

Sulphur sources on the management of *Scaptocoris castanea* (Hemiptera: Cydnidae) on cotton

Fuentes de azufre sobre el control de *Scaptocoris castanea* (Hemiptera: Cydnidae) en algodón

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Abstract: Soil fertility with sulphur sources is an important tool that can be used in the management of pests. Sulphur can induce tolerance or even cause mortality to insect pests. This study evaluated the application of sulphur sources on the population dynamics of *Scaptocoris castanea* (Hemiptera: Cydnidae) and its effects on cotton plants. The experiment had a factorial design consisting of a combination of four concentrations of calcium sulfate (CaSO_4) and four concentrations of ammonium sulphate $[(\text{NH}_4)_2\text{SO}_4]$. There was no correlation between soil humidity (%) and insect infestation (%) (nymphs + adults), except for the treatment without calcium sulfate (0 kg/ha) along with 208 kg/ha of ammonium sulfate. There was a significant difference between average and previous infestation of *S. castanea* with the application of only 1,500 kg/ha of calcium sulfate. The relationship between cotton yield (kg/ha) and ammonium sulfate was best described by a cubic function at concentrations of 750, 1,125 and 1,500 kg/ha of calcium sulfate; on the other hand, a linear function was best adjusted in the absence of calcium sulfate. Sulphur sources influenced the visual appearance of the plants as many plants were found with injuries and/or were reduced in size in those treatments without application of any sulphur sources. These results reinforce the idea that sulphur sources may be useful in the management of *S. castanea* on cotton.

Key words: Control. Brown root stinkbug. Ammonium sulfate. Calcium sulfate.

Resumen: La fertilidad del suelo con fuentes de azufre se presenta como una herramienta importante para ser incorporada en el manejo de las plagas. Este mineral puede conferir tolerancia o mortalidad a los insectos. En este estudio se evaluaron fuentes de azufre sobre la dinámica poblacional de *Scaptocoris castanea* (Hemiptera: Cydnidae) y sus efectos en las plantas de algodón. El experimento tuvo un diseño factorial resultante de la combinación de cuatro dosis de sulfato de calcio (CaSO_4) y cuatro dosis de sulfato de amonio $[(\text{NH}_4)_2\text{SO}_4]$. No hubo correlación entre la humedad del suelo (%) y la infestación de insectos (%) (ninfas + adultos), excepto para el tratamiento sin sulfato de calcio (0 kg/ha) con 208 kg/ha de sulfato de amonio. Hubo una diferencia significativa entre la infestación media y anterior de *S. castanea* con la aplicación de sólo 1.500 kg/ha de sulfato de calcio. La relación entre el rendimiento de algodón (kg/ha) y sulfato de amonio fue mejor descrita por una función cúbica a las concentraciones 750, 1.125 y 1.500 kg/ha de sulfato de calcio. Para esta relación, sólo en ausencia de sulfato de calcio, se describe una función lineal de mejor ajuste. Las fuentes de azufre influyeron en la apariencia visual de las plantas dado que se encontró una cantidad más grande de plantas con lesiones y/o reducción del tamaño en los tratamientos control (sin fuentes de azufre). Los resultados de esta investigación refuerzan el planteamiento de que las fuentes de azufre pueden ser útiles en la implementación de programas de control de *S. castanea* en algodón.

Palabras clave: Control. Chinche marrón. Sulfato de amonio. Sulfato de calcio.

Introduction

The brown root stink bug, *Scaptocoris castanea* (Perty, 1830) (Hemiptera: Cydnidae) is the most common pest species occurring in agricultural areas of the Brazilian Cerrado. Infestations by the brown root stink bug are increasing, both in areas under no-tillage and conventional tillage. This species is predominant in agricultural areas planted with cotton, soybean, rice, corn and pastures (Valério 2005). They occur more commonly in sandy soils, but infestations can also be observed in clayey soils (Silvie *et al.* 2007). The main tactic employed for the control of this pest in cotton has been the use of synthetic insecticides applied to the seeds (Miranda 2010). However, several unsuccessful cases have already been detected in the Brazilian Cerrado, probably due to high selection pressure that has been imposed and possibly triggering cases of re-

sistance. Thus, studies directed towards optimizing the complex management strategies for *Scaptocoris* spp. on cotton are necessary.

