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Original article

Seed treatment strategies combining *Bacillus amyloliquefaciens* and insecticides for management of the emerging soil pest *Scutigerella immaculata* in soybean

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Abstract

Scutigerella immaculata (Symphyla: Scutigerellidae) causes increasing problems in soybean-producing areas of Brazil, particularly in MATOPIBAPA, one of the country's most recent agricultural frontiers. This study evaluated soybean seed treatments for the control of *S. immaculata*, using *Bacillus amyloliquefaciens* applied alone or combined with chemical insecticides. It also assessed pest sampling methods and soybean responses to these treatments. Soybean seeds were treated on the day of planting in the municipality of Açailândia, Maranhão State, Brazil. The experiment was conducted under field conditions in a completely randomized design with nine treatments and four replicates. The treatments (T) were T1: untreated control, T2: fipronil, T3: bifenthrin + imidacloprid, T4: clothianidin, T5: *B. amyloliquefaciens,* T6: clothianidin + fipronil, T7: clothianidin + lambda-cyhalothrin, T8: clothianidin + fipronil + *B. amyloliquefaciens*, and T9: cyantraniliprole + lambda-cyhalothrin + thiamethoxam + abamectin. *S. immaculata* was sampled using potato baits placed in the soil and by direct counting on plant roots. Evaluated parameters included plant height, seedling emergence, seed germination, and fresh root and shoot mass, which showed significant differences between treatments. Direct counting on plant roots detected more individuals than bait sampling. Among the treatments, T7 resulted in the lowest mean number of *S. immaculata* individuals. Pest population peaks occurred between 60 and 90 days after planting. Further studies are needed to clarify the effectiveness of the evaluated biological product and to determine the contribution of soybean seed treatment to the control and integrated management of this emerging pest.

Keywords: biological control, chemical, crop protection, *Glycine max*, Scutigerellidae

Introduction

Scutigerella immaculata Newport, 1844 (Scutigerellidae) belongs to the class Symphyla, a group of edaphic arthropods characterized by the presence of numerous pairs of legs (Fig. 1A) (Jin *et al*. 2023). Morphologically, symphylans are more similar to chilopods (centipedes) and millipedes than to insects (Class Insecta), (Marekl and Shea 2022). This species is of European origin and has a cosmopolitan distribution. It is commonly associated with agricultural and urban environments, where it acts as an underground herbivore (Waterhouse 1968; Joseph 2015). It is typically found in soils rich in organic matter with high moisture, sheltering under stones, in plant litter, decomposing wood, and other humid microhabitats (Fig. 1B). Its feeding behavior is primarily based on the consumption of fine roots, rootlets, coleoptiles, and plant detritus (Umble and Fisher 2003).

In recent years, this feeding habit has been increasingly associated with damage in soybean (*Glycine max* L.) production areas within the MATOPIBAPA region (Maranhão, Tocantins, Piauí, Bahia, and more recently Pará), one of the most rapidly expanding agricultural frontiers in Brazil, characterized by intense soybean cultivation and large-scale agricultural expansion (Fig. 1C) (Santos *et al*. 2023; Araújo *et al*. 2024). The intensification of agricultural land use in this region, combined with the conversion of native ecosystems and the adoption of high-input production systems, has altered soil microbial and faunal communities by reducing biodiversity. These changes may decrease natural biological regulation and create favorable conditions for the establishment and population growth of soil-dwelling pests such as *S. immaculata* (Vanolli *et al*. 2024).

Internationally, *S. immaculata* has been associated with significant losses in agricultural systems, particularly during severe outbreaks as was reported in France after 2010, with intense infestations in maize crops (Joseph 2015). These outbreaks coincided with restrictions and bans on insecticides commonly used in seed treatments, such as imidacloprid, fipronil, and organophosphate compounds, highlighting the importance of chemical management in controlling this group of arthropods (Swenson 1966; Bovi *et al.* 2018).

In Brazil, Loureiro and Galvão (1970) first reported the occurrence of symphylans of the genus *Hanseniella* (Scutigerellidae) causing economic damage to rice crops in Minas Gerais, where they attacked coleoptiles and rootlets. This record remains one of the earliest documented cases of economic losses associated with symphylans in Brazilian agriculture (Morais and Silva 2010). The economic damage caused by *S. immaculata* in crops of agricultural importance is mainly associated with attacks on seeds and roots, resulting in seedling mortality, stand reduction, and delayed vegetative growth (Bateman *et al.* 2023). These symptoms closely resemble those associated with phosphorus deficiency, drought stress, or plant-parasitic nematode infestation. Because the pest remains hidden in the soil and direct signs of infestation are rarely observed without root excavation and soil inspection, accurate field diagnosis is often difficult. (Marchesini *et al.* 2025).

The expansion of agriculture into regions adjacent to biodiversity hotspots, such as the Legal Amazon, has intensified interactions between production systems and natural ecosystems, increasing the occurrence of new phytosanitary challenges (Araújo *et al*. 2024; Ma *et al*. 2025). Although biodiversity provides essential ecosystem services, such as climate regulation, nutrient cycling, and soil structure maintenance, the conversion of these areas into agricultural frontiers has profoundly altered local ecological interactions (Laurance *et al.* 2002).

Chemical seed treatments suppress pests through direct toxic effects, reducing feeding and population establishment. In contrast, *B. amyloliquefaciens* acts indirectly by colonizing the rhizosphere, producing bioactive compounds, promoting plant growth, and inducing systemic resistance, thereby enhancing plant tolerance to soil-dwelling pests (Zalila-Kolsi *et al.* 2023; Vasques *et al*. 2024).

The objective of this study was to evaluate the efficiency of soybean (*Glycine max*) seed treatment (TS) in the control of *S. immaculata*, using *B. amyloliquefaciens* alone and in association with different chemical molecules, as well as to record phytotechnical parameters of soybean plants as a function of the treatments applied.

Materials and Methods

Experimental area and design

Experimental protocols were adapted and conducted at the Itamarati farm, located in the municipality of Açailândia, state of Maranhão (MA), Brazil. The soybean season 2022-2023 was explored (Fig. 2). Because *Scutigerella immaculata* is an emerging pest in soybean production in Brazil and no validated sampling protocol is currently available for this crop in Brazil, the methodology was adapted from sampling approaches previously employed for Symphyla and other soil-dwelling arthropods (Michelbacher 1938; Waterhouse 1968; Joseph 2015).

The study was carried out under field conditions using a Completely Randomized Design (CRD), with nine treatments and four replications. Experimental plots measured 7 m in length and 3 m in width and consisted of six soybean rows per plot. Row spacing was 0.5 m, with a plant density ranging from 12 to 16 plants per linear meter. Each experimental unit corresponded to a used area of 21 m², resulting in a total experimental area of 924 m².

A Completely Randomized Design (CRD) was adopted because preliminary field inspections indicated irregular and aggregated spatial distribution of *S. immaculata* populations throughout the experimental area. Under these conditions, the establishment of homogeneous blocks would not have adequately represented the natural infestation variability of this soil-dwelling pest. Therefore, treatment randomization across the entire experimental area was considered more appropriate to minimize potential spatial bias associated with naturally heterogeneous pest occurrence.

