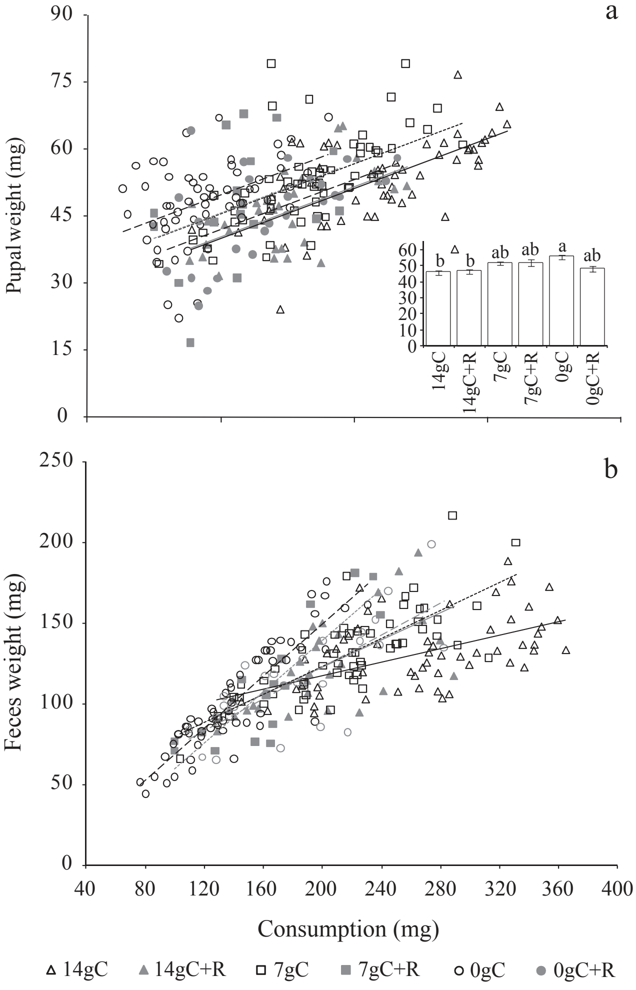

Fig 3 Relationship between consumption (mg) and pupal weight (mg) (a) or feces weight (mg) (b) of *A. gemmatalis* fed on artificial diet containing different concentrations of casein plus 1.30% rutin Inserted graph shows ANCOVA means. (For statistical analysis see Table 4).

