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**CROP SCIENCE**

# Performance of phytosanitary products for control of soybean caterpillar

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**Abstract:** The present work evaluated the efficiency of applied biological control and chemical control of *Chrysodeixis includens*, and the management of this looper caterpillar in the field soybean crop. The experimental design was a randomized complete block design, consisting of six treatments applied only once: two different doses of *Bacillus thuringiensis* (*Bt*), 0.2 and 0.35 L $ha^{-1}$; *Metarhizium rileyi* strain UFMS 02 (Mr), 2.0 and 5.0 kg $ha^{-1}$; insecticide Flubendiamide (Fd) 20 mL $ha^{-1}$; and the control. The reduction of the pest and the percentage of efficiency of the products along the development of the soybean, besides some phytotechnical parameters, were evaluated thirteen days after the application. In general, there was a decrease in the number of caterpillars after thirteen days of spraying, with the *Bt* treatment being 350 mL $ha^{-1}$, which provided the greatest reduction in the population (96.2%) when compared to the control (6.7 %). Regarding efficiency, treatments containing biological products *Bt* (two doses) and Mr 5.0 kg $ha^{-1}$ provided the best results: 95.88, 84.69 and 92.35%, respectively. Among the phytotechnical parameters evaluated, the biological treatments were superior to the chemical treatments in relation to the productivity and the number of pods per plant, not differing statistically among them.

**Key words:** *Bacillus thuringiensis*, Flubendiamide, *Metarhizium rileyi*, soybean looper caterpillar.

## INTRODUCTION

In the latest soybean harvests, the *Chrysodeixis includens* (Walker 1858) (Lepidoptera: Noctuidae, Plusiinae) gained prominence. Previously considered a secondary pest without any economic importance, this looper caterpillar reached principal soybean pest status (Carvalho et al. 2012). The caterpillar has a high polyphagic capacity, with the possibility of survival in 73 species of plants belonging to 29 botanical families, making its control more complex. This factor favors its persistence even at low density until the female finds a host capable of sustaining the development of caterpillars in the agricultural system (Moscardi et al. 2012). Other difficulties for its management are in its habit, because the caterpillars are usually housed in the lower third of the plants, protected from the action of the insecticides, especially with the development of the crop, besides its high voracity and greater tolerance to the insecticides when compared to the soybean caterpillar *Anticarsia gemmatalis* Hübner (1918) (Lepidoptera: Erebidae, Elepidotinae) (Carvalho et al. 2012).

The control of the main soybean pests is guided by the principles of Integrated Pest Management (IPM) and its success depends on several tools, including biological control. These organisms are present in the agroecosystem and are naturally occurring, and deserve attention, such as the entomopathogenic fungus *Metarhizium rileyi* (Farlow) Samson (1974) (Ascomycota: Clavicipitaceae) (Sujii et al. 2002a, b, Carvalho et al. 2012). The *M. rileyi* occurs in more than 30 different species of insects, of which approximately 90% are Lepidoptera, and the caterpillars of the Noctuidae family are among the most susceptible (Ignoffo et al. 1976, Srisukchayakul et al. 2005, Alves & Lopes 2008, Lima et al. 2015), as well as those belonging to the Plusiinae subfamily (Puttler et al. 1976, Ignoffo 1981).

Another well-known microorganism on *C. includens* caterpillars is the bacteria *Bacillus thuringiensis* Berliner (1915) (Eubacteriales: Bacilaceae) (Polanczyk et al. 2000, Pereira et al. 2009). The bacterium produces crystalline protein inclusions that initially act on the absorbent epithelium of the mid-intestine leading to osmotic imbalance resulting in interruption of feeding and subsequently paralysis of the intestine, killing the insect between 2 and 4 days (Habib & Andrade 1998, Bueno et al. 2012).

Sustainable management measures for *C. includens*, such as the application of entomopathogens, should be part of the routine of rural producers, minimizing the selection of resistant populations of the pest. The use is the only mechanism of management and indiscriminately provides the selection of insects resistant to the products used, besides providing deleterious effect on their natural enemies (Carvalho et al. 2012). This interferes with their natural biological control, thus triggering outbreaks of pests previously controlled by entomopathogens (Bueno et al. 2012).

The present study was designed to assess the efficiency of applied biological control and chemical control in the management of *C. includens* and its reflexes on soybean phytotechnical parameters.