The MATOPIBAPA region (Maranhão, Tocantins, Piauí, Bahia, and Pará) has a tropical savanna climate (Aw), with mean annual temperatures of 25–28°C and rainfall ranging from 1.000 to 1.800 mm, concentrated between October and April. Recent studies indicate increasing temperatures and more frequent drought events associated with land-use change and climate variability in this Amazon–Cerrado transition zone (Oliveira Aparecido *et al*. 2023; Siqueira *et al*. 2024). The soils of Açailândia, Maranhão, are predominantly highly weathered acrisols and ultisols, characterized by acidic pH, low natural fertility, high aluminum saturation, and susceptibility to erosion.

The treatments consisted of (T1) untreated control, (T2) fipronil 250 g L^{-1} per 100 kg of seeds, (T3) bifenthrin 135 g L^{-1} + imidacloprid 165 g L^{-1} per 100 kg of seeds, (T4) clothianidin 600 g L^{-1} per 100 kg of seeds, (T5) *Bacillus amyloliquefaciens* strain MBI600, at 600 mL of the pure liquid product per 100 kg of soybean seeds, corresponding to a CFU of $2x10^9$ viable spores/mL , (T6) clothianidin 600 g L^{-1} + fipronil 250 g L^{-1} per 100 kg of seeds, (T7) clothianidin 600 g L^{-1} + lambda-cyhalothrin 250 g L^{-1} per 100 kg of seeds, (T8) clothianidin 600 g L^{-1} + fipronil 250 g L^{-1} + *B. amyloliquefaciens* strain MBI600, at 600 mL of the pure liquid product per 100 kg of soybean seeds, corresponding to a CFU of $2x10^9$ viable spores/mL and (T9) cyantraniliprole 600 g L^{-1} + lambda-cyhalothrin 37,5 g L^{-1} + thiamethoxam 210 g L^{-1} + abamectin 500 g L^{-1} per 100 kg of seeds. The mixtures involved simple, binary, or tertiary mixtures for seed treatment.

Treatment (T2) fipronil 250 g L^{-1}, is a systemic insecticide formulated as a flowable concentrate for seed treatment (FS) and registered in Brazil under MAPA No. 10816. Fipronil acts by contact and ingestion, disrupting γ-aminobutyric acid (GABA)-gated chloride channels in the insect central nervous system, leading to neuronal hyperexcitation and death. The product is classified as Toxicological Category IV (slightly toxic) and Environmental Hazard Class II.

Treatment (T3) consisted of bifenthrin 135 g L^{-1} + imidacloprid 165 g L^{-1} (MAPA Registration No. 6714), formulated for seed treatment. This mixture provided two distinct modes of action: bifenthrin, a pyrethroid, acts by contact and ingestion, affecting voltage-gated sodium channels in nerve membranes; imidacloprid, a neonicotinoid, exhibits systemic activity and binds to nicotinic acetylcholine receptors in the insect nervous system, resulting in hyperexcitation and mortality.

Treatment (T4) acted systemically, being absorbed by treated seeds and translocated within the emerging plant, thereby providing early protection against insects attacking seeds and seedlings during the initial stages of crop development. The product is registered in Brazil under MAPA No. 12812 and is classified as Toxicological Category IV (slightly toxic) and Environmental Hazard Class II.

Bacillus amyloliquefaciens strain MBI600 (T5) is a biological control agent generally formulated as suspension concentrate (SC). It was applied at 600 mL of commercial product per 100 kg of soybean seeds, with CFU of $2x10^9$ viable spores/mL. It is classified as a microbiological product whose mode of action involves preventive protection through competition for space and nutrients, as well as the production of metabolites that inhibit phytopathogenic organisms. The product is registered with MAPA under No. 22718. No significant acute toxicity is expected under typical agricultural exposure conditions.

Treatment (T6) consisted of the combination of clothianidin 600 g L^{-1} and fipronil 250 g L^{-1} applied as seed treatments for soybean. Clothianidin is a systemic neonicotinoid insecticide that is absorbed by the seed and translocated within the plant during early development, providing protection against sucking and chewing insects. Fipronil acts by contact and ingestion, disrupting γ-aminobutyric acid (GABA)-gated chloride channels in the insect nervous system. The combination of these insecticides provided protection against soil-dwelling pests and insects attacking soybean seedlings during the early stages of crop establishment.

Treatment (T7) consisted of the combination of clothianidin 600 g L^{-1} and lambda-cyhalothrin 250 g L^{-1} applied as seed treatment. Clothianidin acts systemically after seed treatment, being absorbed during germination and translocated to the developing seedling, thereby providing protection against insects that attack seeds, roots, and young tissues after emergence. Lambda-cyhalothrin is a pyrethroid insecticide formulated as a capsule suspension (CS) that acts by contact and ingestion, affecting voltage-gated sodium channels in the insect nervous system.

Treatment (T8) consisted of the combination of clothianidin 600 g L^{-1}, fipronil 250 g L^{-1} and *B. amyloliquefaciens* strain MBI600, at 600 mL of the pure liquid product per 100 kg of soybean seeds, corresponding to a CFU of $2x10^9$ viable spores/mL. Clothianidin is a systemic neonicotinoid insecticide that is absorbed during seed germination and translocated within the developing plant. Fipronil acts by contact and ingestion, disrupting γ-aminobutyric acid (GABA)-gated chloride channels in the insect nervous system. The combination targeted pests affecting soybean seeds and seedlings during the early stages of crop establishment.

Treatment (T9) consisted of the combination of cyantraniliprole 600 g L^{-1} + lambda-cyhalothrin 37,5 g L^{-1} + thiamethoxam 210 g L^{-1} + abamectin 500 g L^{-1} applied as seed treatments. Cyantraniliprole, a diamide insecticide, acts primarily by ingestion through activation of ryanodine receptors, leading to muscle paralysis in insects. Thiamethoxam is a systemic neonicotinoid that acts on nicotinic acetylcholine receptors in the insect nervous system, whereas lambda-cyhalothrin is a pyrethroid insecticide that affects voltage-gated sodium channels through contact and ingestion. Abamectin is an avermectin derivative that acts on glutamate-gated chloride channels, affecting insects and certain soil-dwelling pests. This combination targeted insects and other pests that affect soybean seeds and seedlings during the early stages of crop development.

No specific compatibility or cell viability assays were performed prior to planting to confirm the survival of *B. amyloliquefaciens* after mixing between treatments. However, the intermediate biological performance observed in treatments containing *B. amyloliquefaciens* suggested that at least part of the inoculant activity may have been maintained under field conditions. We acknowledge this as an important limitation of the study and recommend that future investigations include compatibility and microbial viability assessments for combined chemical-biological seed treatments. Toxicological and environmental classifications were included to characterize the products evaluated and to justify their selection for field testing against *S. immaculata*.

Seed treatment and trial conduct

The experiment was conducted using soybean seed treatments (ST), carried out on the day of planting. For each treatment, 100 kg of certified soybean seeds, with germination greater than 80%, belonging to a cultivar representative of the MAPITOBAPA region, were used. The treatments were carried out in plastic bags with a capacity of 3 liters, using a volume of 600 mL per 100 kg of seeds.

Parallel to the field trials, the same treatments were installed in 5-liter plastic trays containing sand. These treatments aimed to evaluate germination and initial development of the plants. These trays were kept in an agricultural greenhouse, in a protected environment under controlled growing conditions.

