

Biological control of fungus gnats (*Bradysia matogrossensis*) in tobacco seedlings

Controle biológico do fungus gnats (*Bradysia matogrossensis*) em mudas de tabaco

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ABSTRACT - Tobacco production in Brazil is an essential source of income for many families, especially in the southern region. However, it is subject to attack by pests and diseases, such as the fungus gnats insect. To control this pest, the mite *Stratiolaelaps scimitus* is found in Brazilian soil, which has a predatory potential to fungus gnats. This study aimed to evaluate the use of *S. scimitus* as an insect control agent, compared to chemical control, in the production of tobacco seedlings in the *floating* system. The experiment was carried out on a farm in Crissiumal-RS, in a randomized block design, with four replications: T1= control, T2= chemical control based on Imidacloprid and Azadirachtin, and T3= *S. scimitus*. To analyze the control efficiency, adult insects were captured at different times, and the biological control did not differ from the chemical control. The biological treatment made it possible to reduce the use of chemical products. Concomitantly, questionnaires verified that producers are well accepting the new control model. Therefore, using *S. scimitus* could reduce the proliferation of fungus gnats in the production of tobacco seedlings in the *floating* system, but more studies need to be carried out for a better evaluation.

Keywords: *Stratiolaelaps scimitus*. *Floating*. Pesticide.

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RESUMO - A produção de tabaco no Brasil é uma importante fonte de renda para muitas famílias, principalmente na região sul do País. No entanto, está sujeita ao ataque de pragas e doenças, como o inseto fungus gnats. Como alternativas ao controle dessa praga, encontra-se no solo brasileiro o ácaro *Stratiolaelaps scimitus*, que apresenta potencial predatório ao fungus gnats. O objetivo desse estudo foi avaliar o uso do *S. scimitus* como agente de controle do inseto, comparado ao químico, na produção de mudas de tabaco no sistema *floating*. O experimento foi conduzido em uma propriedade rural no município de Crissiumal-RS, em delineamento em blocos ao acaso, com quatro repetições: T1= controle, T2= químico a base de Imidacloprido e Azadiractina e T3= *S. scimitus*. Para análise da eficiência de controle, foram realizadas capturas de insetos adultos em diferentes épocas, e o controle biológico não diferiu do controle químico. O tratamento biológico possibilitou redução no uso de produtos químicos. De forma concomitante, verificou-se por meio de questionários, que o novo modelo de controle está sendo bem aceito pelos produtores. Portanto, a utilização de *S. scimitus* foi capaz de reduzir a proliferação de fungus gnats na produção de mudas de tabaco no sistema *floating*, porém mais estudos precisam ser realizados para melhor avaliação.

Palavras-chave: *Stratiolaelaps scimitus*. *Floating*. Agrotóxico.

INTRODUCTION

Brazil is the second largest tobacco producer and the largest exporter (SINDITABACO, 2019). In 2019, 297 thousand hectares were cultivated, producing 664 thousand tons of tobacco, generating 5.8 billion reais (AFUBRA, 2019). Tobacco production is vital, enabling high gains in small areas, mainly in small farms, producing 2.1 million direct and indirect jobs (COTRIM et al., 2016).

Tobacco seedlings are produced in a protected environment with the floating system, where styrofoam trays filled with substrate float on water (OLIVEIRA; COSTA 2012). This allows the producer to pay more attention to the seedlings, facilitating the control of pests and diseases and providing greater uniformity of seedlings and high survival rates after transplanting (NEUMANN et al., 2017). On the other hand, this system is more prone to the attack of pests and diseases (ZAMBOLIM et al., 1999), requiring the use of pesticides, which can cause several environmental problems, such as intoxication of farmers, contamination of food, soil, water, and animals, in addition to the emergence of resistant pathogens (MORANDI; BETTIOL, 2009; RIQUINHO; HENNINGTON, 2016). Thus, alternatives are sought to reduce the use of pesticides, such as the use of biological control agents (BARBOSA et al., 2017). Control using mites has grown significantly in recent years (SANTOS, 2017). Some of these mites, called predators, attack harmful organisms (BARBOSA et

al., 2017; SANTOS, 2017; RANGEL; WARD, 2018).