There are some basic strategies in crop production to reduce insect damage. A first strategy is the escape or avoid the pests over time or space. A second is the possibility of tolerance to herbivory, in this case the plant has the greater capacity for recovery of insects damages. The indirect and direct defenses against the pests by the use of chemical or mechanical defenses are other strategies, additionally, the attraction of natural enemies of the pests by the plant with the use of chemical substances it is also possible (Fritz and Simms 1992).

In general, the chemical substances use for defense purposes are mainly secondary metabolites. Among them, the production of protease inhibitors (PIs) from plants against in-

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sect proteinases, such as peptides that act as protease inhibitors (PIs), is part of the defense mechanism of plants against pests and pathogens (Silva-Filho and Falco 2000). Calcium sulfate (gypsum) and ammonium sulphate are major sources of sulphur. Applications of calcium sulfate in the form of gypsum are of capital importance for cotton production in the Brazilian Cerrado (Prochnow and Blair 2010). Calcium deficiency in tropical soils, with or without aluminum toxicity, often occur not only in the topsoil, but also in the subsurface. Calcium sulfate (gypsum) enables the increase of calcium, decreased phytotoxicity caused by aluminum and distribution of cationic nutrients into the deeper layers of the soil, which promotes root growth, allowing the plant more tolerance to certain pests, diseases and drought situations (Tanaka and Mascarenhas 2002). The roots of cotton plants are stimulated by the application of nitrogen in the soil in the form of ammonium sulphate before sowing (Carvalho 2007). In cotton, Miranda *et al.* (2011; 2012) found that sulphur may contribute to increase tolerance to attacks by *S. castanea*. Therefore, the present study aimed to evaluate the interactions of sulphur sources via ammonium sulfate and calcium sulfate on the population dynamics of *S. castanea* and quantitative and qualitative parameters of cotton plants in the "Cerrado" of Goiás, 2010/2011 season.

Materials and methods

This research was conducted in the experimental area of the "Fundação Goiás", "Santa Helena de Goiás", Goiás State, Brazil, with brown root stink bug infestation proven. Cotton was planted in December 2010. Intensive cultivation, except those related to the treatments used in this study, was carried out as recommended by Embrapa Cotton. The experiment had a factorial and consisted of 16 treatments resulting from the combination of two factors: four concentrations of calcium sulfate (CaSO_4): 0, 750, 1,125 and 1,500 kg/ha, and four concentrations of ammonium sulphate $[(\text{NH}_4)_2\text{SO}_4]$: 0, 104, 208 and 312 kg/ha. The control consisted of 0 kg/ha of sulphur sources. As fixed factor was included the insecticide imidacloprid (3.5 kg/ha), applied in spray at planting.

The experimental design consisted of a randomized blocks with four replications. Each plot consisted of eight rows of 9 m length, spaced 90 cm, using the four central lines as area designated to evaluations. At the time of the experiment, immediately after trenching, prior sampling was performed to recording the insect population density in its nymphal and adult stages.

Calcium sulfate (CaSO_4) was applied without incorporation in total area about 30 days before planting. The planting was done with cotton fertilizing and planter machine, formulation and dosage as defined treatments. After planting, the ammonium sulfate was applied manually in total area, without incorporation. Considering the maximum concentration of ammonium sulfate (312 kg/ha), corresponding to 62 kg/ha N, the remaining plots received the same way 62 kg/ha N, adding to what was applied in the form of complementation with urea, so that only the effect of "S" was evaluated.