The parameters evaluated in the field and in protected cultivation were: (i) plant height (cm), measured at 50 plants per plot at 10, 20, 30, 40 and 50 days after planting (DAP); (ii) emergence in a sandbox at 5, 10, 15 and 20 DAP; (iii) vigor and phytotoxicity, by mean comparative visual notes to the control, at 5, 10, 15 and 20 DAP; (iv) germination (%). The assessment (v) number of individuals of *S. immaculata*, sampled by means of baits or direct count in the roots following the methodology of Benjamin and Nielsen (2004), at 20, 30, 45, 60 and 90 DAP; (vi) fresh mass of the root and shoot of the plants at 20, 45 and 60 DAP; and (vii) productivity, obtained by harvesting three rows of 5 m per plot.

Statistical analysis and modeling

Data were screened for outliers using BoxPlot graphs and tested for normality by the Lilliefors test and histogram inspection in SAEG® software (Ribeiro Junior and Melo 2009). Counts of *S. immaculata* obtained from bait traps and direct root sampling were transformed using $\sqrt{(x + 1)}$ when normality assumptions were not met. Transformation adequacy was assessed by comparing coefficients of variation (CV), as proposed by Reed *et al*. (2002). ANOVA and mean comparisons were performed using transformed data, whereas figures are presented on the original scale.

Polynomial regression models were fitted according to the temporal behavior of the evaluated variables. To minimize overfitting, models were visually inspected by comparing observed and predicted values (Hawkins 2004), and those with the highest coefficients of determination (R^2) were validated using the holdout cross-validation method in SigmaPlot® 11. The models were described by the general equation: [$y = \beta_0 + \beta_1x + \beta_2x^2 + \cdots + \beta_nx^n + \varepsilon$] where ($y$) is the response variable, (x) is days after planting (DAP), (β) are regression coefficients, and (ε) is the residual error.

Cluster analysis based on Euclidean distance was performed to classify treatments according to seedling emergence using SAEG® software. Biological variables were subjected to ANOVA, and means were compared by Tukey's test ($P \leq 0.05$). Additionally, the temporal response of plant height was evaluated using range, standard deviation, and coefficient of variation to determine whether extended assessment periods improved the sensitivity for detecting treatment effects.

Results

Plant height evaluations conducted at later developmental stages showed progressively greater data dispersion and variability, indicating higher sensitivity for detecting treatment effects at 30 and 40 DAP compared with earlier evaluations (Table 1).

The distribution of plant height as a function of days after planting (DAP) is shown in Figure 3. From 30 DAP onward, a greater dispersion of values was observed, particularly at 40 DAP, compared with the evaluations conducted at 10 and 20 DAP (Figs. 3A and B).

Mean comparisons for soybean plant height at 10, 20, 30, and 40 DAP are shown in Figure 4. No significant differences between treatments were detected at 10 DAP ($F = 1.26$; $P = 0.080$) (Fig. 4A). From 20 DAP onward, significant differences in plant height were observed between treatments ($F = 4.26$; $P = 0.004$) (Fig. 4B).

Treatment effects became more pronounced at 30 and 40 DAP, indicating that later evaluations were more sensitive for detecting differences in soybean growth (Figs. 4C and D).

Hierarchical cluster analysis based on Euclidean distance revealed the formation of at least four distinct groups regarding soybean seedling emergence following seed treatment (Fig. 5A). Treatments T7 and T9 formed one group characterized by higher emergence values, whereas T6, T5, and T4 constituted an intermediate group. Treatments T3 and T2 formed a third group, while the untreated control (T1) was positioned as an isolated group with the lowest emergence. These groupings were confirmed by analysis of variance, which showed significant differences in seedling emergence between treatments ($F = 18.26$; $P = 0.04$) (Fig. 5B). Treatments T7 and T9 resulted in the highest emergence values, followed by intermediate values observed for T6, T5, and T4, whereas T3, T2, and the untreated control exhibited the lowest emergence rates.

Seedling vigor did not differ between treatments, with all treatments reaching 100%, suggesting low sensitivity of this parameter under the evaluated conditions (Fig. 5C). As for phytotoxicity symptoms, there were also no significant differences between treatments, with no symptoms observed, regardless of any of the nine ST applied to soybean seeds in the pre-planting (Fig. 5C).

Significant differences in seed germination were observed between treatments ($F = 20.45$; $P = 0.04$) (Fig. 5D). Treatments T7 and T9 exhibited the highest germination rates, exceeding 98% and 96%, respectively (Fig. 5D). Treatments T2, T3, T4, T5, T6, and T8 showed intermediate germination values, ranging between 90% and 94%, whereas the untreated control (T1) presented germination values below 88% (Fig. 5D).

The number of *S. immaculata* individuals differed significantly between treatments for both sampling methods, including bait traps ($F = 34.78$; $P = 0.04$) and direct root counts ($F = 43.23$; $P = 0.03$) (Fig. 6). The untreated control consistently exhibited the highest number of *S. immaculata* individuals across both sampling methods used (Figs. 6A and B), including baited traps and direct root counts. When sampling was performed using bait traps, no individuals of *S. immaculata* were detected in treatments T3, T4, T5, T6, and T7 (Fig. 6A). In contrast, direct root counting revealed that treatment T7 resulted in the lowest mean number of *S. immaculata* individuals compared with the other treatments (Fig. 6B).

Population growth dynamics of *S. immaculata*, based on direct root counts, were described using quadratic polynomial regression models across the different sampling times (Fig. 6C). The regression curves, as well as the adjusted coefficient of determination, for each treatment are presented as follows: (T1) $y= 2.45-0.07x+0.0001x^2$ and adjusted $R^2= 0.98$, (T2) $y= 1.14-0.01x+0.0003x^2$ and adjusted $R^2= 0.96$, (T3) $y= 2.20-0.07x+0.0009x^2$ and adjusted $R^2= 0.94$, (T4) $y= 1.99-0.06x+0.0008x^2$ and adjusted $R^2= 0.94$, (T5) $y= 1.44-0.02x+0.0005x^2$ and adjusted $R^2= 0.97$, (T6) $y= 1.75-0.04x+0.0006x^2$ and adjusted $R^2= 0.94$, (T7) $y= 1.58-0.03x+0.0005x^2$ and adjusted $R^2= 0.94$, (T8) $y= 1.83-0.05x+0.0007x^2$ and adjusted $R^2= 0.98$ and, finally, (T9) $y= 1.45-0.02x+0.0004x^2$ and adjusted $R^2= 0.99$.

All treatments exhibited high coefficients of determination (adjusted R^2 ranging from 0.94 to 0.99), indicating a strong fit between observed and estimated values. In all treatments, pest population growth was relatively low during the initial sampling periods and increased more markedly at later stages, particularly between 60 and 90 DAP (Fig. 6C).

Significant differences in fresh root mass were observed between treatments at 20 DAP ($F = 67.89$; $P = 0.03$), 45 DAP ($F = 56.32$; $P = 0.03$), and 60 DAP ($F = 80.21$; $P = 0.03$) (Figs. 6A, C and E). At 20 DAP, treatment T5 resulted in the lowest root fresh mass (Fig. 7A). At 45 DAP, lower root fresh mass values were observed for treatments T9, T4, T2, and the untreated control (Fig. 7C). At 60 DAP, the untreated control (T1) presented the lowest root fresh mass of all treatments (Fig. 7E).

