AGRICULTURAL
SCIENCE & TECHNOLOGY

Article

Persistence and Efficacy of *Bacillus thuringiensis*-Based Biopesticide against *Chrysodeixis includens* in Soybeans: A 3-Year Field Study

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ABSTRACT: This study aimed to evaluate the persistence of *Bacillus thuringiensis* (Bt)-based biopesticide and its efficacy against *Chrysodeixis includens*. The field trials were organized in a factorial design, with three application times (8:00 AM, 12:00 PM, and 6:00 PM) and collection times after application (0, 24, 48, and 72 h). Applications were made using a CO_2-pressurized backpack sprayer at 100 L ha^{-1} at the respective times. Leaves were collected from the upper third of the soybean plants and brought to the laboratory for bioassay preparation, where 48 s-instar larvae per treatment were assessed for mortality. Applications performed in the early morning resulted in higher caterpillar mortality and greater persistence of the active toxin over time. However, pest control decreased as the exposure time of Bt-sprayed leaves to field climatic conditions increased. The control was greatest immediately after application and within the first 24 h, indicating a decline in the residual effectiveness of the product over time.

KEYWORDS: *soybean looper, application technology, biological control, agricultural sustainability*

INTRODUCTION

The soybean looper, *Chrysodeixis includens* (Walker, [1858]) (Lepidoptera: Noctuidae) is one of the insect pests with significant potential for economic damage in soybean crops (*Glycine max* L. Merr.), especially in South America.[1] Biopesticides containing *Bacillus thuringiensis* (Bt), referred to here as Bt biopesticides, are commonly used to control this pest,[2] and the implementation of genetically modified crops that express Bt insecticidal proteins has further aided its management.[3]

Bt biopesticides are indispensable tools to complement or replace chemical pesticides, owing to their higher target specificity and lower selection pressure for resistance.[4−6] It must be pointed out that the use of biological pest control agents is an important element of agricultural sustainability, allowing farmers to cultivate crops and commodities without chemical residues. This not only increases the market value of their produce but also helps in the environmental protection and the preservation of ecosystem services.[7,8]

B. thuringiensis is a Gram-positive, spore-forming, rod-shaped bacterium present in different ecosystems, including water, soil, insects, dust, and tree leaves.[9,10] The key characteristic of Bt is the production of parasporal crystals with insecticidal activities against various orders of insects, mites, nematodes, and protozoa during the vegetative and sporulation phases.[11,12] The infection process begins when a susceptible insect consumes a spore-crystal complex. Alkaline pH in the insect midgut solubilizes the crystal complex, and intestinal proteases activate protoxins. These activated toxins bind to specific receptors in the midgut epithelium, forming pores and causing cell lysis, eventually leading to the target's death as the spores germinate.[13]

However, under field conditions, Bt biopesticides are prone to degradation. Factors such as extreme temperatures, prolonged exposure to ultraviolet (UV) radiation, rainfall, dew, and soil pH can reduce the efficacy of insecticidal proteins by degrading spores and crystals.[14,15] UV radiation generates free radicals that lead to the oxidation of amino acids, particularly histidine and tryptophan.[16,17] Tryptophan residues in Cry insecticidal proteins are vital for the toxic effects of Bt.[18]

Field persistence evaluation of Bt is crucial for its effectiveness and broader use as a pest control agent.[19] Reduced persistence can lead to higher production costs owing to the need for frequent reapplication or the adoption of alternative control strategies.[20,21] Although various strategies have been developed to protect Bt from environmental conditions,[22,23] quantifying its field persistence, especially in terms of efficacy over time, is essential for developing strategies to enhance its persistence. It is worth noting that most Bt persistence studies have been conducted in the northern hemisphere,[24−26] where the climate conditions differ significantly from those of this study. Therefore, this study aimed to evaluate the efficacy and persistence of Bt biopesticides when applied at different times to soybean crops to control soybean looper (*Chrysodeixis includens*).