## MATERIALS AND METHODS

The present study was performed at the commercial area of the Triângulo de Prata Farm (18°29'26"S and 52°34'32"W), located in the Brazilian state of Goiás at the Chapadão do Céu municipality. The soybean cropland field sitting at an altitude of 847 m was sown on October 21, 2014 and harvested on February 15, 2015. The soybean cultivar, with RR technology, Anta 82, was sown using line spacing of 0.45 and 18 seeds per meter. The area was prepared in the no-tillage system, on cultural remains of *Zea mays* L. (Poales: Poaceae) and adopted the technical recommendations for the crop.

The experimental area was composed of four blocks, containing 24 plots which were 20 meters long by 6.3 meters wide (14 rows spaced at 45 cm), totaling 6 per block. All blocks were separated from each other by an interval of 2 m (safety area), and to avoid any border effect in the plots, the 8 central lines were used as an useful area, neglecting 2 meters in lengthwise direction and 3 lines on each side.

The experimental design was a randomized complete block (DBC) composed of six treatments, all with single dose: control (without product), *B. thuringiensis* var. *kurstaki* (strain HD-1) (Bt) at doses of 0.2 and 0.35 L $ha^{-1}$; *M. rileyi* (isolated UFMS 02) (Mr), at the concentration of $1.0 \times 10^9$ conidia $mL^{-1}$ at the doses of 2.0 and 5.0 kg $ha^{-1}$ (conidia + rice) and the insecticide Flubendiamida (Fd ) at the dose of 2.0 mL $ha^{-1}$ (14.8 gia $ha^{-1}$) (Agrofit 2020).

The bacterial suspensions and the insecticide solution were prepared using commercially available registered products, as recommended by the manufacturer (Agrofit

2020). The fungus *M. rileyi* was multiplied according to the methodology adapted from Loureiro et al. (2005), mixed with distilled water and 0.01% adhesive spreader. The control treatment received only distilled water mixed with the adhesive spreader.

After the emergency, sampling was carried out with a beat cloth (measuring 1.0 × 1.0 m) every 7 days, at 6 meters of the central lines of the plot, at three points (useful area), disregarding the border, to verify the presence of live, dead and parasitized caterpillars.

The treatments were applied when an average of 13 caterpillars m-¹ (maximum of insects obtained as a function of the pluviometric regime of the region, Figure 1). There was only one application of product in the total area of each plot when the crop was in the reproductive stage, pod formation (R3 and R4) after 16 h and temperature ranging from 23 to 27 °C using a costal nozzle sprayer with adjustable volume for 150 L ha$^{-1}$.

Thirteen days after the application of the treatments, the sampling was performed in the useful area, quantifying the number of live caterpillars. In addition, we evaluated the number of pods and grains, mass of 1,000 grains in addition to productivity to verify the effect of caterpillar management on these parameters. For these evaluations, an area of 5 m in length and two central lines of the crop were considered in each plot. After harvesting, grain moisture was corrected to 13%, estimating yield.

Data obtained on insect mortality and phytotechnical parameters were submitted to analysis of variance, and the comparison between means of treatments was performed by the Scott-Knott test ($p \leq 0.05$). The percentage of caterpillar reduction and efficiency were calculated using the Henderson & Tilton equation (1955). For the estimation of productivity, the