Similarly, shoot fresh mass differed significantly between treatments at 20 DAP ($F = 38.25$; $P = 0.04$), 45 DAP ($F = 72.41$; $P = 0.04$), and 60 DAP ($F = 77.21$; $P = 0.04$) (Figs. 7B, D and F). At 20 DAP, treatment T5 resulted in lower shoot fresh mass values than the other treatments (Fig. 7B). At 45 DAP, treatments T7 and T1 exhibited the lowest shoot fresh mass (Fig. 7D), whereas at 60 DAP, T7 and T1 presented the highest and lowest shoot fresh mass values, respectively (Fig. 7F).

Soybean yield did not differ significantly between the seed treatment strategies evaluated for protection against *S. immaculata* ($F = 10.81$; $P = 0.06$) (Fig. 8). Regardless of treatment, yield values were higher than the current national average for soybean production in Brazil.

In addition to treatment effects, the temporal distribution of plant height data demonstrated that evaluations conducted at later stages of crop development provided greater sensitivity for detecting differences between seed treatments. Although standard protocols adopted by Sumitomo Chemical Brasil Indústria Química S/A prioritize evaluations at 10 and 20 DAP, the present results indicated that assessments at 30 and 40 DAP obtained more pronounced biological responses, as reflected by increased data dispersion and variability. These findings support the inclusion of extended evaluation periods in future seed treatment assessment protocols.

Discussion

The present study addressed two complementary aspects of soybean seed treatment (ST). The first focused on phytotechnical parameters associated with germination and early seedling establishment, including effects on final yield. The second investigated the effectiveness of ST strategies for the management of *Scutigerella immaculata*, an emerging soil-dwelling pest that has raised increasing concern among soybean growers in the MATOPIBAPA region. The high clay content may have provided favorable moisture retention and refuge conditions for *S. immaculata*, contributing to population persistence throughout the crop cycle. Likewise, soil pH may influence the establishment and metabolic activity of *Bacillus* spp. in the rhizosphere, potentially affecting the expression of plant growth-promoting traits.

Seed treatment technologies have long been adopted in soybean production systems and contribute to crop establishment and productivity, particularly through inoculation with beneficial microorganisms (Brzezinski *et al*. 2015; Souza *et al*. 2016). Among these, *B. amyloliquefaciens* has attracted considerable attention due to its ability to colonize the rhizosphere, promote plant growth through nutrient mobilization and phytohormone production, and induce systemic resistance against biotic stresses (Backer *et al*. 2018; Rabbee *et al*. 2019). Additionally, *Bacillus* spp. produces bioactive metabolites, including lipopeptides and antimicrobial compounds, which can modulate rhizosphere microbial communities and contribute to the suppression of soil-borne pests and pathogens (Caulier *et al*. 2019). *Bacillus* based products can be easily incorporated into conventional seed-treatment programs, facilitating large-scale adoption in soybean production systems (Backer *et al*. 2018; Lamichhane *et al*. 2020). Thus, beyond pest management, the inclusion of *B. amyloliquefaciens* was justified by its potential to improve root development, plant vigor, and tolerance to root herbivory (Backer *et al*. 2018; Fabiano *et al*. 2018).

Despite the widespread use of chemical and biological products in seed treatment, knowledge regarding the biological responses of soybean plants to exposure to xenobiotic compounds remains limited, particularly when synthetic insecticides are applied. In the present study, insecticides from different chemical groups pyrazoles, pyrethroids, neonicotinoids, and anthranilamides were evaluated, as well as biological products of direct origin, such as *B. amyloliquefaciens*, and indirect origin, such as avermectins. These products were tested individually and in binary or tertiary mixtures. The use of mixtures has been increasingly reported as a relevant strategy for pest management (Gandini *et al.* 2020) and for maintaining crop performance under field conditions (Zandoná *et al.* 2019).

Although treatments containing *B. amyloliquefaciens* did not consistently result in the lowest pest densities, their intermediate performance suggests indirect plant-mediated effects rather than direct toxicity to *S. immaculata*. *Bacillus* species are known to promote plant growth through mechanisms such as phytohormone production, nutrient solubilization, and induction of systemic resistance. In addition, modulation of the rhizosphere microbial community may enhance root vigor and tolerance to herbivory. Therefore, the contribution of *Bacillus*-based treatments may be more closely related to improved plant resilience than to direct suppression of soil arthropods.

The complexity of interpreting plant and pest responses observed in this study is closely related to the combined use of products with distinct chemical and biological modes of action in seed treatment. Even so, when defining the treatments used, it was assumed that this approach would provide a greater approximation between the experimental conditions adopted and the current agricultural reality in Brazil. In this context, the mixture of products of different natures has become an increasingly frequent practice in the field. This practice has relevant positive points, both from the operational point of view and in terms of savings in the number of applications made (Gazziero 2015; Rakes *et al.* 2017).

With respect to plant height, particularly at 30 and 40 days after planting (DAP), it was not possible to precisely determine why treatments T3 and T4 improved plant growth compared with the untreated control, although their responses were lower than those observed for the most effective treatments.

This response does not appear to be directly associated with inefficiency in controlling *S. immaculata.* Other treatments with comparatively lower pest suppression, such as T5 and T8, also did not rank among the most effective treatments based on root sampling. Nevertheless, most treatments resulted in greater and statistically similar plant height values throughout the evaluation period. These findings are consistent with previous studies reporting that certain seed treatment formulations may influence soybean growth dynamics during early development (Ribeiro *et al.* 2014; Brzezinski *et al.* 2015; Fabiano *et al.* 2018).

In the present study, the lower initial infestation levels observed during early soybean development likely contributed to improved root establishment and greater plant tolerance to herbivory throughout subsequent growth stages. This may explain why some treatments still showed differences in *S. immaculata* populations between 60 and 90 DAP, even after the expected residual activity period of seed-applied products. In addition, *Bacillus amyloliquefaciens* based treatments may have promoted indirect physiological effects through rhizosphere modulation, induced systemic resistance, phytohormone production, and enhanced root vigor, which can persist beyond the initial protection window. Previous studies have demonstrated that seed treatments may produce long-term indirect effects on plant physiological performance and root-associated microbial interactions, even when direct pesticidal activity declines over time (Fabiano *et al*. 2018; Lamichhane *et al*. 2020). Therefore, the late-season population increase of *S. immaculata* observed in this study likely reflected the progressive reduction of direct seed treatment protection combined with ecological and physiological interactions established during the early stages of soybean development.

The hierarchical clustering of treatments based on seedling emergence revealed at least four distinct response groups, which were subsequently confirmed by mean comparison tests. These results indicate that seedling emergence exhibited a degree of plasticity depending on the nature of the seed treatment applied. Similar responses have been reported in studies evaluating the physiological quality of treated soybean seeds (Ferreira *et al.* 2016; Pereira *et al.* 2020). Notably, seedling emergence and germination proved to be more sensitive indicators of treatment effects than seed vigor, which remained unchanged between treatments, as did visual phytotoxicity symptoms.

An important observation was that untreated seeds consistently exhibited lower germination and emergence rates than treated seeds, highlighting the advantage of seed treatment for early crop establishment. This represented an advantage for crop establishment under field conditions. On the other hand, between those treatments, TS, there were differences in these biological responses. T7 was the treatment that led to better germination and emergence values of soybean seedlings than the others evaluated. Neonicotinoid and pyrethroid-based products have been investigated when exposed to vegetable seeds, with distinct responses observed (Bortoletto *et al.* 2017; Bork *et al.* 2018; Lamichhane *et al.* 2020).