Received: April 17, 2025
Revised: June 2, 2025
Accepted: June 4, 2025

Table 1. Average Temperature (°C) and Relative Humidity (%) during the Application Time[a]

	2021		2022		2023	
Application time (h)	Temperature (°C)	Relative Humidity (%)	Temperature (°C)	Relative Humidity (%)	Temperature (°C)	Relative Humidity (%)
08:00 AM	18	93	22	82	19	84
12:00 PM	20	93	23	74	26	54
06:00 PM	27	63	32	43	18	96

[a]The wind speeds during the applications ranged from 3 to 10 km h^{-1}. Precipitation of 36.4 mm was recorded 48 h after application in 2021; 0.6 mm within the first 24 h in 2022; and 4.4 mm after 48 h in 2023.

MATERIALS AND METHODS

Experimental Characterization. Experiments were conducted at the Federal University of Santa Maria (UFSM) in Santa Maria, Brazil, using the glyphosate-resistant transgenic soybean cultivar NA 5909 RR with no Cry protein expression. The soybeans were grown using no-until practices with 0.50-m row spacing and a plant population of 300,000 plants per hectare. Each treatment was applied to plots of 60 m^2, from which leaflets were collected for analysis.

The study was repeated thrice during the 2020/21, 2021/22, and 2022/23 growing seasons. A completely randomized design was employed in a factorial arrangement, with an additional control treatment. The first factor was the application time (08:00 AM, 12:00 PM, and 06:00 PM), and the second factor was the sampling time after application (0, 24, 48, and 72 h). An additional treatment (control) without product application was also included. The control data were used to calculate Abbott's corrected mortality (%).

Treatment Application. The treatments were applied on March 19, 2021, February 21, 2022, and April 4, 2023, when the soybean plants were at the R4 growth stage.[27] Applications were made using a CO_2-pressurized backpack sprayer equipped with a 3.0 m boom containing six hollow cone nozzles. Magno MGA 90° 01 series nozzles were used in 2021 and 2022, whereas Teejet TXA 80° 01 VK series nozzles were used in 2023. Nozzles were spaced 0.5 m apart, calibrated to deliver a spray volume of 100 L ha^{-1}, producing fine to very fine droplets.

Weather conditions during the application and sampling periods (Table 1) were monitored using data from the National Institute of Meteorology (INMET) automatic weather station located less than 1 km from the experimental site.

Insect Rearing and Bioassay Setup. The *Chrysodeixis includens* caterpillars were reared on an artificial diet[28] until pupation. Pupae were individually placed in inverted 25 mL plastic cups on moistened filter paper until adult emergence. The adults were then transferred to PVC cages lined with yellow paper for oviposition. Throughout their adult stage, they were provided with a 10% honey solution administered via capillary cotton wool in plastic containers within the cages. After hatching, the neonate larvae were fed soybean leaves until the bioassay began.

The mortality bioassay was conducted by feeding *C. includens* caterpillars with soybean leaves sprayed with the Bt biopesticide Dipel (Sumitomo Chemical Brasil Indústria Química S.A., Maracanaú/CE, Brazil) according to their respective application schedules. The sprayed leaves from the upper third of the plant were collected, placed in plastic bags, and transported to the laboratory. In the laboratory, the caterpillars were placed on a gelatinous mixture of 2.5% carrageenan inside disinfected 100 mL plastic pots (Coposan, Orleans, SC, Brazil). Each pot was infested with two second-instar (L2) larvae (5−7 mm in length) and received a single leaf, on which the larvae remained and fed until the end of evaluations. For each year (2021, 2022, and 2023) and treatment, 84 larvae were used in 42 containers measuring 6.8 cm top diameter x× 4.5 cm bottom diameter × 4.1 cm height. Thus, each treatment presented 6 replicates, consisting of 14 larvae.

The pots were sealed and maintained in a controlled environment at approximately 25 °C and 65% relative humidity. Leaf collection and caterpillar infestation were repeated 24, 48, and 72 h postapplication to evaluate the residual effect of the product on caterpillar mortality. Mortality was evaluated 5 days after infestation, with caterpillars deemed dead if they did not move when touched.