Weekly sampling in each plot was made from plant emergence (first week) until 13th week (91 days after plant emergence), with the count of live insects present in each sample. Therefore, mini-trenches were opened to 30 x 20 x 60 cm, one per plot. The sampling methodology used was adapted from studies performed by Alvarado (1989). The water content of

the soil was determined weekly by drying up sub-samples to 105 °C. The sample volume was 180 cm³, corresponding to the amount of soil present in an area of 30 x 30 x 20 cm layer of soil. To verify the effect of treatments on vegetative growth and reproductive stadium were observed the number of plants with symptoms of attack. At the end of the cycle, productivity (seeds and fiber) was quantified by collecting four central rows of each plot and the root system of the plants was assessed through manual opening of trenches (a portion of the depth of 100 cm).

Statistical analyzes were conducted using analytical software (SAS Institute 2002). The following variables were studied: number of insects per sample, insect infestation, productivity and number of plants with attack symptoms. These variables were submitted to normality test (Shapiro-Wilk and Kolmogorov D) and homogeneity of variances test (Bartlett's test). The data were subjected to analysis of covariance - ANCOVA, and transformed into $(x + 0.5)^{1/2}$. A model was used to test the significance of the effects of the blocks and divide the treatments in two main effects (calcium sulfate, ammonium sulfate) and interaction (calcium sulfate *versus* ammonium sulfate) by use of PROC GLM (SAS Institute 2002).

For statistical analyses of the population dynamics of the insects in each treatment, a polynomial regression analysis was used, in this case the analyses were conducted by applying the procedure GENMOD (SAS Institute 2002). Other data were subjected to regression analysis, using the PROC REG (SAS Institute 2002). Previous infestation and mean were compared by t test ($P < 0.05$). Pearson correlation analysis was performed to identify the relationship between soil moisture and insect infestation (nymphs + adults).

Results and discussion

Interactions significant by concentrations of calcium sulfate and ammonium sulfate concentrations were observed in the population fluctuation of nymphs + adults ($F_{15,24} = 309$, $P < 0.01$), soil moisture (%) ($F_{15,24} = 498$, $P < 0.01$), average infestation ($F_{15,24} = 3,021$, $p < 0.01$); attacked plants expressing reduced size ($F_{15,24} = 102$, $P < 0.01$), yellowish plants ($F_{15,24} = 1,002$; $P < 0.01$) and productivity ($F_{15,24} = 876$, $P < 0.01$). Therefore, there is dependence of levels of calcium sulfate and ammonium sulfate in the expression of these variables.

Population peaks were observed in the 9th evaluation in most treatments, except in conditions of absence sulphur (0 kg/ha $[(\text{NH}_4)_2\text{SO}_4]$ or 0 kg/ha CaSO_4), and when applied calcium sulfate, with ammonium sulfate following concentrations 208 kg/ha $[(\text{NH}_4)_2\text{SO}_4]$ + 0 kg/ha (CaSO_4); 208 kg/ha $[(\text{NH}_4)_2\text{SO}_4]$ + 750 kg/ha (CaSO_4); 0 kg/ha $[(\text{NH}_4)_2\text{SO}_4]$ + 1,500 kg/ha (CaSO_4); 208 kg/ha $[(\text{NH}_4)_2\text{SO}_4]$ + 1,500 kg/ha (CaSO_4) and 312 kg/ha $[(\text{NH}_4)_2\text{SO}_4]$ + 1,500 kg/ha (CaSO_4). For these conditions the highest population peaks were found in the following periods: 8th (6.66 insects), 4th (11.32 insects), 2nd (8.00 insects), 2nd (14.33 insects), 2nd (6.67 insects) and 2nd (17.67 insects), respectively. There was no directional pattern of the occurrence of *S. castanea* depending on the treatment, because the regression models tested were not significant.