The superior performance of T7 may have been associated with the complementary modes of action of neonicotinoids and pyrethroids. Neonicotinoids exhibit systemic properties, allowing translocation within plant tissues and providing protection to emerging roots and shoots, whereas pyrethroids act primarily through contact and ingestion, producing rapid neurotoxic effects on exposed arthropods. The combination of these two chemical groups may therefore result in both immediate suppression of soil-dwelling individuals and extended residual protection during early seedling establishment. Such complementary activity could explain the consistent reduction of *S. immaculata* observed in root sampling under this treatment.

The experimental design did not allow inference regarding potential synergistic or antagonistic interactions between seed treatment components affecting seedling emergence. The results indicate that seedling emergence was indeed a more sensitive parameter to the treatments used than, for example, seed vigor, in addition to the visual signs of phytotoxicity that did not clash between treatments.

Regarding pest sampling, direct counting of *S. immaculata* on soybean roots proved to be more effective than the use of potato bait traps, despite being a more labor-intensive and destructive method. Soil-dwelling pests often present challenges for sampling due to their cryptic behavior and spatial heterogeneity (Benjamin and Nielsen 2004). Therefore, direct root sampling should be prioritized when accurate estimates of *S. immaculata* populations are required. Potato bait traps may serve as a complementary monitoring tool, particularly under moderate-to-high infestation levels, but their lower sensitivity at low population densities should be considered. The absence of individuals in bait traps, as observed in some treatments, should not be interpreted as complete pest suppression, since root sampling revealed the presence of *S. immaculata* in those same treatments.

Among the evaluated seed treatments, T7 consistently resulted in the lowest incidence of *S. immaculata* based on direct root counts. Neonicotinoid- and pyrethroid-based insecticides have been widely recognized as effective tools for managing herbivorous pests in soybean systems (Joseph *et al.* 2017). Nonetheless, it is important to emphasize that all seed treatment strategies evaluated in this study provided some degree of pest suppression relative to the untreated control, and none should be considered ineffective under the tested field conditions.

The results suggested that future management protocols for *S. immaculata* should consider the use of binary mixtures, particularly those combining neonicotinoids and pyrethroids, as demonstrated by the performance of T7. In addition to pest suppression, this treatment also promoted favorable plant development, as evidenced by increased root and shoot fresh mass during early growth stages, which may contribute to improved crop establishment.

Although early-season pests are known to cause stand reduction and yield losses in soybean (Sentelhas *et al.* 2015), such effects were not observed in the present study, as grain yield did not differ between treatments. These findings indicated that seed treatment alone may not be sufficient to fully eliminate *S. immaculata* populations in the soil. The results highlighted the importance of integrating seed treatment with broader crop protection strategies. Sustainable management programs increasingly rely on integrated approaches that combine chemical, biological, cultural, and monitoring-based tactics to maintain pest populations below economic thresholds (Czembor *et al*. 2025).

The yield levels observed across all treatments exceeded the national average for soybean production in Brazil, suggesting that the experimental conditions, including soil preparation, cultivar selection, crop management, and edaphoclimatic characteristics of the Açailândia region, were favorable for high productivity. Maranhão has been increasingly recognized as an important agricultural frontier for soybean expansion in Brazil (Martinelli *et al.* 2017).

Overall, seed treatment contributed to improved protection of the soybean root system during the early stages of development, as indicated by reduced initial pest populations, differences in root fresh mass, and enhanced germination and emergence in specific treatments. However, the sampling strategy and experimental design did not allow for assessment of *S. immaculata* dynamics throughout the entire crop cycle. It is, therefore, hypothesized that this pest exhibits behavior similar to other early-season soil pests, a hypothesis supported by the absence of yield differences between treatments despite variations in early pest incidence.

Although several seed treatments improved early vegetative parameters, including emergence, plant height, and root and shoot fresh mass, these responses did not translate into significant differences in soybean yield. This result suggested that the initial suppression of *S. immaculata* was sufficient to improve early crop establishment, but not enough to alter final productivity under the environmental and infestation conditions evaluated. Soybean plants exhibit considerable physiological plasticity and compensatory growth capacity following early root injury, which may partially explain the absence of yield differences between treatments. Similar findings have been reported in studies demonstrating that improvements in early plant development do not always result in proportional yield gains under moderate pest pressure or favorable environmental conditions (Lamichhane *et al*. 2020; Zandona *et al*. 2019). Therefore, although complex seed treatment mixtures such as T9 may have contributed to early pest suppression and plant protection, their economic viability should be considered cautiously and may depend on local infestation intensity, environmental conditions, and cost-benefit relationships within integrated pest management programs.

Overall, the results indicated that seed treatment played a critical role in mitigating early-season damage caused by *S. immaculata*, particularly during the establishment phase of soybean. However, its effects appeared to be temporally constrained, reinforcing the need to integrate chemical and biological seed treatments with broader integrated pest management (IPM) strategies. Such strategies may include crop rotation, soil management practices, and monitoring-based decision-making to ensure sustainable long-term suppression of soil-dwelling pests.

Conclusions

The seed treatment (ST) significantly influenced phytotechnical parameters associated with the early establishment of soybean, including plant height, seedling emergence, seed germination, and root and shoot fresh mass, particularly from 30 days after planting (DAP) onward.

Seed treatments reduced *Scutigerella immaculata* populations during the early stages of crop development; however, these effects did not translate into significant yield increases under the conditions evaluated. Further studies are needed to determine whether yield benefits may occur under different infestation levels or environmental conditions.

Root sampling should be prioritized for accurate detection of *S. immaculata*, while potato bait traps may serve as a complementary monitoring tool, especially under moderate-to-high infestation levels. Among the evaluated treatments, the mixture of clothianidin + lambda-cyhalothrin (T7) resulted in the lowest mean number of *S. immaculata* individuals, whereas treatments containing *Bacillus amyloliquefaciens*, applied alone or in combination, showed intermediate performance, indicating potential complementary use within integrated pest management programs.

Despite differences in early pest incidence and vegetative development between treatments, soybean yield did not differ and remained above the national average, suggesting that seed treatment primarily mitigated early damage caused by *S. immaculata* without necessarily translating into yield differences under the conditions evaluated. Therefore, seed treatment should be considered an important, but not standalone, strategy for the management of this emerging pest and should be integrated with additional agronomic practices throughout the crop cycle.

Population growth of *S. immaculata* was more pronounced between 60 and 90 DAP than on earlier sampling dates.

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Table 1. Dispersion metrics associated with soybean plant height evaluations conducted on different days after planting (DAP), aiming to assess the temporal sensitivity of seed treatment effects under field conditions.

DAP	Standard deviation	Range amplitude (cm)	Coefficient of variation (%)
10	0.79	4	6.44
20	1.62	5	7.66
30	3.41	11	11.34
40	5.25	18	13.49

DAP = days after planting. Increasing dispersion metrics over time indicate greater sensitivity of later evaluations (30–40 DAP) for detecting differences among soybean seed treatment strategies.

