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Effect of the flavonoid rutin on the biology of *Spodoptera frugiperda* (Lepidoptera: Noctuidae)

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ABSTRACT. The fall armyworm *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) is a major pest of maize crops in Brazil. The effects of plant metabolites on the biology and behavior of insects is little studied. The aim of the study was to evaluate the activity of rutin on the biology of the *S. frugiperda* by using artificial diets containing rutin. The study evaluated four treatments: regular diet (control group) and diets containing 1.0, 2.0 and 3.0 mg g^{-1} of rutin. The following biological variables parameters of the larvae were evaluated daily: development time (days), larval and pupal weight (g) and viability (%), adult longevity and total life cycle (days). A completely randomized experimental design was used with 25 replication. The rutin flavonoid negatively affected the biology of *S. frugiperda* by prolonging the larval development time, reducing the weight of larvae and pupae and decreasing the viability of the pupae. The addition of different concentrations of rutin prolonged the *S. frugiperda* life cycle. The use of plant with insecticidal activity has the potential with strategy in IPM.

Keywords: fall armyworm, insecticide plant, secondary metabolite, flavonoid.

Efeito do flavonoide rutina na biologia de *Spodoptera frugiperda* (Lepidoptera: Noctuidae)

RESUMO. A lagarta *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) é a principal praga da cultura do milho no Brasil. A ação de substância química de defesa de planta, na biologia e comportamento de inseto é pouco estudada. O objetivo do trabalho foi avaliar a ação de rutina e os efeitos na biologia da lagarta *S. frugiperda*, utilizando-se dieta artificial acrescida com este flavonoide. O trabalho consistiu de quatro tratamentos: dieta normal (testemunha) e dieta com adição de 1,0; 2,0 e 3,0 mg g^{-1} de rutina. As lagartas foram avaliadas diariamente, observando os parâmetros biológicos: período, peso e viabilidade larval, período, peso e viabilidade pupal, longevidade de adultos e ciclo total. Utilizou-se o delineamento experimental inteiramente casualizado com 25 repetições. O flavonoide rutina mostrou-se influenciar de forma negativa a biologia de *S. frugiperda* por prolongar o período larval, diminuir o peso larval e pupal e viabilidade pupal. A adição de rutina nas diferentes concentrações prolongou o ciclo de *S. frugiperda*. O uso de planta com atividade inseticida tem potencial como estratégia em MIP.

Palavras-chave: lagarta do cartucho, planta inseticida, metabolito secundário, flavonoide.

Introduction

The fall armyworm *Spodoptera frugiperda* (J.E Smith) (Lepidoptera: Noctuidae) is a major pest species of maize crops (Cruz, Figueiredo, Oliveira, & Vasconcelos, 1999; Pereira et al., 2002). *Spodoptera frugiperda* is a polyphagous species that infests cotton (Campos, Boiça-Júnior, Valério Filho, Campos, & Campos, 2012; Jesus, Boiça Junior, Alves, & Zanuncio, 2014), rice, millet, sorghum (Busato et al., 2004), soybean (Boiça Junior, Souza, Neves, Ribeiro, & Stout, 2015) and other crops. In Brazil, one of the factors that contribute to failures in the control of *S. frugiperda* is the large number of hosts caused by the succession of crops with different phenologies (Sá, Fonseca, Boregas, & Waquil, 2009; Barros, Torres, & Bueno, 2010).

The larvae of *S. frugiperda* feed on maize leaves and ears, reducing the photosynthetic capacity of the plant and its production because of damage to the reproductive structures (Lima, Ohashi, Souza, & Gomes, 2006). This damage depends on the plant phenological state, infestation period and intensity of pest infestation (Cruz et al., 1999).

Spodoptera frugiperda control has been mainly performed with chemical insecticides (Yu, Nguyen, & Abo-Elghar, 2003). To reduce the use of chemical controls, alternative techniques are being studied. Among these alternatives, plant resistance to insects and the use of plant metabolites with insecticidal effects have shown promising results in integrated pest management programs (Pereira et al., 2002; Hoffmann-Campo, Ramos Neto, Oliveira, & Oliveira, 2006; Jesus et al., 2014).