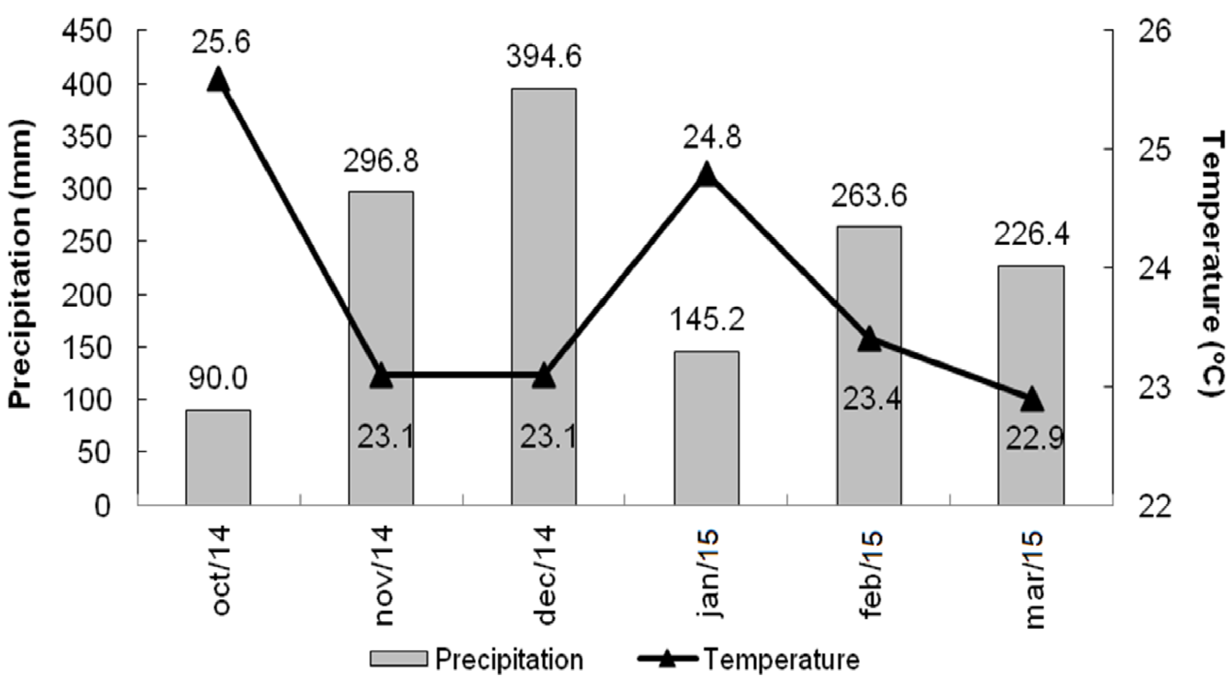


**Figure 1.** **Mean temperature (°C) and rainfall (mm), recorded during the conduction of the experiment. Information obtained between 2014 and 2015 at the meteorological station of the Cerradinho Bioenergia S/A Plant, Chapadão do Céu municipality, Brazilian state of Goiás.**

methodology proposed by Lee & Herbek (2005) was adapted as follow: the number of pods, grains and productivity were transformed by Ln (x), and the mass of 1,000 grains in $(x + 1.0)^{0.5}$, for the correction of normality.

## RESULTS

The most significant reductions in infestation were observed for the Bt (dose-independent) and *M. rileyi* (5.0 kg ha$^{-1}$). The same was observed for the *M. rileyi* efficiencies (2.0 kg ha$^{-1}$). In turn, the Flubendiamide insecticide provided a lower efficiency than the treatments mentioned, but higher than the control (Table I, Figure 2).

Regarding the phytotechnical parameters, the management of *C. includens*, reflected only on the number of pods per plant and productivity. It was found that all biological treatments provided better performance relative to the insecticide and the control (Table II, Figure 2).

## DISCUSSION

The treatments with Bt provided reduction of the number of insects and efficiency above 80% (Table I). These results resemble those observed by Polanczyk et al. (2000), which analyzed two subspecies of *Bt*, *B. thuringiensis thuringiensis* strain 4412 and *B. thuringiensis aizawai* strain HD68, on second instar larvae of *Spodoptera frugiperda* Smith and Abbot (Lepidoptera: Noctuidae), obtained mortality of 80.4 and 100%, respectively.

The toxic activity of *B. thuringiensis* is closely linked to host characteristics such as intestinal pH, enzyme complex and specific receptors (Berlitz et al. 2006), which may have contributed to the infection of *C. includens* caterpillars, and consequently their death. In addition, there is a wide range of *cry* proteins and at least ten have been specifically identified for *B. thuringiensis* var. *kurstaki* strain HD-1, providing an extremely efficient bio-insecticide against caterpillars (US 2016).