Figure descriptions

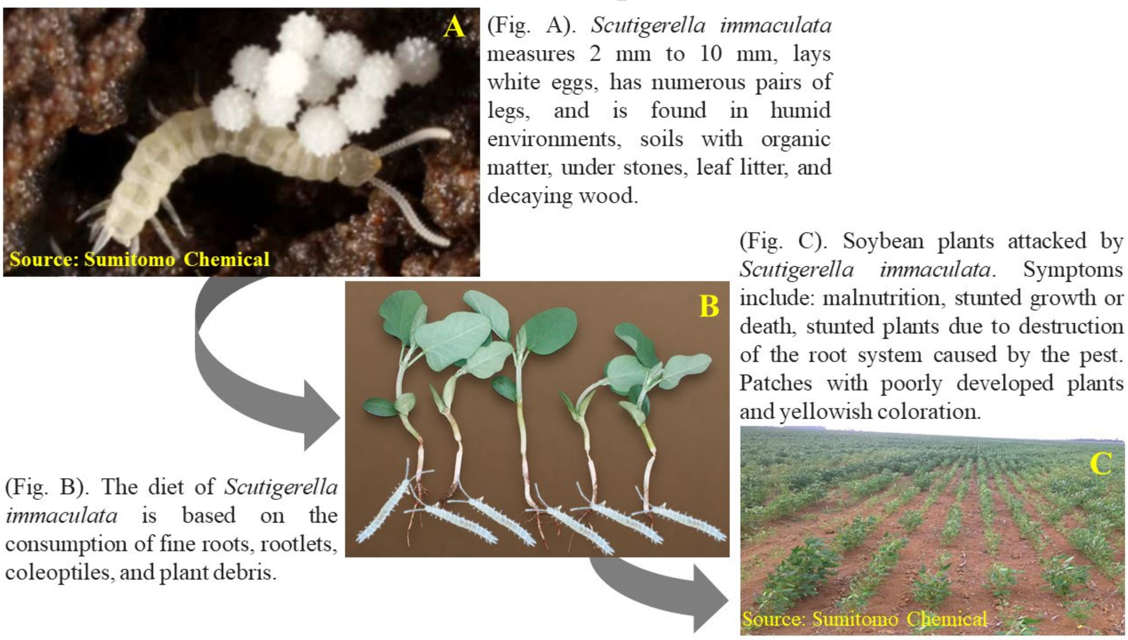


Fig. 1. Geographic location of the experimental area (Farm Itamarati) in the municipality of Açailândia, Maranhão State, Brazil, where the field experiment on the management of *Scutigerella immaculata* in soybean was conducted. Descriptions of the treatments used in Figs. 2–7 are provided in the Materials and Methods section.

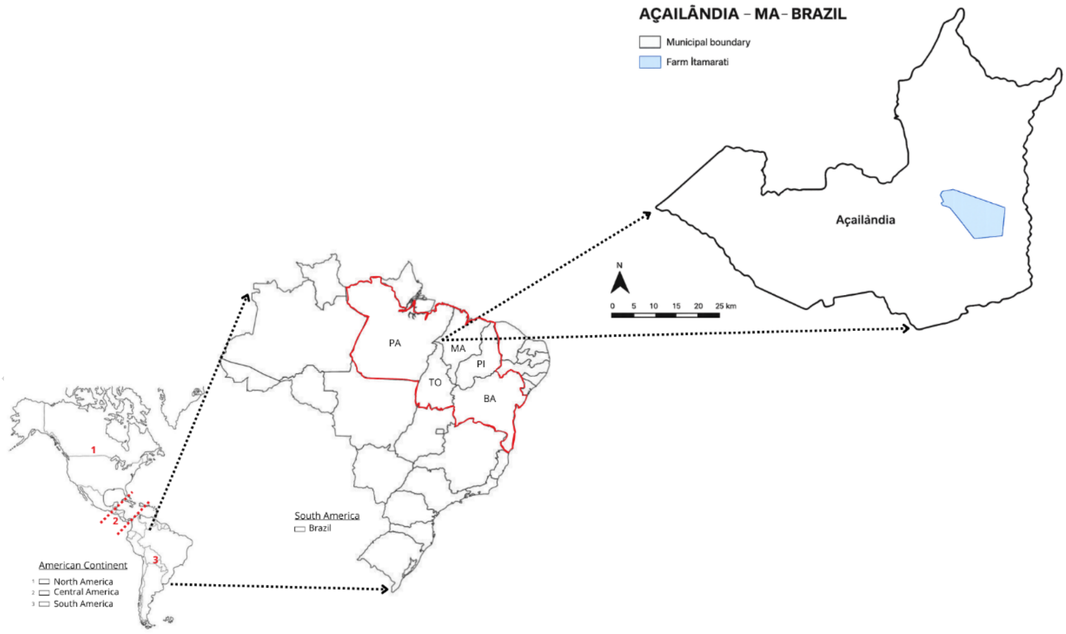


Fig. 2. Plant height distribution of soybean plants at 10, 20, 30 and 40 days after planting (DAP) under different seed treatment strategies. (A) Distribution of observed values. (B) Temporal variation in plant height.

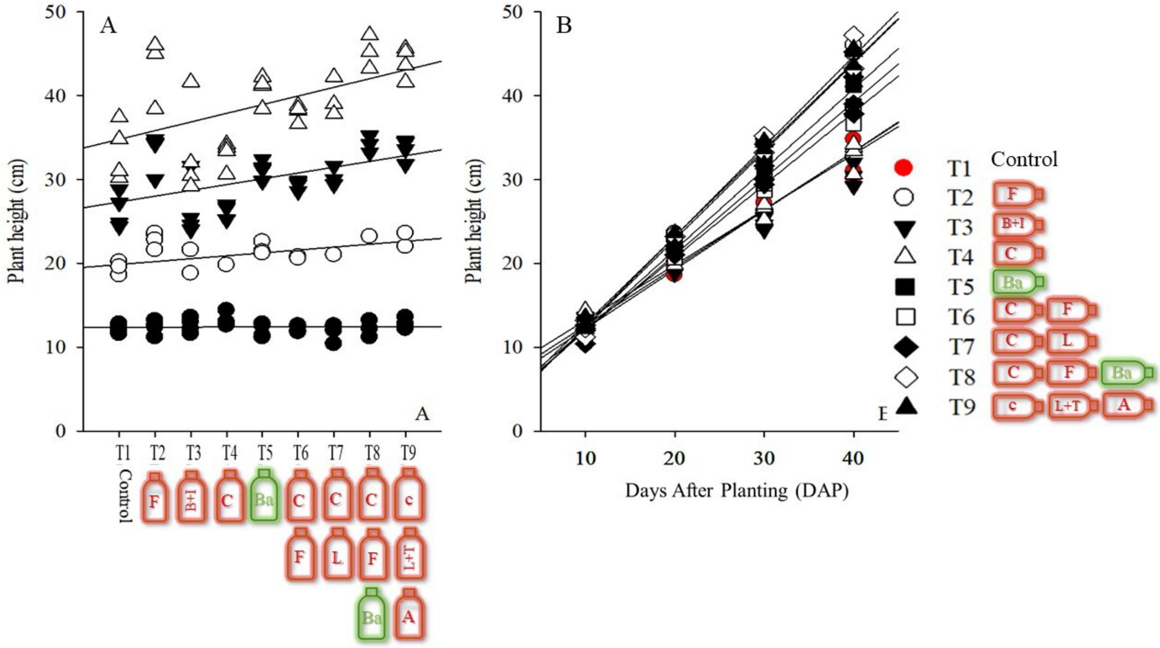


Fig. 3. Mean plant height (cm ± SE) of soybean plants at 10 (A), 20 (B), 30 (C), and 40 (D) DAP under different seed treatments. Means followed by the same letter do not differ according to Tukey's test ($P \leq 0.05$).