Resistance can be express by morphological characteristics or the presence of chemical metabolites (allelochemicals), such as alkaloids, flavonoids, terpenoids, sterols etc., present in the plants (Piubelli, Campo, Moscardi, Miyakubo, & Oliveira, 2005; Hoffmann-Campo et al., 2006; Magarelli, Lima, Silva, Souza, & Castro, 2014).

The activity of plant chemical substances has been promising, and new components with insecticidal potential have been discovered, and they have the potential for use in pest management for crops of agricultural importance (Pereira et al., 2002; Deota & Upadhyay, 2005; Rajamma, Dubey, Sateesha, Tiwan, and Ghosh, 2011; Tavares, Pereira, Freitas, Serrão, & Zanuncio, 2014).

Rutin is a flavonoid that can be used in plant protection against insects, especially lepidopterans, because of its anti-nutritional effects (Harborne & Grayer, 1993; Salvador et al., 2010; Tavares et al., 2014). In soybean, the flavonoids rutin and genistin were identified in several parts of the plant (Piubelli et al., 2005; Hoffmann-Campo et al., 2006; Lucci & Mazzafera, 2009).

Rutin can prolong the life cycle of *Anticarsia gemmatalis* Hubner, 1818 (Lepidoptera: Noctuidae) and cause higher larval mortality when it is added to the insect diet (Gazzoni, Hulsmeyer, & Hoffmann-Campo, 1997; Hoffmann-Campo et al., 2006; Piubelli, Hoffmann-Campo, Moscardi, Miyakubo, & Oliveira, 2006). Larvae of *Manduca sexta* Linnaeus, 1763 (Lepidoptera: Sphingidae) fed a diet containing rutin showed negative growth performance (Stamp & Skrobola, 1993).

The addition of 2% rutin to the diet of *Trichoplusia ni* Hubner, 1803 (Lepidoptera: Noctuidae) negatively affected the survival, behavior, and physiology of the insect (Hoffmann-Campo, Harbone, & Mcaffery, 2001). In *S. frugiperda,* the metabolite astilbin from the *Dimorphandra mollis* (Fabaceae - Mimosoideae) plant reduced the larval weight and prolonged the larval and pupal stages (Pereira et al., 2002).

Therefore, the current study aimed to evaluate whether diets containing the flavonoid rutin affected the biology of *S. frugiperda*.

Material and methods

Spodoptera frugiperda mass rearing

The methodology proposed by Cruz (2000) with adjustments (Jesus et al., 2014) was used to obtain the *S. frugiperda* larvae. Briefly, male and female moths were maintained in polyvinyl chloride (PVC) tubes (10 cm diameter; 21.5 cm height) that were internally coated with bond paper sheets for egg laying and covered with voile at the top to prevent insect escape.

Cotton pads soaked in 10% honey solution were placed on top of the cages for moth feeding and changed every two days. The bond paper sheets with egg masses were recovered daily, and the egg masses were cut from the sheets with the aid of scissors and then transferred to plastic containers (100 mL) containing 5 g of the artificial diet (Kasten Júnior, Precetti, & Parra, 1978). These containers were covered and maintained in a climate-controlled room (temperature 27 ± 2°C, 70 ± 10% relative humidity (RH) and 12 hours photophase).

The larvae were individually housed when they reached the second instar (3 days, approximately 4 mm) because of their cannibalistic habits. The individuals were placed in 50 mL plastic cups containing 5 g of artificial diet, and the cups were sealed with acrylic lids and maintained in a climate-controlled room until the pupae developed. Subsequently, the pupae were segregated using sexual dimorphism as a parameter to differentiate males and females (Luginbill, 1928), and seven pairs of male and female moths were maintained in each cage.

Purification and structural characterization of rutin

Technical grade rutin was purchased and purified in the laboratory by recrystallization using methanol as the solvent.

After recrystallization, the isolated crystals were characterized by nuclear magnetic resonance spectroscopy (NMR H^1, C^{13}) (Figure 1).

The spectra were obtained at the Laboratory of Nuclear Magnetic Resonance (Laboratório de Ressonância Magnética Nuclear - RMN) of the Chemistry Institute (Instituto de Química - IQ) of the Federal University of Goiás (Universidade Federal de Goiás - UFG).