For entomopathogenic fungi, when a larger amount of conidia germinates, both invasion and colonization of the insect's body are faster and more efficient, making it difficult to proliferate other competing microorganisms that could hinder its sporulation (Neves & Hirose 2005). According to Ignoffo (1981), the complete cycle of the *M. rileyi* fungus on *Trichoplusia ni* Hübner (1802) (Lepidoptera: Noctuidae) varies from 8 to 12 days at 25 °C, with the most favorable

**Table I. Means (±EP) of *Chrysodeixis includens* caterpillars (Lepidoptera: Noctuidae) on soybean leaves before and after spraying with biological and chemical products, control efficiency and reduction of insect numbers.**

| Treatment | NIVaA¹ | NIVdA¹ | %Red¹ | %E¹ |
|---|---|---|---|---|
| Bt 350 mL ha$^{-1}$ | 13.0 ± 0.61 a | 0.5 ± 0.79 a | 96.15 | 95.88 |
| Mr 5.0 kg ha$^{-1}$ | 14.0 ± 0.35 a | 1.0 ± 0.61 a | 92.86 | 92.35 |
| Bt 200 mL ha$^{-1}$ | 14.0 ± 0.94 a | 2.0 ± 0.25 a | 85.71 | 84.69 |
| Mr 2.0 kg ha$^{-1}$ | 13.0 ± 0.35 a | 3.50 ± 0.25 b | 73.08 | 71.15 |
| Fd 20 mL ha$^{-1}$ | 15.0 ± 0.79 a | 3.75 ± 0.50 b | 75.0 | 73.21 |
| Testemunha | 15.0 ± 0.61 a | 14.0 ± 0.65 c | 6.67 | – |
| C.V. % | 10.86 | 30.21 | – | – |

***Means followed by the same letter in the column do not differ from each other by the Scott-Knott test at the 5 % probability. ¹NIVaA = the number of live insects before application; NIVdA = number of live insects after application; % Red = percentage of insect reduction; % E = percentage of efficiency.**

temperature being around 26 °C (Alves 1998). This factor corresponds to that observed in the present study (Figure 1). At the moment of application of the phytosanitary treatments, the soybean plants presented a large amount of leaves. According to Alves & Lecuona (1998), this large amount of leaves is favorable to the development of the fungus *M. rileyi*.

Puttler et al. (1976) worked with the *M. rileyi* fungus, obtaining for *T. ni* 52% mortality under laboratory conditions. Ignoffo (1981) observed 67 % mortality for this same species in the field; in addition, it also observed a reduction in the reproductive capacity of adults, favoring their management with other measures based on IPM. Alves et al. (1978) obtained mortality between 50 and 60 % for the same cotton caterpillar in the field.

The treatments with biological insecticides presented a greater reduction in the number of caterpillars and better efficiency in detriment to the chemical treatment, except for the treatment Mr 2.0 kg $ha^{-1}$ (Table I). To be considered economically viable, a phytosanitary product should provide at least 80 % efficiency in controlling a pest (Tomquelski & Martins 2007).

Martins & Tomquelski (2015) conducted field trials with the Flubendiamide insecticide at doses of 12 and 14.4 grams of active ingredient per hectare for large (> 1.5 cm) and small (<1.5 cm) of *C. includens*. For the lower dosage, the values were less than 80%, regardless of the size of the caterpillars. For the higher dosage, values above 80% efficiency were obtained for small caterpillars only after the first evaluation (2 days after application of treatments) and, for large caterpillars, at the evaluations performed at 4 and 7 days after application.

Indicative of the lower effect of the chemical treatment may be related to some factors. The location of the pest in the plant, in the middle and lower thirds, provides benefit of the umbrella effect due to the leaves, favoring less contact between the active ingredient and