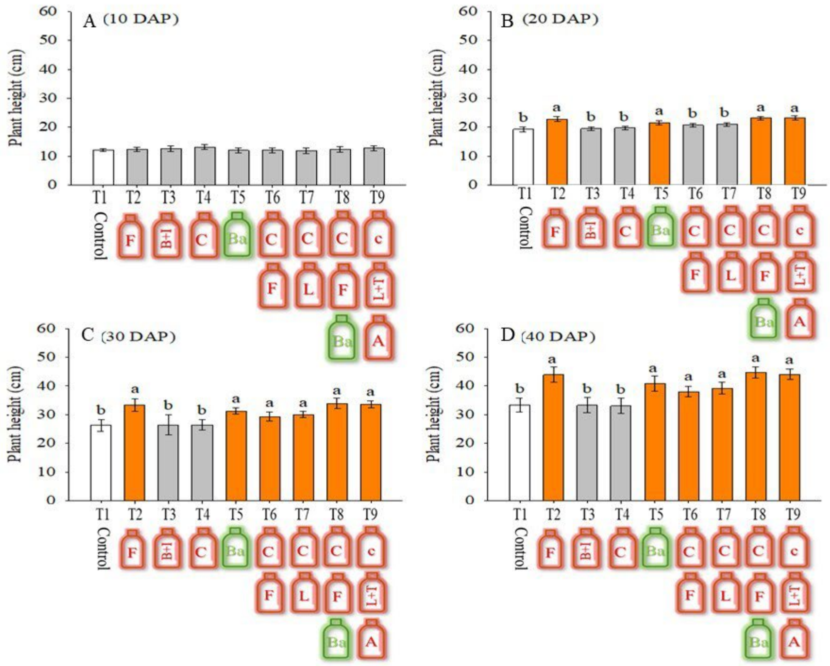


Fig. 4. Seed quality responses under different seed treatments. (A) Hierarchical clustering of treatments based on Mahalanobis distance. (B) Seedling emergence. (C) Seedling vigor and phytotoxicity. (D) Seed germination. Means followed by the same letter do not differ according to Tukey's test ($P \leq 0.05$).

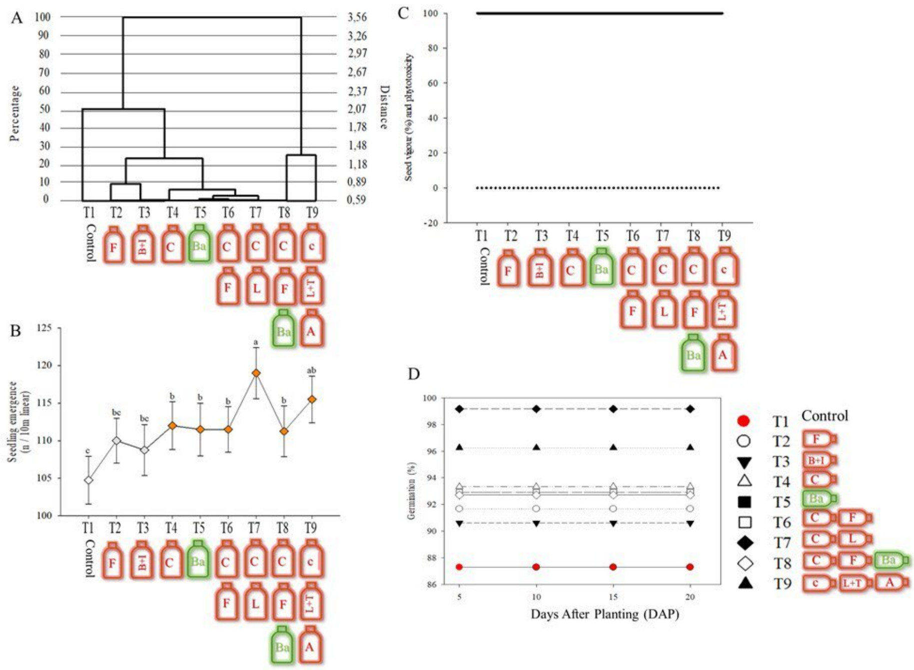


Fig. 5. Population density of *Scutigerella immaculata* in soybean under different seed treatments. (A) Potato bait traps. (B) Direct root counts. (C) Population dynamics determined by polynomial regression. Means followed by the same letter do not differ according to Tukey's test ($P \leq 0.05$).