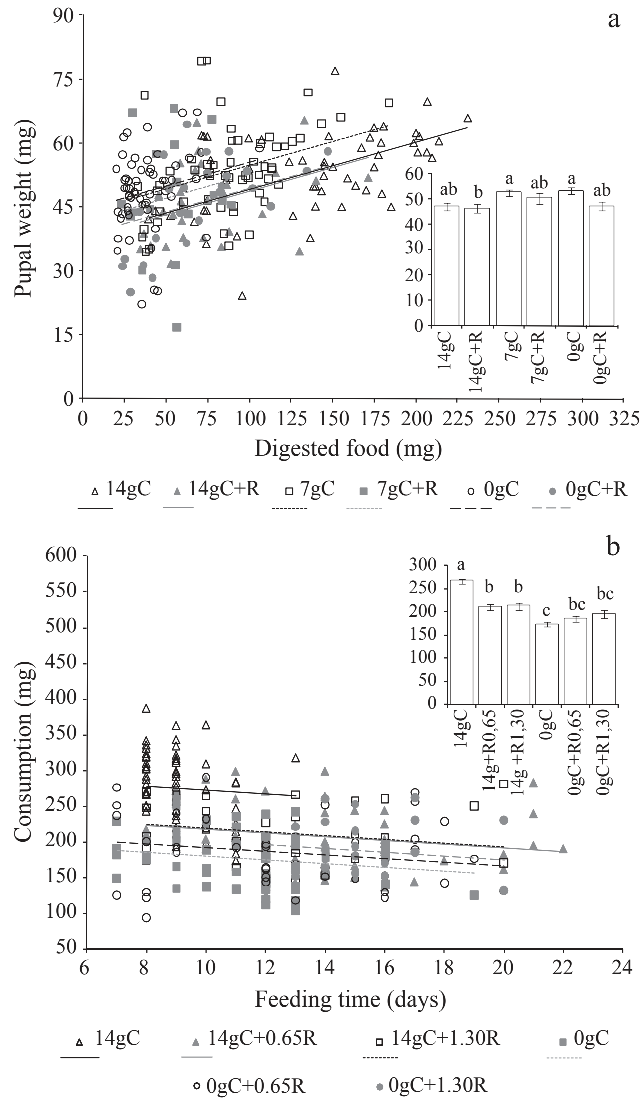

Fig 4 Relationship between weight of digested food (mg) and pupal weight (mg) of *A. gemmatalis* fed on artificial diet containing different concentrations of casein plus 1.30% rutin (a), and relationship between feeding time (days) and consumption (mg) of *A. gemmatalis* fed on artificial diet containing different concentrations of casein plus plus 0.65% and 1.30% rutin (b). Inserted graph shows ANCOVA means. (For statistical analysis see Tables 4 e 6, respectively).

the feeding time covariate (Table 6a), in its relationship to pupae weight and to food consumption. Additionally, no interaction between treatment and food consumption (covariate), in the conversion of ingested food into biomass evaluated by the relationship consumption x pupa weight (Table 6c), nor of conversion of digested food into biomass (digested food x pupa weight, Table 6e) was detected. As, in this last case, there was no significant effect of the covariate (Table 4f), the data obtained by ANOVA were accepted as valid.

The insects' consumption (Fig 4a) was negatively related to feeding time; in other words, those insects fed for a longer period (14gC + 0.65%R, 14gC + 1.30%R, 0gC + 0.65%R, and 0gC + 1.30%R) did not show a proportional increase in the quantity of food consumed. The ANCOVA mean (graph insert, Fig 4a) indicates that the insects fed a standard diet (14gC) consumed more when compared to the other treatments.

In the evaluation of the conversion efficiency of ingested food by ANCOVA (Fig 5a), a positive relationship between the factors was observed; the more the larvae consumed, the higher the pupae weight. Insects fed the standard diet (14gC) were most efficient in converting food into biomass; the least efficient insects were those fed the 0gC+1.30%R diet, followed by the 14gC + 1.30%R diet (graph insert, Fig 5a).

The food consumption (covariate) and the treatments significantly affected the feces weight; the amount of feces produced by the insects was dependent on an interactive effect between the covariate and treatments. In the dispersion graph (Fig 5b), again a positive relationship between the

Table 5 Means (±SE) of dry weight of pupae, consumed diet, feces and feeding time of *Anticarsia gemmatalis* fed on artificial diet containing different concentrations of casein (C) and 0.65% and 1.30% rutin (R).

Diet	Weight (mg)			Feeding time (days)
	Pupa	Consumed diet	Feces weight	
14gC	59.58 ± 0.88 a	274.33 ± 5.69 a	140.98 ± 4.11 a	9.05 ± 0.36 d
14g+0.65%R	46.02 ± 0.10 c	206.74 ± 6.41 b	113.92 ± 4.63 bc	14.06 ± 0.40 ab
14g+1.30%R	42.64 ± 1.24 c	211.29 ± 7.98 b	131.25 ± 5.77 ab	12.90 ± 0.50 bc
0gC	50.90 ± 0.91 b	175.33 ± 5.84 c	107.45 ± 4.22 c	11.46 ± 0.37 c
0gC+0.65%R	44.50 ± 1.11 c	184.55 ± 7.11 bc	122.02 ± 5.14 bc	12.56 ± 0.45 bc
0gC+1.30%R	37.25 ± 1.36 d	187.27 ± 8.72 bc	124.53 ± 6.30 abc	15.04 ± 0.55 a
F value	54.65***	36.57***	7.74***	26.34***

Means followed by the same letter on the column are not significantly different by Tukey and Kramer, at 5% probability; *** P < 0.001.

factors could be observed. However, the tendency curve of the graph indicates that the insects fed a standard diet (14gC) retained more food by producing smaller amounts of feces, even when they increased their consumption. Therefore, they were more efficient in assimilating food, which probably explains their better overall performance.