One of the pests that attack seedlings is the insect called fungus fly, also known as fungus gnats, a term in English that corresponds to different species of dipterans from the families Sciaridae, Keroplatidae, Mycetophilidae, among others. These species are pests that attack mushroom crops and seedling nurseries (DUARTE; CUNHA; MORAES, 2018), and losses can reach 19.5% in production (RADIN et al., 2009). In Brazil, the most common species is *Bradysia matogrossensis*, whose adult individuals are flies measuring just over 2 mm, with dark wings and long antennae. The larvae damage the roots of seedlings, leaving them more vulnerable to attack by root diseases (DUARTE; CUNHA; MORAES, 2018). Damage to tobacco crops can occur both by the adult fly, which transmits pathogenic fungi and by the larvae that feed on the roots of the seedlings (OLIVEIRA; COSTA, 2012), which may cause death or deformation of the seedlings, reducing the number of plants (BARBOSA et al., 2017).

As an alternative for the management of fungus gnats, predatory mites can be used, mainly from the Laelapidae family, such as *Stratiolaelaps scimitus*, which in Europe are used to control thrips, dipterans (*Sciaridae*), and mites (*Astigmatina*) and in Brazil, it is efficient in controlling *Bradysia matogrossensis* larvae (CASTILHO; MORAES;

NARITA, 2010), as in mushrooms (CASTILHO et al., 2009) and azalea seedlings (POLETTI, 2010).

Thus, this study aimed to evaluate the use of *Stratiolaelaps scimitus* as a control agent for fungus gnats in the production of tobacco seedlings.

MATERIALS AND METHODS

The study was carried out on a farm producing tobacco in a floating system located in Crissiumal, Rio Grande do Sul (RS), under geographic coordinates 27°26'27.3" S and 54°12'05.0" W. The climate classification according to Koppen is subtropical - Cfa (KÖPPEN, 1948), with temperatures between -3 to 18 °C in the coldest months, and an average above 10 °C in the hottest months, with no defined dry season (humid every year) and hot summer, with an average monthly temperature above 22°C (KÖPPEN, 1948).

This study evaluated three treatments, adopting a randomized block design, with four replications: T1= control, T2= chemical control based on Imidacloprid and Azadirachtin, and T3= *S. scimitus*. The dosages and volumes of spray used followed the recommendations of the manufacturers (Table 1).

Table 1. Dosages and recommended volumes of pesticides and biological insecticides used in treatments tested on tobacco seedlings.

Agricultural Defensive	Dosage	Volume of spray
Fungicide		
Cuprous oxide	2 kg ha ⁻¹	1000 L/ha
Iprodione	1 kg ha ⁻¹	40 L/50 m ²
Mancozeb+Matalaxil - M	1.25 grams/m ²	Enough volume to water evenly *
Insecticide		
Imidacloprid	15 g/50 m ²	40 L/50 m ²
Azadirachtin	500 ml/100 L of water	50 ml/tray
Biological Insecticide		
<i>Stratiolaelaps scimitus</i>	2000 to 5000 mites/m ²	200 to 500 ml/m ²

*Mancozeb+Matalaxil fungicide – M, the information in the package insert mentions that the volume of solution must be sufficient to water uniformly; for this purpose, the dosage of 100 ml/tray was used in the experiment.

Source: Recommendations contained in the package inserts of each product.

The installation of the treatments took place in a standard shed structure, with dimensions of 8 x 32 m, being protected on the sides by an agricultural screen with 65% transparency (Sombrite®) and covered with a laminated screen of 75%. Each treatment was allocated in different sheds (but with the same environmental and exposure conditions) to avoid possible interference; whose experimental units were composed of four styrofoam trays (replications) with 200 cells each, totaling 800 tobacco seedlings in each treatment. Each tray, separately, was evaluated as a repetition. The trays had dimensions of 67 cm in length, 34 cm in width, and 5.2 cm in

height. The cells of the trays had internal dimensions of 2.7 x 2.7 cm.