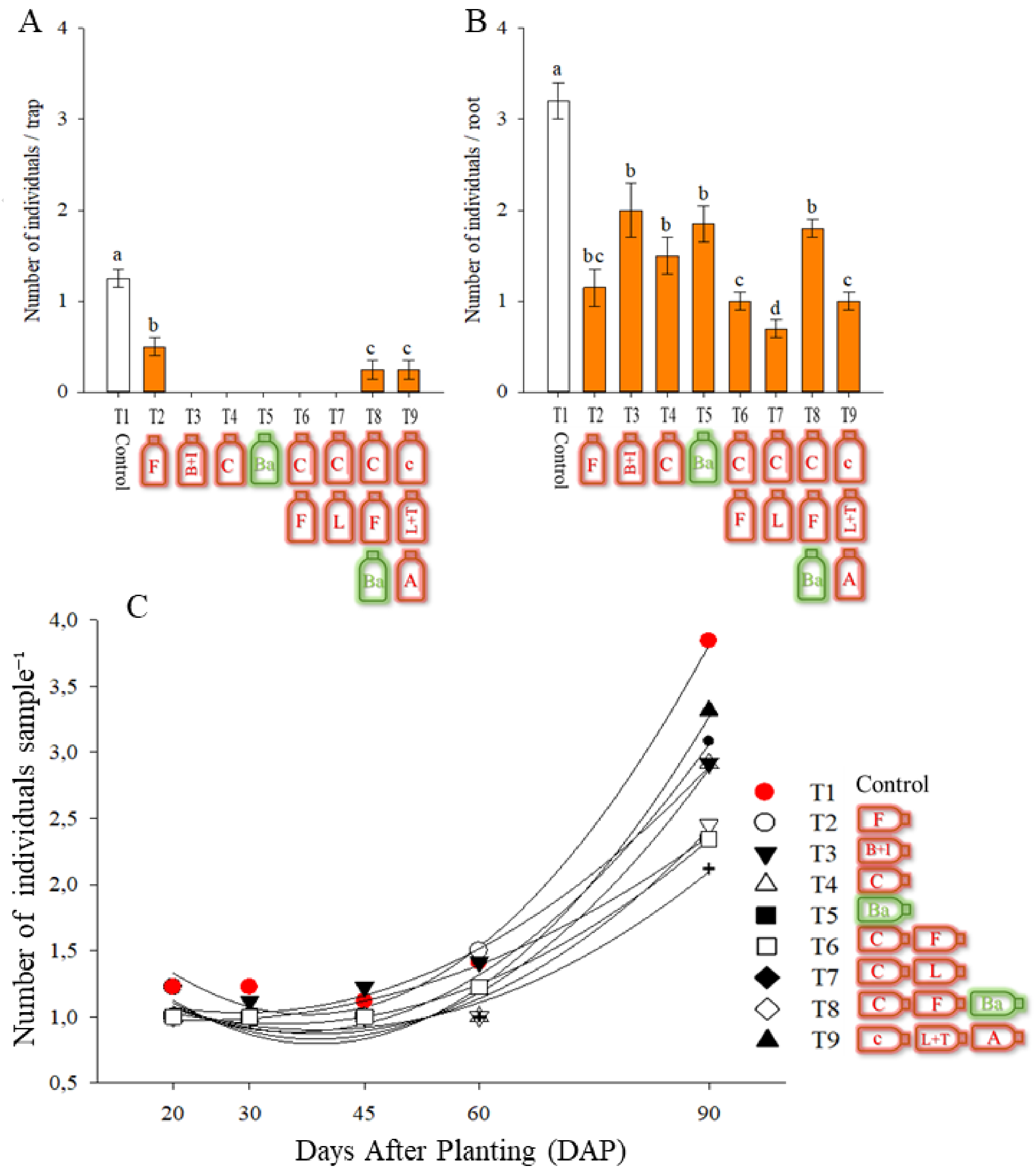


Fig. 6. Root fresh mass at 20 (A), 45 (C), and 60 (E) DAP; shoot fresh mass at 20 (B), 45 (D), and 60 (F) DAP. Means followed by the same letter do not differ according to Tukey's test ($P \leq 0.05$).

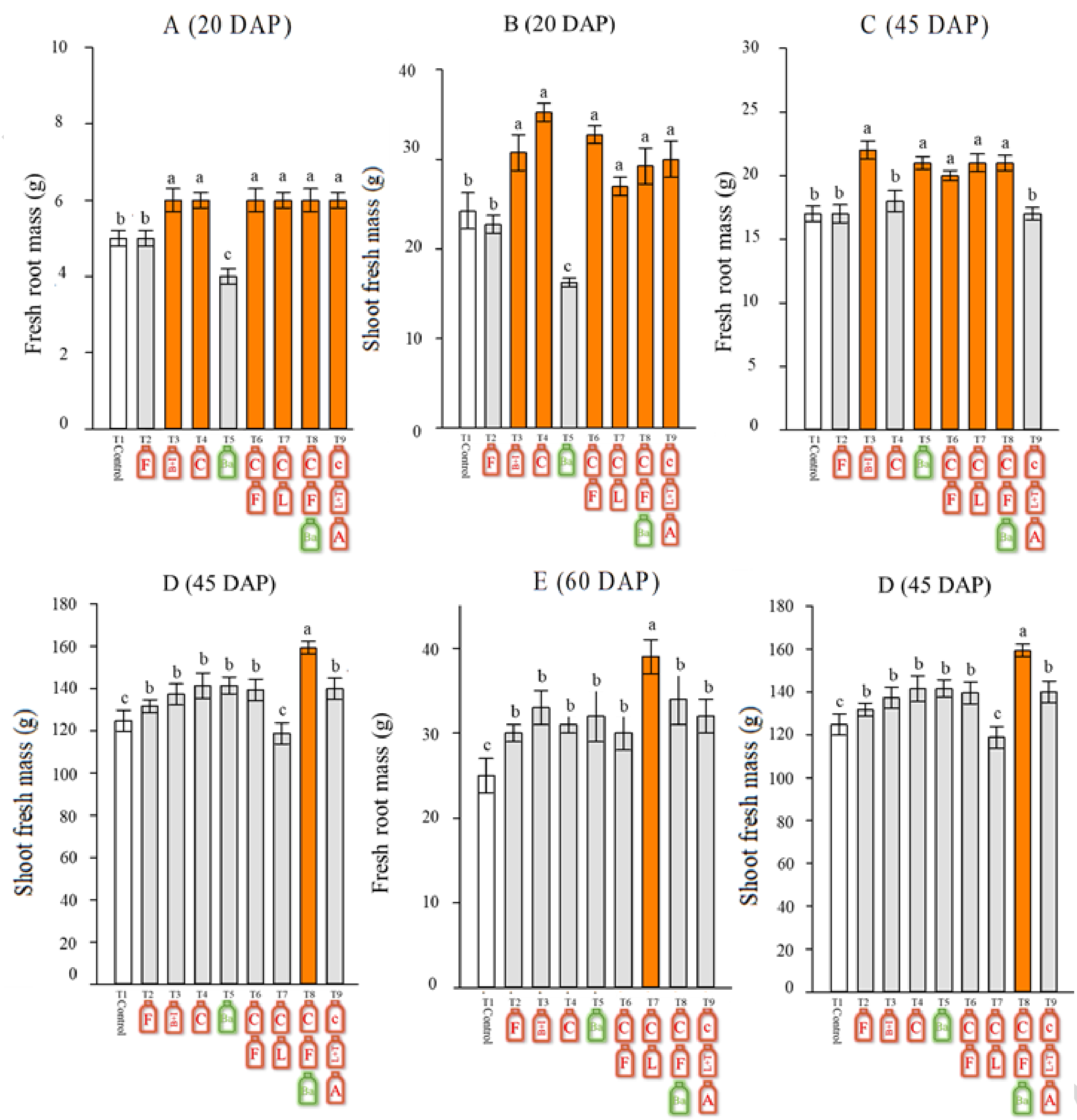
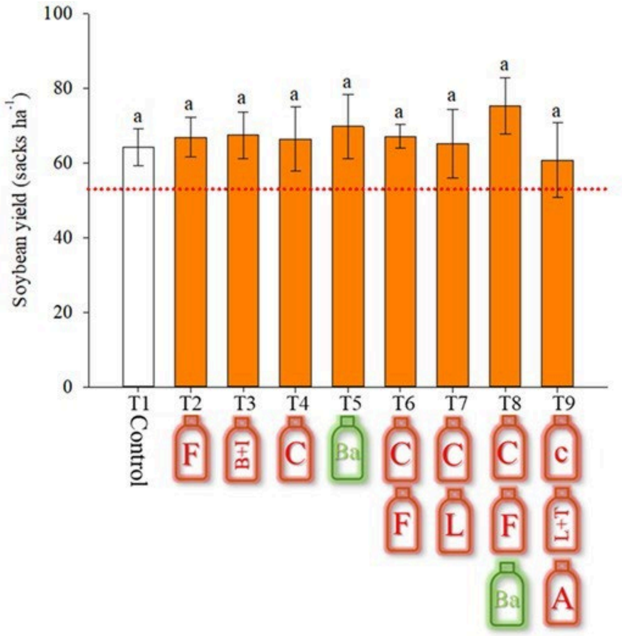


Fig. 7. Soybean yield (60 kg sacks ha^{-1}; mean ± SE) under different seed treatments in fields infested by *Scutigerella immaculata* (Symphyla: Scutigerellidae). Means followed by the same letter do not differ according to Tukey's test ($P \leq 0.05$). The horizontal red line indicates the current average soybean yield in Brazil (56 sacks ha^{-1}).


Soybean yield (sacks ha-1)
100
80
60
40
20
0
a
T1
T2
T3
T4
T5
T6
T7
T8
T9
Control
F
C
Ba
L
A