Tabela 6 Analysis of covariance (ANCOVA) adjusting *Anticarsia gemmatalis* pupae weight and consumed diet by covariates feeding time (a, b); pupae weight and feces weight by covariate consumed diet (c, d); and pupae weight by covariate digested food (e, f).

Variation	GL	F value		
		Pupae weight	Consumed diet	Feces weight
(a) Feeding time (covariate)	1	0.01 NS	6.94**	-
Treatment	5	5.01***	3.10**	-
Feeding time x treatment	5	2.14 NS	1.44 NS	-
Residual	251	-	-	-
(b) Treatment	5	38.30***	27.33***	-
Feeding time	1	0.01 NS	6.88**	-
Residual	256	-	-	-
(c) Consumed diet (covariate)	1	11.22**	-	318.73***
Treatment	5	0.71 NS	-	1.25 NS
Consumed diet x treatment	5	1.84 NS	-	2.98*
Residual	251	-	-	-
(d) Treatment	5	37.59***	-	-
Consumed diet	1	11.04**	-	-
Residual	256	-	-	-
(e) Digested food (covariate)	1	1.35 NS	-	-
Treatment	5	2.99*	-	-
Digested food x treatment	5	1.58 NS	-	-
Residual	251	-	-	-
(f) Treatment	5	35.97***	-	-
Digested food	1	1.34 NS	-	-
Residual	256	-	-	-

NS Non significant; *P < 0.05; **P < 0.01; *** P < 0.001

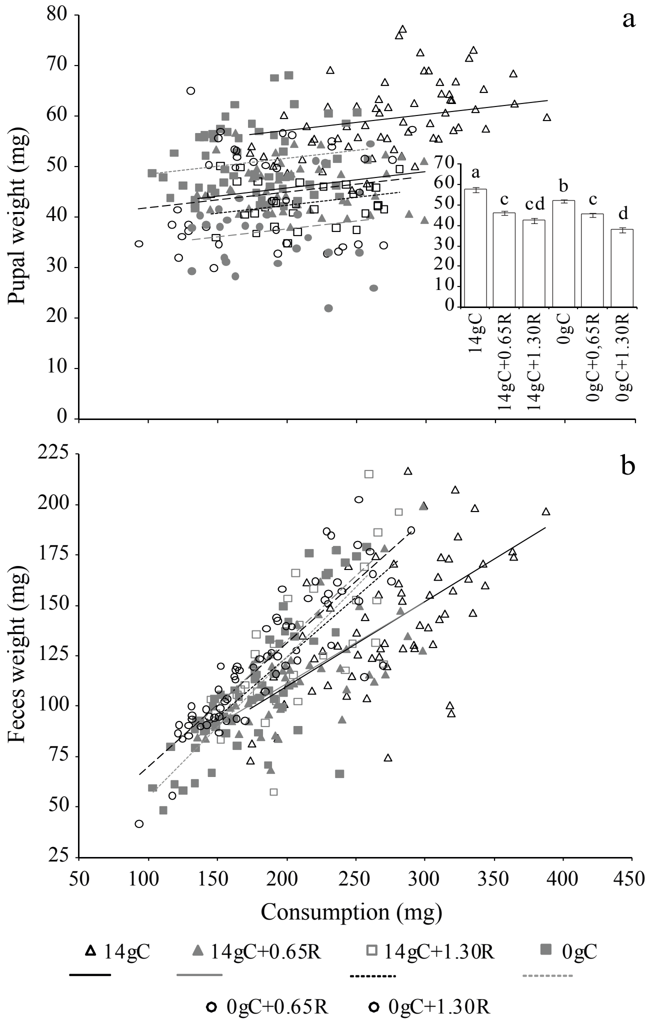

Fig 5 Relationship between consumption (mg) and pupal weight (mg) (a) or feces weight (mg) (b) of *A. gemmatalis* fed on artificial diet containing deferent concentrations of casein plus 0.65% and 1.30% rutin. Inserted graph shows ANCOVA means. (For statistical analysis see Table 6).