In all treatments, the fungicides Cuprous Oxide, Iprodione, and Mancozebe + Matalaxil-M were used to control diseases. Imidacloprid and Azadirachtin insecticides were used only in the chemical treatment, and the biological insecticide was used only in the biological treatment (Table 1).

In all cases, the recommendations contained in the package inserts of the applied products (Table 1) were respected, proportionally to the treatment area, except for

biological control, in which the adopted dosage of biological insecticide was 10,000 mites, or 1000 mL, for 60 trays. Thus, the volume used in the experiment was 16.67 mL/tray, equivalent to 73 mL/m². This dosage is recommended by the tobacco culture integrator company since there is no recorded recommendation for this crop in the package insert.

The application of the biological insecticide was carried out only at the time of tobacco sowing. For this, the contents of the package were previously shaken for homogenization, followed by the distribution of 67 mL of the product directly on the substrate in the four trays of the biological treatment, which corresponds to approximately 667 predatory mites in inert material (vermiculite), as the manufacturer declares 10,000 mites per 1000 mL.

The conduction system in the production of seedlings in the floating system was the same for the three treatments, using the substrate based on pine bark in the trays. For fertilization, water-soluble fertilizer with the NPK formulation 20-10-20 was used, which was released into the tank water in each treatment two times. The first fertilization was after germination, that is, 14 days after sowing, when the equivalent of 333 kg ha⁻¹ was released into the water of the floatings, and the second moment of fertilization was carried out at 24 days after sowing, with the release of the dose equivalent to 416 kg ha⁻¹. The fertilizer was dissolved in a watering can and spread over the water in the treatment tank. The volume of water used at the time of sowing was 10 liters/tray in each treatment, being replenished 40 days after sowing, with a volume of 5 liters/tray in each treatment.

Tobacco cultivar BAT 2101 was used, whose sowing was performed manually for the three treatments, placing it in half of the tray, that is, in 100 cells, one seed, and in the other cells, two seeds. This was done in all repetitions. At 20 days after sowing, seedlings were pricked out in the repetitions of each treatment, leaving only one plant per cell where the two seeds had germinated and transplanting seedlings to the cells where germination eventually did not occur since the minimum germination guaranteed by the manufacturer is 80%.

To capture and later count the insects (fungus gnats), yellow sticky traps were used on the trays, as indicated by Barbosa et al. (2017). The manufacturer recommends using between 100 and 200 traps/ha. However, to determine the maximum number of individuals present in the trays (repetitions) area, two traps were placed in each treatment, suspended by string. The trays in the opposite direction were used as a reference, using 10 cm of height from the base of the trays, and later, the development of the seedlings was monitored, always maintaining the height of the base (FREIRE et al., 2007; BARBOSA et al., 2017).

The survey of the number of adult insects of fungus gnats took place 15, 30, and 45 days after tobacco sowing. After collection in each period, the traps were collected and taken to the laboratory of the State University of Rio Grande do Sul (UFRGS), Três Passos unit, for counting with a magnifying glass.

The identification of adult insects of fungus gnats was carried out based on the following characteristics: Adult insects are tiny flies, about 1 to 3 mm long, with antennae and long legs, the antennae being moniliform, pair of transparent wings, shiny black head and elongated, whitish to the transparent body, very similar to mosquitoes (FREIRE et al., 2007).

At 50 days after sowing, the roots of the tobacco seedlings were sampled, collecting 15 seedlings from each repetition, totaling 60 seedlings per treatment. The plants sampled for the evaluation of the roots were collected in the center of the trays to avoid the influence of borders.