Based on the soil humidity (%) there was no correlation between soil moisture (%) and insect infestation (%) (nymphs + adults), except for treatment without calcium sulfate (0 kg/ha) and 208 kg ha⁻¹ of ammonium sulfate ($r = 0.7086$, $P < 0.05$) (Table 1). There was a tendency in reducing insect

Table 1. Correlation coefficients between soil moisture and infestation of *Scaptocoris castanea* (nymphs + adults) as a function of concentrations of calcium sulfate and ammonium sulfate, Santa Helena de Goiás - GO, 2010/2011 season.

| CaSO ₄ (kg/ha) | (NH ₄) ₂ SO ₄ (kg/ha) | | | |
|---------------------------|---|-----------------------|-----------------------|-----------------------|
| | 0 | 104 | 208 | 312 |
| 0 | 0.2308 ^{ns} | 0.3768 ^{ns} | 0.7086* | 0.0015 ^{ns} |
| 750 | -0.0086 ^{ns} | -0.6033 ^{ns} | 0.1645 ^{ns} | 0.4162 ^{ns} |
| 1,125 | 0.5066 ^{ns} | 0.5076 ^{ns} | -0.2674 ^{ns} | 0.0440 ^{ns} |
| 1,500 | 0.0440 ^{ns} | 0.3001 ^{ns} | -0.1720 ^{ns} | -0.3787 ^{ns} |

Correlation analysis conducted with data generated from a serious time with data from soil moisture and relative infestation (%) (nymphs + adults) between December 1st, 2010 and March 25th, 2011.

infestation in most treatments when reducing precipitation (periods: 4 and 5) (Fig. 1). Oliveira and Malaguido (2004) observed such behavior in the study of the distribution of *S. castanea* in the soil profile throughout the year in different locations, with the highest concentration of these insects in the rainy season. Nardi *et al.* (2007) found in pastures that the number of adult *S. carvalhoi* was dependent on rainfall distribution, noting a higher incidence with increasing precipitation.

The interaction between levels of sulphur sources on the stages of *S. castanea* (nymph or adult) was not significant. Therefore the results of infestation of both stages (nymphs + adults) were combined and analyzed together. A cubic function was the one that best explained this behavior, and the models described 87.60, 88.80, 70.80 and 89.30% of the variation in average infestation of *S. castanea* treatments 0 ($y = -0.0005x^2 + 0.022x + 4.126$); 750 ($y = -0.000005x^2 + 0.027x + 4.040$); 1,125 ($y = -0.0001x^2 + 0.040x + 4.700$), and 1,500 kg ha⁻¹ of calcium sulphate ($y = 0.0001x^2 - 0.05x + 8.855$) (Fig. 2).

Only in the treatment 1,500 kg/ha of calcium sulphate was observed significant difference in infestations (%) of *S. castanea* between the previous infestation and infestation after the application of sulphur sources (over cotton cycle). In this condition occurred reducing the infestation of this pest, registering the smallest insect infestations (below 2%), which were significantly lower ($P < 0.05$) than previous infestations. Therefore, the results revealed that the use of 1,500 kg/ha of calcium sulfate, independent of the concentration of ammonium sulfate (104, 208 or 312 kg/ha), contributed positively lowering rates of infestation population *S. castanea* (Fig. 3).

Several factors may be related with the reduction of infestation found in this study, for instance, as non preference for plants with balanced nutritional stadium (Miranda *et al.* 2011) or constitutive barriers on the plant supplied by sulphur (Dubuis 2004; Hell and Kruse 2007) or the insecticidal effect of these compounds (Malaguido *et al.* 1999). Thus, further studies are necessary to elucidate the cause of infestation by lower sulphur sources adopted, especially the sulphate and calcium concentration of 1,500 kg/ha.