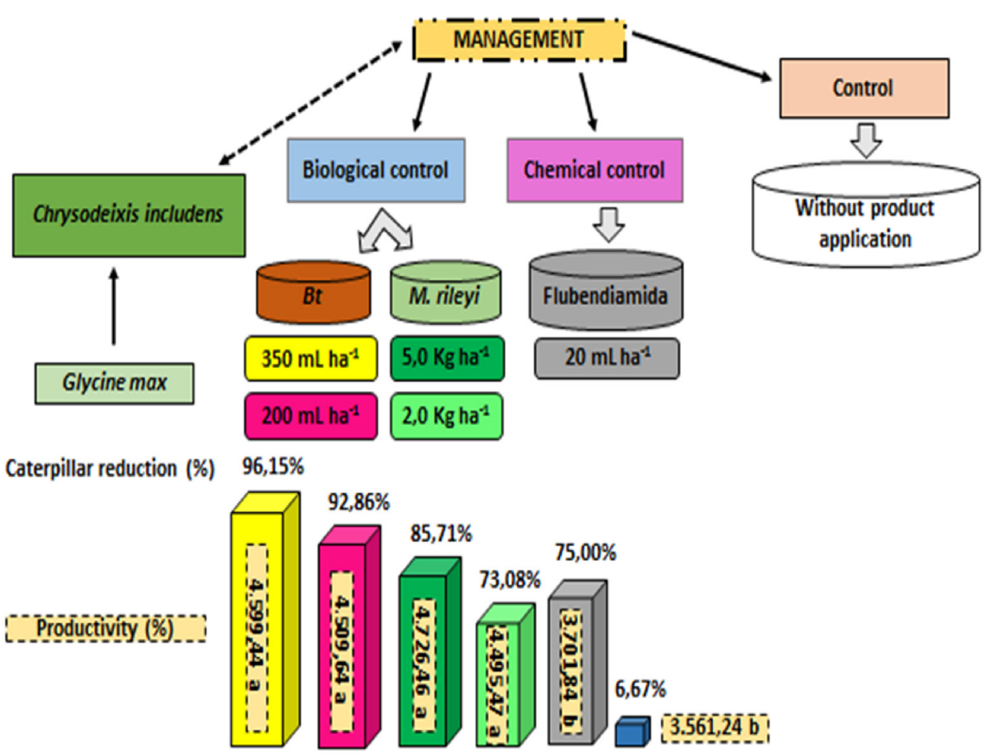


**Figure 2.** Performance of biological products as a function of phytosanitary treatments.

the pest (Carvalho et al. 2012). There is also high tolerance of this species to chemical molecules (Sosa-Gómez & Omoto 2012) and the possibility of selection of resistant individuals, as reported by Mascarenhas & Boethel (2000).

Another point to consider is the mode of action of the tested insecticide. Regarding the phytotechnical parameters, the management of *C. includens* reflected only on the number of pods per plant and productivity. It was found that all biological treatments provided better performance relative to the insecticide and the control (Table II). In general, pesticides, minerals and organics penetrate more or less the tissues of plants, especially when associated with certain surfactants. Thus, they act on their metabolism presenting action on the main physiological processes, such as respiration, perspiration and photosynthesis (Chaboussou 2006). The muscular contraction of the insects depends on the vesicular release of calcium ions, which are activated by means of rianodine receptors (RYR) (Lahm 2000), composed of four identical subunits, forming the calcium canals, located in the sarcoplasmic reticulum of the muscles and non-muscular cell endoplasmic reticulum (Gullan & Cranston 2005).

Diamides, the chemical group of Flubendiamide, activate the irregular release of calcium stores from the cells, acting on the RYR, which binds troponin, and changes its configuration causing it to detach from the tropomyosin, followed by the release of the site of actin binding in myosin resulting in muscle contraction (Campbell et al. 1987, Satelle et al. 2008). As a result of the intoxication by diamidas, the insect suffers a sudden cessation of feeding, lethargy, paralysis and, finally, death (Hanning et al. 2009).

The $Ca^{+2}$ similar to RYR canals are found in membranes of the vacuole, endoplasmic reticulum and vesicles of plant cells, such as Inositol Triphosphate ($IP_3R$) receptors and cyclic ribosomal adenosine diphosphate (cADPRR) receptors, induced by Inositol Triphosphate ($IP_3$) and Adenosine diphosphate cyclic ribosome (cADPR), respectively, are opened, allowing the passage of $Ca^{+2}$ ions to the cytosol (Inácio et al. 2011, Maathuis 2011).

Among the functions of the $Ca^{+2}$ in the plant tissue are cell division and extension, an extremely important process for the growth of root and pollen tubes. The $Ca^{+2}$ is stored in the endoplasmic reticulum, in the chloroplasts

**Table II. Number of plants $m^{-1}$ (NP ± EP), number of pods $plant^{-1}$ (NVP ± EP), number of $pods^{-1}$ (NGV ± EP) and mass of 1,000 grains (M1000 G ± EP) and soybean yield (kg $ha^{-1}$) (P ± EP).**