After collection, the roots were washed and separated from the rest of the plant, packed in a plastic bag, and transported to the UFRGS laboratory, Três Passos unit, where they were allocated separately for treatment and repetition in paper bags, weighed, and placed in an oven with forced air circulation, at 60 °C, until the constant mass is obtained (SILVA-OLAYA; CERRI; CERRI, 2017). Then, the roots of each treatment were weighed on a precision scale to determine the dry mass of the roots. The amount of use of insect control products was also calculated through the mediation of the dosages applied in the treatments to compare the total amount applied in the treatment with biological control and chemical treatment, respectively.

In addition to the plant parameters evaluated, the degree of satisfaction and acceptance of farmers in using the predatory mite *S. scimitus* was qualitatively analyzed, through the application of questionnaires, with closed questions, applied to tobacco producers. In this way, we sought to identify the difficulties encountered by the producers, in addition to positive and negative points and the percentage of acceptance of the use of the studied biological control, contributing to the analysis of the acceptance of the product. The questionnaire was applied to 15 farms in the same municipality where the study was carried out, Crissiumal/RS, which used the predatory mite *Stratiolaelaps scimitus* in the 2018/2019 harvest.

The data for the parameters evaluated in the plants were subjected to analysis of variance, and the Tukey test, according to the procedures available in the Sisvar statistical package (FERREIRA, 2019), and the other data from the questionnaire was interpreted in percentages qualitatively.

RESULTS AND DISCUSSION

The results of trap collections showed variations between treatments and collection times concerning the number of adult insects (Table 2). Between 15 and 45 days ranged from 8 to 32 adult insects captured by the traps. The largest number of insects collected occurred 30 days after sowing tobacco, with 32 adult insects captured in the control treatment, which did not receive any pest control product.

Table 2. Number of adult insects of fungus gnats in different treatments and times of tobacco seedling production.

Treatment	Period			Overall average of the period
	15 days	30 days	45 days	
Control	22.0 bA*	32.0 cB	17.5 bA	23.8 b
Chemical	9.0 aA	16.0 aB	9.5 aA	11.5 a
Biological	8.0 aA	21.3 bB	8.3 aA	12.5 a
CV (%)	16.5			

*Means followed by the same lowercase letter in the column and uppercase in the row do not indicate statistical difference by the Tukey test at 5% probability.

In the average occurrence of adult insects of fungus gnats, the treatments that received chemical or biological control reached similar results, and both reduced the occurrence of adult insects concerning the control treatment (Table 2).

The largest number of individuals of the adult insect observed in the treatments was 30 days after sowing. According to (OLIVEIRA; COSTA, 2012), this may be associated with the floating system, which is conducive to developing fungus gnats. For up to 30 days, the tobacco seedlings did not cover the substrate, leaving it exposed, and the adult insects looked for this place to lay their eggs. These results are consistent with those of Poletti (2010), in a study carried out in Holambra (SP) in the commercial production of azalea seedlings, in which the collection carried out at 30 days also showed a greater number of individuals of the adult insect fungus gnats. Duarte, Cunha and Moraes (2018) report in their study with strawberries, carried out in different municipalities, that the occurrence of fungus gnats was higher in the initial period of rooting, demonstrating that production in a protected environment can be favorable to the development of the pest, as it is humid and rich in decomposing organic matter.

At 30 days after sowing, the application of a chemical product showed a better result than the biological product, which can be explained by the immediate action of pesticides on pests, superior to the response of biological products, as

corroborated by Coppi (2018). However, the average result among all the times collected revealed that the occurrence of the adult insect fungus gnats was higher in control, showing that the predatory mite *S. scimitus*, used in the biological treatment, affected the pest without showing the statistical difference with the conventional chemical products, thus presenting an effect similar to that presented by the chemical products used to control fungus gnats. This result corroborates with Castilho et al. (2009), who observed that the smallest number of adults of *Bradysia matogrossensis* captured was in the chambers where the predatory mite *S. scimitus* had been released. Poletti (2010), in a study in Holambra, SP, found that the insect *B. matogrossensis*, in the commercial production of azalea seedlings, was about three times lower in the area where the predatory mite *S. scimitus* was released. Santos (2017) describes the results as promising in studies where *S. scimitus* was used as a controller of *B. matogrossensis*.