Once isolated, the rutin crystals were characterized by NMR H^1 (Figure 2a) and C^{13} spectra (Figure 2b) and compared with data from the literature to verify the purity of the rutin.

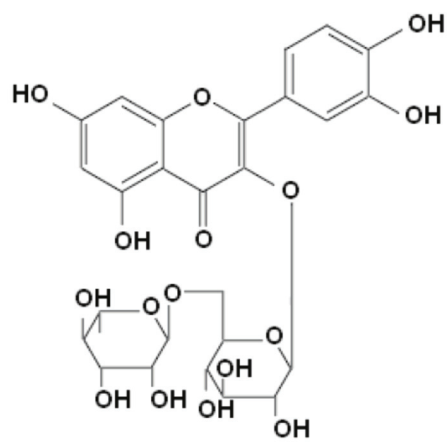

Figure 1. Chemical structure of rutin (quercetin 3 - *O* rhamnosyl glucoside).

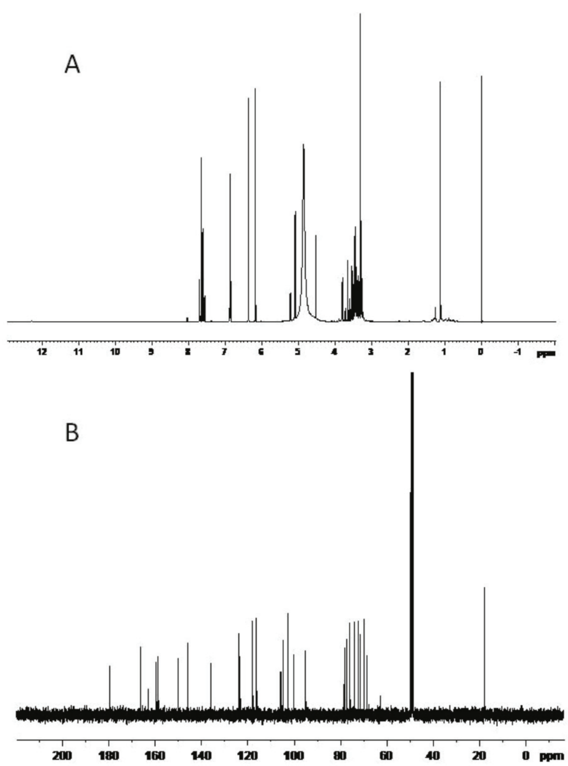

Figure 2. Spectra of rutin H^1 (A) and rutin C^{13} (B).

Biology of *Spodoptera frugiperda* fed rutin

The experimental design used in the current study was completely randomized with 4 treatments and 25 replicates, and each plot consisted of a 50 mL plastic cup. Five grams of the diet containing the flavonoid rutin at concentrations of 0 (control), 1, 2 and 3 mg g^{-1} and one newly hatched caterpillar were placed in each of the cups to monitor the pest life cycle.

The following biological parameters were evaluated: larvae and pupae development time and viability, weight of 10 day old larvae, weight of 24 hours old pupae, longevity and adult total life cycle.

Statistical analysis

The data were subjected to an analysis of deviance (Anodev), and the regression models were fitted with linear predictors containing polynomial effects of the first, second and third degrees for the rutin concentration. A Poisson distribution (Poisson regression) was assumed for the parameters larvae and pupae development time, total life cycle and adult longevity. For larval and pupal viability, a binomial distribution (binomial regression) was assumed. The weight of the larvae and pupae fit a Gaussian model. The nominal level of significance was 5%. The statistical analyses were performed using the software R version 3.0.3 (R Core Team, 2014).

Results and discussion

The development time of larvae and pupae of *S. frugiperda* can be observed in Figure 3. As shown, these parameters were affected by the different concentrations of rutin and there was a linear effect ($p < 0.001$) of rutin concentration on the larval development time, with higher concentrations of rutin inducing longer larval development time. A concentration of 3.0 mg g^{-1} of rutin prolonged the larval development time by 8 days on average compared with the control group (0 mg g^{-1}).