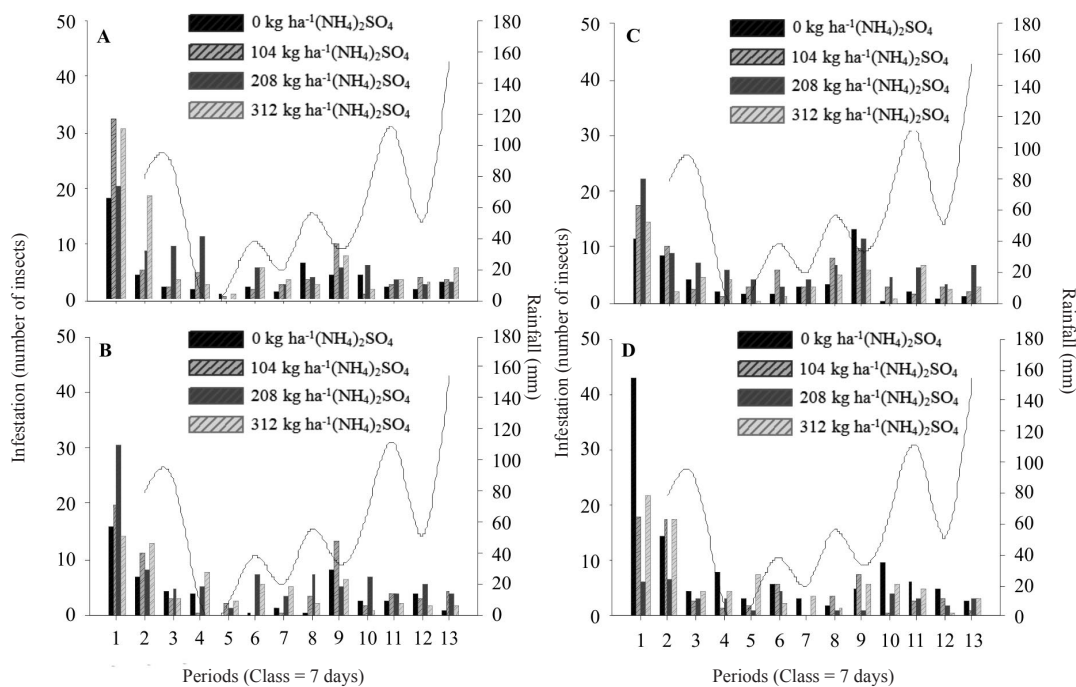


Figure 1. Rainfall (mm) (line) and population fluctuation (absolute infestation) (bars) of nymphs + adults of *Scaptocoris castanea* depending on the time of evaluation, and sources of sulphur: ammonium sulfate and calcium sulphate: A = 0, B = 750, C = 1,125 and D = 1,500 kg/ha. Santa Helena de Goiás - GO, 2010/2011 season. Curves generated by means of series of 13 evaluations (weeks).

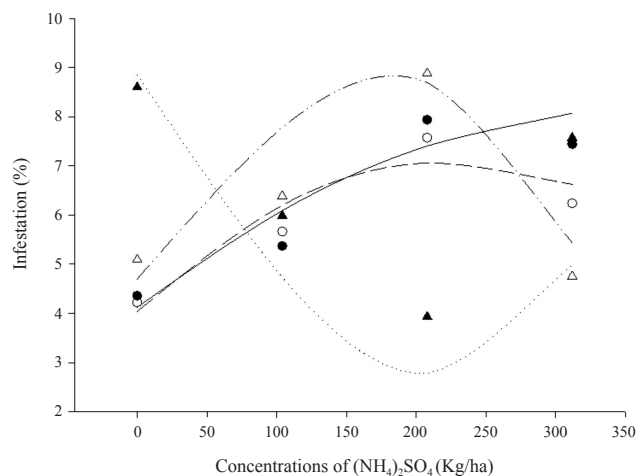


Figure 2. Infestation mean relative (%) of *Scaptocoris castanea* (insects / sample) throughout the cotton cycle, for different concentrations of ammonium sulfate. Calcium sulfate: 0 kg/ha (●), 750 kg/ha (○), 1,125 kg/ha (△), and 1,500 kg/ha (▲). Santa Helena de Goiás - GO, 2010/2011 season.