The results obtained in this study, as well as those reported in the literature, are promising, as they allow the reduction of the occurrence of fungus gnats during the production of tobacco seedlings, thus preserving the seedlings of the bed and the attack of other pathogens (OLIVEIRA; COSTA, 2012; DUARTE, 2018), in addition to reducing the use of pesticides in the production of tobacco seedlings.

The dry mass of plant roots ranged between 0.11 and 0.14 g/plant (Table 3).

Table 3. Dry mass of roots of tobacco seedlings collected at 50 days after sowing.

Treatment	Dry mass of roots (g/plant)
Control	0.13 a*
Chemical control	0.11 a
Biological control	0.14 a
CV (%)	17.19

*Means followed by the same letter in the column do not differ statistically by Tukey's test at a 5% probability of error.

Based on the results, there is no statistical difference, but in absolute numbers, the plants of the biological treatment produced greater root mass 50 days after sowing; however, without statistically significant differences between treatments. Even with the incidence of the pest, this attack

was insignificant because, depending on the degree of attack, the damage may not be observed or relevant (COPPI, 2018). Crops have a certain level of tolerated pests, with the degree of infestation determining the occurrence of damage (SILVA; SOBRINHO, 2017).

In a study with strawberry plants in Eldorado do Sul, RS, Radin et al. (2009) observed the death of 19.5% of the plants, and in all of them, there was the presence of larvae of fungus gnats. This demonstrates the importance of biological control of this pest, avoiding this type of attack by larvae and the consequent death of plants. In other studies (POLETTI,

2010; DUARTE; CUNHA; MORAES, 2018), the occurrence of the fungus gnats and its damage was also identified; however, the root density of the plants was not evaluated in any of these studies, showing that more studies related to this theme need to be developed.

The total amount of product use can be seen in Table 4.

Table 4. Amount of pesticides used in the treatments during the production of tobacco seedlings.

Products	Treatments		
	Control	Chemical	Biological
Cuprous oxide	1.2 g	1.2 g	1.2 g
Iprodione	0.5 g	0.5 g	0.5 g
Mancozeb+Matalaxil – M	6.0 g	6.0 g	6.0 g
Imidacloprid	0.0	1.0 g	0.0
Azadirachtin	0.0	5.0 ml	0.0
Total	7.7 g/m ²	8.7 g + 5 ml/m ²	7.7 g/m ²

In general, a reduction in the use of chemicals was obtained, specifically Imidacloprid and Azadirachtin, which had a 100% reduction in their use. In the current production model of tobacco seedlings in the floating system, more products with fungal action are used than insecticides. The result shows a positive effect in reducing the use of pesticides, as the biological control agent can replace chemical insecticides, keeping pest infestation under control. The study by Viera-Arroyo et al. (2020) emphasizes the importance of

replacing chemical inputs with biological ones, providing alternative elements for agricultural production, and reducing pesticides and pest resistance.

As for the results of the field research carried out with the application of questionnaires to producers about the use of *S. scimitus*, there was 80% satisfaction among satisfied and delighted producers with the use of biological control (Table 5).

Table 5. Results of the questionnaire were applied to farmers who used the predatory mite *Stratiolaelaps scimitus* in the production of tobacco seedlings in the 2018/2019 harvest.