Discussion

The highest mortality rates were observed for insects fed diets with half the concentration of casein in relation to the standard diet used for mass rearing of *A. gemmatalis*, or without added casein, but containing rutin. According to Stamp (1994), flavonoids generally affect defoliating insects mainly during instar changes and *A. gemmatalis* showed an accentuated mortality rate during their moulting to the last instar. During this stage, insects generally increase food consumption and will, consequently, ingest a higher amount of allelochemicals. Flavonoids incorporated to artificial diets also caused higher mortality of sixth instars of *A. gemmatalis* (Gazzoni *et al* 1997). In this particular case, flavanoids were suggested to alter the activity of enzymes and hormones, blocking biochemical pathways and reducing the assimilation of essential substances and nutrient storage.

The prolongation of the feeding time of *A. gemmatalis* larvae fed on rutin-diet may have been a tentative to uptake the required nutrients to enhance survival. However, *A. gemmatalis* fed diets with different rutin concentrations in comparison to rutin-free diets were also shown to have reduced food consumption (Hoffmann-Campo *et al* 2006, Piubelli *et al* 2006), indicating the larva attempted to avoid poisoning by eating smaller meals, for a prolonged time, to obtain the ideal weight for pupation. Although this strategy allows them to survive, the prolonged larval development will certainly make them largely prone to the attack of their natural enemies in field conditions, increasing their mortality risks.

In general, our results showed that larvae fed a diet containing rutin in any concentration consumed less food and were less efficient in converting food to biomass, as was also the case for larvae on a casein-free diet without added rutin. During choice-tests with *Schistocerca americana,* Bernays & Raubenheimer (1991) observed that insects on low-protein diets supplemented with rutin fed smaller portions of food for longer periods, while in long term experiments, rutin negatively affected not only *S. americana* growth, but also the conversion of ingested food to biomass (Bernays *et al* 1991), as demonstrated in here for *A. gemmatalis*. The lower consumption of the caterpillars in treatments without casein is probably due to the lack of phagostimulatory effects attributed of this protein (Parra 1979), as observed for *Ostrinia nubilalis* (Beck 1956).

A shorter feeding time, higher larval and/or pupal weight, and lower mortality rates are parameters that indicate an adequate growth rate for insects (Soo Hoo & Fraenkel 1966). *Anticarsia gemmatalis* fed on diets containing rutin, especially on the 7gC and 0gC casein diets, did not show adequate growth rates compared to insects fed standard diets with rutin. Furthermore, the effect of the flavonoid became more pronounced only when insects were fed an unbalanced diet (with a low concentration or lack of casein). Our results did not corroborate those of Duffey & Stout (1996), who stated that high-energy proteins such as casein can accentuate the negative effects of phenolic compounds (chlorogenic acids and rutin) to lepidopteran larvae. Considering the results obtained so far, we can suggest that with an adequate concentration of casein, there is no interactive effect of this protein with the flavonoid rutin on the biology of the velvetbean caterpillar, and thus, casein does not increase the adverse effects of rutin on *A. gemmatalis* larvae.

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References

Banzatto D A, Kronka S N (1992) Experimentação agrícola. Funep, Jaboticabal, 247p.

Beck S D (1956) Nutrition of the European corn borer, *Pyrausta nubilalis* (Hübn.). IV. Feeding reactions of the first instar larvae. Ann Entomol Soc Am 49: 399-405.

Bernays E A, Howard J J, Champagne D, Estesen B J (1991) Rutin: a phagostimulant for polyphagous acridid *Schistocerca americana*. Entomol Exp Applic 60: 19-28.