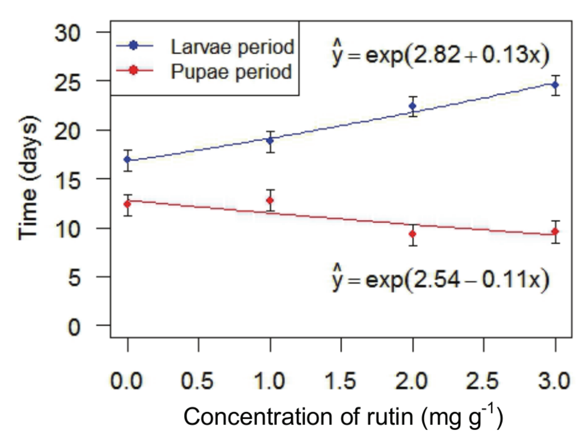

Figure 3. Development time (days) of the larvae and pupae of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) fed an artificial diet containing different concentrations of rutin.

A linear effect ($p = 0.0059$) of rutin concentration on pupal development time was also observed; however, the effect was opposite to that of the larvae, with lower concentrations of rutin prolonging the developmental time of *S. frugiperda* pupae.

The shorter development time and lower weight of *S. frugiperda* larvae fed diets containing rutin may be associated with lower food intake by the caterpillars. This phenomenon may be related to the allelochemicals acting as feeding deterrents and digestion inhibitors and forming free radicals (Pereira et al., 2002; Salvador et al., 2010).

The weight of the larvae and pupae of *S. frugiperda* fed artificial diets containing different concentration of rutin can be observed in Figure 4. A linear effect ($p < 0.001$) was observed for the rutin concentrations on the larval and pupal weight.

Higher rutin concentrations correlated with lower weights in the *S. frugiperda* larvae and pupae (Figure 4). In the control group (0 mg g^{-1} rutin), the mean larval and pupal weights were 0.37 and 0.29 g, respectively, whereas at a rutin concentration of 3 mg g^{-1}, the mean larval weight drastically decreased to 0.15 g and mean pupal weight decreased to 0.23 g.

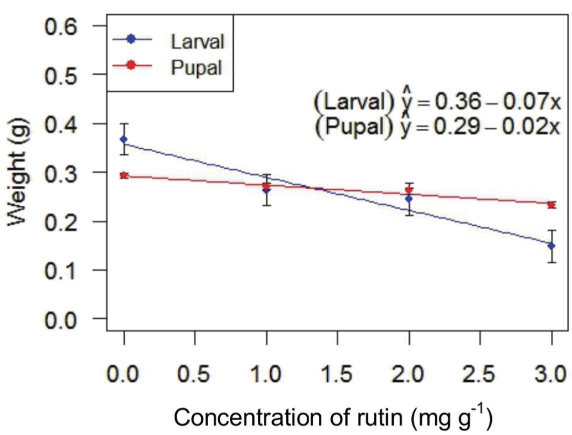

Figure 4. Weight (g) of the larvae and pupae of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) fed an artificial diet containing different concentrations of rutin.

Many flavonoids can act as feeding deterrents for phytophagous insects, even at relatively low concentrations (Harborne & Grayer, 1993; Pereira et al., 2002). The deterrent effect is related to astringency, which is caused by phenolic compounds precipitating proteins (Appel & Maines, 1995). Reduced food intake and its consequent deleterious effects were also observed in *A. gemmatalis* fed artificial diets containing rutin (Hoffmann-Campo et al., 2006; Piubelli et al., 2006).

The nutritional quality of the diet without the flavonoid rutin was likely suitable for *S. frugiperda* larval development. Therefore, the adverse effects observed in the larvae fed diets containing rutin most likely occurred because of the presence of toxins in the artificial diets (Harborne & Grayer, 1993; Hoffmann-Campo et al., 2006; Piubelli et al., 2006; Salvador et al., 2010). These toxins may have produced damage in the digestive tract of *S. frugiperda* that was similar to the damage in the midgut of *A. gemmatalis* fed soybean genotypes containing the flavonoid rutin (Salvador et al., 2010).