The effect of sulphur sources used was evident in the visual appearance of the plants evaluated. The models adopted confirmed biggest trends of plants with injuries and/or reduced size in treatments without such sources of sulphur (0 kg/ha of ammonium sulfate and calcium sulfate) (Fig. 4). It is believed that the absence of such sources of sulphur could induce mineral imbalance in the plant, because the use of mineral fertilizers and pesticides soluble interferes with the process of proteosynthesis and carbohydrate metabolism,

bringing the plant to accumulate amino acids and reducing sugars in the tissues (Medeiros *et al.* 2003).

Sulphur is an essential element for plant growth. Sulfur compounds such as phytoalexins and glucosinolates, may be important enhancers of the protection of plants to adverse agents such as arthropods and plant pathogens (Dubuis 2004; Hell and Kruse 2007, Bohinc *et al.* 2012). The self-defense system of the plant is more efficient in good nutritional conditions; hence it is likely that the use of sulphur sources provides better nutritional conditions for the cotton plant, allowing a greater tolerance.

A nutritional deficiency resulting from an imbalance in the number of macro and micronutrients can cause changes in the metabolism of the plant, causing the state of proteolysis predominate in the tissue. In this case the pests obtain soluble substances necessary for their nutrition. On the other hand, when there is a nutritional balance in the plant, one or more elements act beneficially on the metabolism by stimulating protein synthesis, resulting in a low content of substances soluble nutritional requirements trophic not corresponding to the pest, and the plants thus less attractive to insects and pathogens (Medeiros *et al.* 2003).

The relationship between yield of cotton (fiber + seeds) (kg/ha) and ammonium sulfate was best described by a cubic function, in concentrations of 750 kg/ha ($y = -0.000005x^3 + 0.034x^2 - 4.297x + 2.454$, $R^2 = 0.987$), 1,125 kg/ha ($y = -0.0001x^3 + 0.060x^2 - 5.659x + 2.290$, $R^2 = 0.987$) and 1,500 kg/ha ($y = -0.011x^2 + 4.795x + 2.268$, $R^2 = 0.982$) of calcium sulfate (Fig. 5). Only in the absence of calcium sulfate there is a linear relationship ($y = 1.431x + 2.115$, $R^2 = 0.964$) between productivity and concentrations of ammonium sulfate. The trend lines confirmed that the productivity of seed cotton (kg/ha) was significantly lower in the