Bernays E A, Raubenheimer D (1991) Dietary mixing in grasshopers: changes in acceptability of different plant secondary compounds associated with low levels of dietary protein (Orthoptera: Acrididae). J Insect Behav 5: 545-556.

Dixon R A, Steele C L (1999) Flavonoids and isoflavonoids – a gold mine for metabolic engineering. Trends Plant Sci 4: 394-400.

Duffey S S, Stout M J (1996) Antinutritive and toxic components of plant defense against insects. Arch Insect Biochem Physiol 32: 3 -37.

Gazzoni D L, Hulsmeyer A, Hoffmann-Campo C B (1997) Efeito de diferentes doses de rutina e quercitina na biologia de *Anticarsia gemmatalis*. Pesqu Agropecu Bras 32: 673- 681.

Greene G L, Leppla N C, Dickerson WA (1976) Velvetbean caterpillar: a rearing procedure and artificial medium. J Econ Entomol 69: 487-488.

Harborne J B, Grayer R J (1993) Flavonoids and insects, p. 589-618. in Harborne J B (ed), The flavonoids, advances in research since 1986. London, Chapman and Hall, 676p.

Hoffmann-Campo C B, Harbone J B, Mcaffery A R (2001) Pre-ingestive and post-ingestive effects of soya bean extracts and rutin on *Trichoplusia ni* growth. Entomol Exp Applic 98: 181-194.

Hoffmann-Campo C B, Oliveira E B, Moscardi F (1985) Criação massal da lagarta da soja *Anticarsia gemmatalis*. EMBRAPA-CNPSo, Documentos 10. Londrina, 23p.

Hoffmann-Campo C B, Ramos Neto J A, Oliveira M C N de, Oliveira L J (2006) Detrimental effect of rutina on *Anticarsia gemmatalis*. Pesq Agropec Bras 41: 1453-1459.

Horton D R, Redak R A (1996) Further comments on analysis of covariance in insect dietary studies. Entomol Exp Appl 69: 263-275.

Panizzi A R, Parra J R P (1991) Ecologia nutricional de insetos e sua aplicação no manejo de pragas. Manole/CNPq, São Paulo, 359p.

Parra J R P (1979) Biologia dos Insetos ESALQ, Piracicaba, 383p., mimeographed.

Piubelli G C, Hoffmann-Campo C B, Moscardi F, Miyakubo S H Oliveira M C N de (2005) Are chemical compounds important for soybean resistance to *A. gemmatalis*? J Chem Ecol 31: 1509-1524.

Piubelli G C, Hoffmann-Campo C B, Moscardi F, Miyakubo S H, Oliveira M C N de (2006) Baculovirus resistant *A. gemmatalis* responds differently to dietary rutin. Entomol Exp Appl 119: 53-60.

Raubenheimer D, Simpson S J (1992) Analysis of covariance: an alternative to nutritional indices. Entomol Exp Appl 62: 221-231.

SAS Institute (1996) SAS Propriety Software Release 6.12. SAS-STAT User´s guide, Vol II. SAS Institute Inc., Cary, N C, 1686p.

Simmonds M S J (2001) Importance of flavonoids in insect-plant interactions: feeding and oviposition. Phytochemistry 56: 245-252.

Soo Hoo C F, Fraenkel G (1966) The selection of food plants in a polyphagous insect, *Prodenia eridania* (Cramer). J Insect Physiol 12: 693-709.

Stamp N E (1994) Simultaneous effects of potassium, rutin and temperature on performance of *Manduca sexta* caterpillars. Entomol Exp Appl 72: 135-143.

Stamp N E, Skrobola C M (1993) Failure to avoid rutin diets results in altered food utilization and reduced growth rate of *Manduca sexta* larvae. Entomol Exp Appl 68: 127-142.

Vanderzant E S (1966) Development, significance and application of artificial diet for insect. Ann Rev Ent 19: 139-405.

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