Questions	Answer Options	Response (%)
How satisfied are you with the use of <i>Stratiolaelaps scimitus</i> ?	Dissatisfied	20
	Satisfied	67
	Very satisfied	13
How easy is it to use biological control?	Bad	0
	Good	60
	Excellent	40
How was the infestation of the adult insect of fungus gnats?	Low	60
	Medium	33
	High	7
Was any damage to the seedlings caused by fungus gnats, such as damaged initial leaves, dead seedlings, leaf deformation, and poor rooting?	Yes	13
	No	87
Would you recommend using <i>Stratiolaelaps scimitus</i> for other producers?	Yes	80
	No	20
Do you intend to continue using <i>Stratiolaelaps scimitus</i> ?	Yes	80
	No	20
In your opinion, what is the main difficulty observed in the use of <i>Stratiolaelaps scimitus</i> ?	Had no difficulties	47
	Delivery logistics	47
	Living with the adult insect	6
In your opinion, what is the main positive point of using <i>Stratiolaelaps scimitus</i> ?	Reduction in the use of chemicals	53
	Non-toxic product	20
	Practicality in using the product	27

Among the fifteen producers interviewed, 67% reported being "satisfied" with using the predatory mite, 13% responded "very satisfied", and only 20% reported being "dissatisfied". This result is due to the excellent efficiency of biological control, as according to the reports of the producers, the occurrence of the adult insect fungus gnats was reduced with the use of *S. scimitus* as a pest controller.

In a study carried out in Maringá, PR, Chagas et al. (2016) observed that among the 11 producers interviewed, all stated that the expected results with the use of biological control were achieved, being considered a viable alternative among producers.

When asked how easy it was to work with *S. scimitus*, 60% of the producers answered that it was "good", and 40% said it was "excellent".

Regarding the infestation of the adult insect of fungus gnats, 87% of the interviewed producers did not observe damage caused by the insect, and 60% responded that the infestation was low, which reinforces the action of the predatory mite.

This justifies the recommendation of using biological control by 80% of the interviewed producers, who informed that they intend to continue using the product based on *S. scimitus*. A point that draws attention concerns the difficulties encountered by the owners interviewed regarding using *S. scimitus*. Among the 15 farms interviewed, 47% said delivery logistics are a problem because the product is a live mite that cannot be stored for a long time, making distribution difficult.

The current way adopted by the company that works with this product in the study region is to deliver it to all producers who use it simultaneously. According to the producers, this practice is used to reduce the cost of freight; however, it ends up making it challenging to plan the individual property since the dates of tobacco sowing between the producers are not the same, which can compromise the use of the organic product. According to the information in the package insert, *S. scimitus* must be stored between 8 and 10 °C and has a shelf life of 21 days. Thus, using living organisms often requires special care in their handling since the shelf life and storage must be respected according to the recommendations manufacturer (MORANDI; BETTIOL, 2009).

The main positive point mentioned by 53% of the interviewed producers regarding the use of *S. scimitus* was the reduction in the use of chemical products, which corroborates the results of this study in which it was possible to reduce the use of pesticides by up to 11%. The concern of these producers about the excessive use of pesticides in the agricultural environment is evident, and this is an opportunity to reduce their use, being a technique well accepted by these farmers.

Barbosa et al. (2017) and Chagas et al. (2016) mention in their studies that the great advantage of using biological controllers is that it is a product that does not harm the environment and the health of producers. This point of view differs from that of Silva (2017). In a study carried out with 107 strawberry producers in the Betinho Settlement (DF), there were no alarming effects on health and safety, noting the

absence of factors that impact the quality of food life of the strawberry producer due to the use of pesticides. On the other hand, Sousa (2016) carried out an exploratory study with producers from the District of Cuncas, in the municipality of Barro (CE), and found that the extensive chronic damage that the pesticide brings to the environment, biodiversity, and man himself, must be worked on through a paradigm shift in agriculture to reduce the use of agrochemicals gradually.

The results obtained in this study, whether due to the effectiveness of biological control and the satisfaction of producers concerning the use of *S. scimitus*, demonstrate that it is possible to reduce the use of chemical products to control fungus gnats and, consequently, minimize the impacts about the environment.

CONCLUSIONS

In a floating system for the production of tobacco seedlings, the use of the biological control agent *Stratiolaelaps scimitus* had a similar effect to the chemical in the control of the fungus gnats, being possible to reduce the number of pesticides used and is well-accepted by farmers.

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