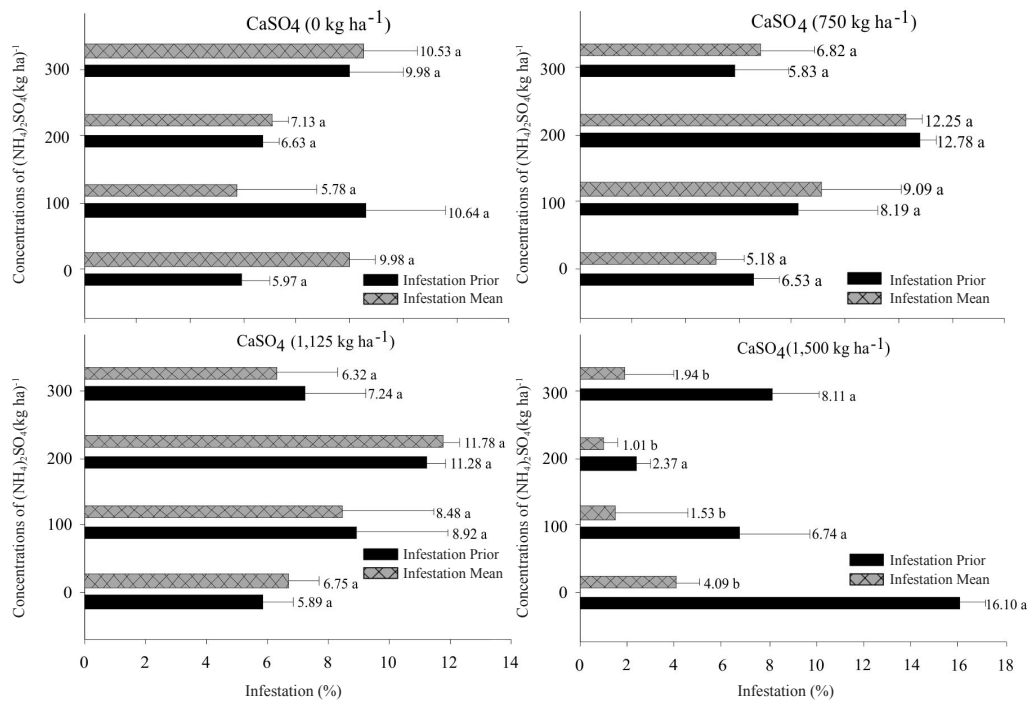


Figure 3. Infestation prior and median *Scaptocoris castanea* (nymphs + adults) as response of concentrations of calcium sulfate and ammonium sulfate, Santa Helena de Goiás - GO, 2010/2011 season. Means followed by the same letter (in the same concentration of calcium sulfate or ammonium sulfate) do not differ by t test ($P = 0.05$). Original data, analysis of co-variance (ANCOVA) was conducted with data transformed $(x + 0.5)^{1/2}$.

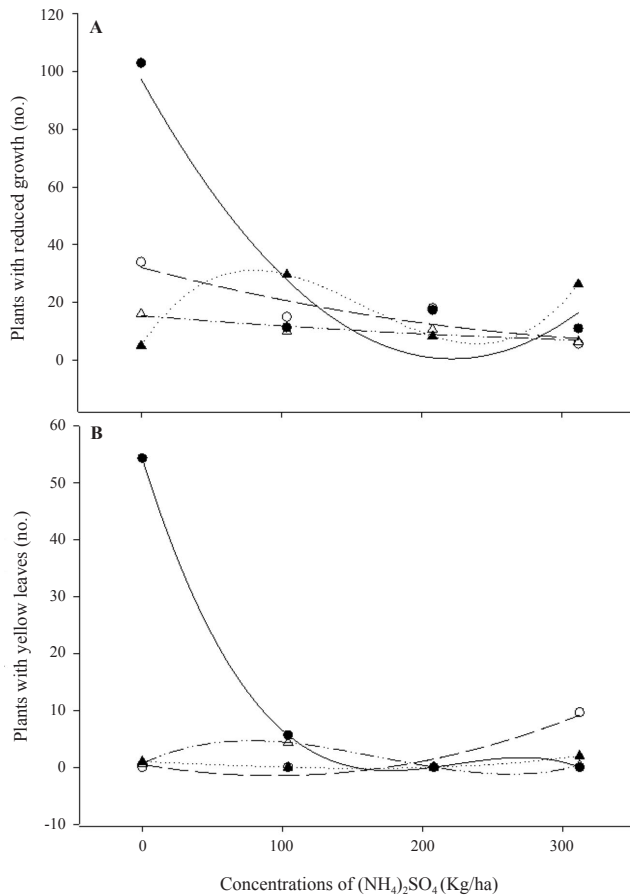


Figure 4. Number (no.) plants with reduced size (A): Calcium sulphate: 0 (—●—) ($y = 0.002x^2 - 0.875x + 97.5$, $R^2 = 0.90$), 750 (---○---) ($y = 0.00009x^2 - 0.126x + 32.13$, $R^2 = 83.20$), 1,125 (---▲---) ($y = 0.00005x^2 - 0.039x + 15.41$, $R^2 = 0.857$) and 1,500 kg/ha (---▲---) ($y = 0.000005x^3 - 0.006x^2 + 0.731x + 5$, $R^2 = 0.967$), and yellowish (B): calcium sulphate: 0 (—●—) ($y = -0.00006x^3 + 0.003x^2 - 0.794x + 54.33$, $R^2 = 0.97$), 750 (---○---) ($y = 0.0001x^2 - 0.041x + 0.483$, $R^2 = 0.933$), 1,125 (---▲---) ($y = -0.006x^3 - 0.001x^2 + 0.114x + 0.66$, $R^2 = 0.98$) and 1,500 kg/ha (---▲---) ($y = 0.07x^3 - 0.011x + 1$, $R^2 = 0.96$), during the attack *Scaptocoris castanea*, Santa Helena de Goiás - GO, 2010/2011 season.

absence of treatment with ammonium sulfate and calcium sulfate (Fig. 5).