The biological parameters of *S. frugiperda* pupae were affected by rutin concentrations in the diet, and the negative effects on the pupal stage were most likely related to the larval-pupal metamorphosis during ecdysis (Gazzoni et al., 1997). This suggests that rutin had a negative effect on the activity of enzymes and hormones, blocked biochemical pathways and reduced the assimilation of essential substances or the formation of reserves in the insect (Salvador et al., 2010; Tavares et al., 2014).

A significant effect on the larval viability was not observed ($p > 0.05$) for any of the rutin concentrations (Figure 5); however, an effect was observed for pupal viability ($p < 0.001$), although only the cubic effect was significant ($p < 0.001$). All of the pupae that were fed diets containing 2 mg g^{-1} rutin developed to adults, whereas insects fed diets containing 1 and 3 mg g^{-1} rutin had the lowest pupal viability (57.14%).

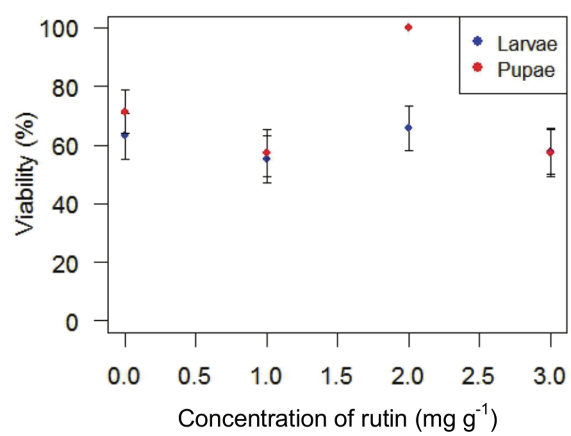

Figure 5. Viability (%) of the larvae and pupae of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) fed an artificial diet containing different concentrations of rutin.

A linear effect ($p < 0.001$) was observed for the total life cycle of *S. frugiperda* fed diets with different rutin concentrations, with concentrations of 3 mg g^{-1} prolonging the life cycle by ten days on average compared with insects from the control group (0 mg g^{-1}) (Figure 6). The adult longevity was not affected ($p > 0.05$) by the different rutin concentrations.

These data corroborate those obtained by Hoffmann-Campo, Ramos Neto, Oliveira, and

Oliveira (2006), Piubelli, Hoffmann-Campo, Moscardi, Miyakubo, and Oliveira (2006) and Salvador et al. (2010) who studied the biological parameters of *A. gemmatalis* fed diets containing rutin. These authors observed a negative effect of rutin on the feeding and biology of this insect at the larval stage and pupal stage, including reduced weight of pupae fed diets containing the flavonoid (Hoffmann-Campo et al., 2006; Salvador et al., 2010).

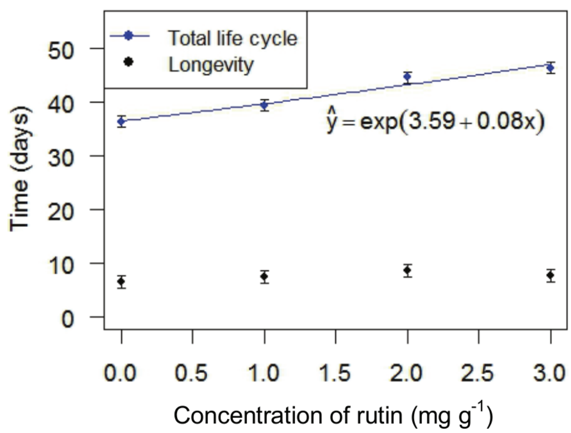

Figure 6. Total life cycle (days) and longevity (days) of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) fed an artificial diet containing different concentrations of rutin.

In general, a negative effect on the biology of *S. frugiperda* was observed at the highest concentrations of the flavonoid rutin. The total life cycle of the insect was prolonged, which is a method used by insects to overcome dietary deficiencies and store more lipids for direct use by the adults (Panizzi, 1991).

Conclusion

The flavonoid rutin negatively affected the biology of *S. frugiperda* by prolonging the larval development time, reducing the larval and pupal weight and decreasing the pupal viability.

The addition of different concentrations of rutin prolonged the life cycle of *S. frugiperda*; therefore, the use of rutin is indicated in future studies evaluating the control of *S. frugiperda*.

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