| Treatment | NP | NVP | NGV[1] | M 1000 G | P |
|---|---|---|---|---|---|
| Bt 350 mL $ha^{-1}$ | 13.20 ± 0.237 a | 39.20 ± 1.82 a | 2.47 ± 0.024 a | 0.162 ± 0.004 a | 4.599.44 ± 286.61 a |
| Mr 5.0 kg $ha^{-1}$ | 13.48 ± 0.716 a | 38.70 ± 4.09 a | 2.42 ± 0.034 a | 0.164 ± 0.003 a | 4.509.64 ± 294.6 a |
| Bt 200 mL $ha^{-1}$ | 13.30 ± 0.197 a | 42.70 ± 4.19 a | 2.36 ± 0.048 a | 0.159 ± 0.002 a | 4.726.46 ± 472.23 a |
| Mr 2.0 kg $ha^{-1}$ | 13.63 ± 0.343 a | 38.58 ± 2.50 a | 2.46 ± 0.025 a | 0.157 ± 0.003 a | 4.495.47 ± 260.99 a |
| Fd 20 mL $ha^{-1}$ | 13.73 ± 0.188 a | 33.52 ± 1.824 b | 2.45 ± 0.039 a | 0.153 ± 0.006 a | 3.701.84 ± 83.94 b |
| Flubendiamide | 13.63 ± 0.096 a | 31.75 ± 1.68 b | 2.49 ± 0.008 a | 0.149 ± 0.003 a | 3.561.24 ± 164.13 b |
| C.V. % | 4.90 | 3.98 | 2.97 | 0.39 | 1.53 |

***Means followed by the same letter in the column do not differ from each other by the Scott-Knott Test at 5% probability level.**

and in the vacuole in which it appears in the concentration 105 times greater than in the cytosol, where the concentration of $Ca^{+2}$ is extremely low, being maintained between 0.1 and 0.2 μmol $L^{-1}$, which is essential for the cell, since it prevents phosphate precipitation, avoids competition with $Mg^{+2}$, for the binding sites and is a prerequisite for the performance of $Ca^{+2}$ as a secondary messenger, making the $Ca^{+2}$ an important regulatory function, including in the balance between anions and cations and in the osmotic regulation of the cell (Furlani 2004, Inácio et al. 2011).

According to O'Brien & Ferguson (1997), calcium is involved in the programmed death of the plant cell resulting from the disorganization of functions such as the loss of membrane selective permeability and the non-operation of the signaling mechanisms in which calcium operates as a messenger, leading to several cytological events where cell death begins by loss of compartmentalization of calcium increased in its content in the cytosol (Malavolta 2006).

Therefore, the effect generated in the number of pods and consequently in the soybean yield, even if there was no difference in relation to the level of protection exerted between Flubendiamida and Mr 2.0 kg $ha^{-1}$, may have occurred due to the effect of the first in relation to the calcium canals, increasing its content in phytotoxic form and leading to abortion of the pods and consequently their reduction. This hypothesis requires specific tests that elucidate this possibility.

In general, the biological products tested did not allow the *C. includens* caterpillars to cause a reduction in soybean yield, even though the lower dose of Mr (2.0 kg $ha^{-1}$) had significantly lower control efficiency than the best treatments (Table II). This fact can be related to the tolerance of soybean plants to the reduction of leaf area, around 30% (Gallo et al. 2002), thus conserving the source-drain relationship between this leaf mass and the full development of the pods (Taiz & Zeiger 2013) and favoring the translocation of photoassimilates (Majerowicz 2004).

The present work highlights the potential of *B. thuringiensis* and *M. rileyi* fungi to control the populations of *C. includens*. Research of this nature is scarce in the scientific literature, requiring more studies involving number of applications, different doses of entomopathogens and higher insect densities to propose a microbial control program of *C. includens*.

We conclude the application of *B. thuringiensis* at doses of 200 and 350 mL $ha^{-1}$ and *M. rileyi* at 5.0 kg $ha^{-1}$ dose were the most efficient in reducing the number of *C. includens* caterpillars. In turn, the productivity and number of pods per plant were higher for biological treatments than for the chemical.

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## Author contributions

F.S.P.B. developed the project, assembled the experiment, collected and analyzed the data and wrote the manuscript. E.S.L. helped, guided and oversaw the writing of the project and assembly of the experiment; provided funds for the purchase of materials; and reviewed the manuscript. L.G.A.P. helped elaborate on the project, provided the necessary laboratory materials and reviewed the manuscript. P.M.D. collaborated on the analysis and interpretation of data and revised the manuscript. J.E.J. and L.A.A. cooperated in the assembly and evaluation of the experiment. A.A.N. reviewed the manuscript.