The soil fertility is an important tool to be incorporated into the management of *S. castanea*. The growing of cotton roots are stimulated by the application of gypsum (Tanaka and Mascarenhas 2002) and by use of nitrogen in the form of ammonium sulphate before the sowing (Carvalho 2007). This good growing of cotton roots is important in the conference of tolerance to pest attacks. Moreover, ammonium sulfate, depending on its concentration, can accelerate plant development and enhance their ability to withstand the attack of the pest. However, when used in excess, this soluble compound may inhibit the process of protein synthesis and increase the presence of free amino acids in plants, which favors the infestation of sucking arthropods (Tokeshi 2002).

In recent studies, Miranda *et al.* (2011) analyzed the influence of the use of sulfur compounds on the population of *S. castanea* on cotton crop. The authors found no evidence of the influence of gypsum or sulfur fertilizers applied to the soil, in isolation, inducing population reduction of *S. castanea*.

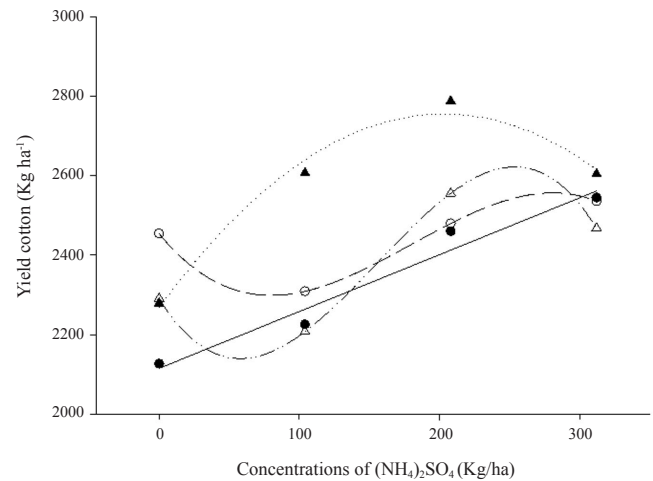


Figure 5. Yield of cotton (seeds and fiber) (kg/ha) data observed (dots) and estimated (lines) for different concentrations of calcium sulfate and ammonium sulfate. Santa Helena de Goiás - GO, season 2010/2011. Calcium sulfate: 0 (—●—) ($y = 1.431x + 2115$, $R^2 = 0.964$), 750 (---○---) ($y = -0.000005x^3 + 0.034x^2 - 4.297x + 2454$, $R^2 = 0.987$), 1,125 (---▲---) ($y = -0.0001x^3 + 0.060x^2 - 5.659x + 2290$, $R^2 = 0.987$) and 1,500 kg/ha (---▲---) ($y = -0.011x^2 + 4.795x + 2268$, $R^2 = 0.982$).

However, the study concluded that the use of such sources of sulfur (calcium sulfate and ammonium sulfate) promotes tolerance of cotton plants to insect attack. The results of this study revealed that there is not a pattern of directional temporal distribution of *S. castanea* in all treatments. There is not a linear relationship between relative humidity of the soil (%) and insect infestation (%) (nymphs + adults) only found this relation on the treatment that did not receive calcium sulfate (0 kg kg/ha) with addition of 208 kg/ha of ammonium sulfate. Concentrations of ammonium sulfate between 120 and 320 kg/ha, when combined with a concentration of 1,500 kg/ha of calcium sulfate, promote lesser number of attacked plants, and increase in productivity of cotton. Furthermore, the concentration of 1,500 kg/ha of calcium sulfate, independent of the concentration of ammonium sulfate, also favors smaller infestations *S. castanea*.

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