STATISTICAL ANALYSIS

Individual statistical analysis was performed for each year. The Shapiro-Wilk test was used to verify the normality of the data. When necessary, square root (x) or square root (x + 0.5) transformations were applied to normalize the data, and these transformations are indicated in the results table. The homogeneity of variances was assessed using Levene's test. Subsequently, analysis of variance (ANOVA) was conducted, followed by Scott-Knott's test at a 5% significance level to compare the means of different factors. All statistical analyses were conducted using R software.[29] The agronomic efficiency of the treatments was calculated using Abbott's equation.[30]

RESULTS

In all three years of the experiment, there was a significant interaction between application time and the interval (in hours) between application and leaf collection for infestation. In 2021, a higher number of dead caterpillars was observed when Bt was applied at 8:00 AM, particularly when leaves were collected shortly after application (0 and 24 h). At this time, the number of dead caterpillars was significantly higher compared to applications at 12:00 PM and 6:00 PM. The effect of the collection time was also evident, as a progressive decrease in caterpillar mortality was observed with increasing intervals between application and collection (Table 2).

In 2022, a similar trend was observed, although an exception occurred when the application at 6:00 PM resulted in the highest mortality at time zero, surpassing even the 8:00 AM treatment. Nevertheless, over time, mortality rates decreased more sharply for the 12:00 PM and 6:00 PM applications, particularly 72 h after application. In 2023, the results were again consistent with the general trend of greater efficacy when Bt was applied in the early morning. The number of dead caterpillars decreased more rapidly when the insecticide was applied at 12:00 PM and 6:00 PM, especially at 72 h postapplication.

When analyzing the three years together, a consistent pattern was evident: applications performed at 8:00 AM resulted in the highest caterpillar mortality rates, particularly in the earlier collection intervals (0 and 24 h). In contrast, applications carried out at 12:00 PM and 6:00 PM were generally less effective, especially at 48 and 72 h after application, where the number of dead caterpillars was significantly lower. This indicates that, regardless of year or environmental variations, Bt application during early morning hours leads to greater biological activity and prolonged persistence on treated plant surfaces.

Considering that the leaves were collected after application and transferred to the laboratory for infestation under controlled temperature and humidity conditions, the observed

Table 2. Mean Mortality of *Chrysodeixis includens* (N = 14 Larvae per Treatment) in Response to Dipel Applications at Different Times of Day and Leaf Collection Intervals—Combined Data from 2021, 2022, and 2023[a]

Year 2021*			
	Application Time (h)		
Collection Time (h)	8:00 AM	12:00 PM	6:00 PM
0	12.3 Aa	11.2 Ab	11.0 Ab
24	11.8 Aa	10.7 Ab	7.7 Bc
48	4.7 Ba	3.3 Bc	4.0 Cb
72	2.3 Ca	2.0 Ca	2.3 Da
CV (%)	4.62		
Year 2022*			
	Application Time (h)		
Collection Time (h)	8:00 AM	12:00 PM	6:00 PM
0	10.5 Ab	8.83 Ac	11.8 Aa
24	6.7 Ba	6.0 Bb	7.3 Ba
48	4.0 Cb	6.0 Ba	6.2 Ca
72	1.8 Db	3.5 Ca	2.0 Db
CV (%)	5.28		
Year 2023**			
	Application Time (h)		
Collection Time (h)	8:00 AM	12:00 PM	6:00 PM
0	10.0 Aa	7.2 Ab	5.7 Ac
24	9.7 Aa	4.3 Bb	4.2 Bb
48	6.5 Ba	4.0 Bb	4.2 Bb
72	4.7 Ca	2.5 Cb	2.3 Cb
CV (%)	5.72		

[a]Means followed by the same lowercase letter in the row and uppercase letter in the column are not significantly different (Scott-Knott's test at $p \leq 0.05$). *Square root transformation (x + 0.5) and **square root transformation (x).

effects are not related to differential ingestion by caterpillars in the field, but rather to the quality of Bt deposition and its preservation on the leaf surface at the time of application. In this context, the environmental data recorded (Table 1) indicate that applications performed in the early morning occurred under milder temperatures and higher relative humidity conditions that tend to reduce Bt degradation by abiotic factors such as heat and radiation, while also favoring better adhesion and distribution of the product on the leaf surface. Conversely, applications performed at midday or late afternoon were exposed to higher temperatures and/or lower humidity, which may have accelerated Bt degradation or impaired its fixation on the plant surface before leaf collection, ultimately reducing its efficacy even under standardized laboratory conditions.

When estimating mortality using Abbott's equation, the bioinsecticide's effectiveness in pest control is confirmed. For the leaf samples collected immediately after application, an average efficacy of over 60% was achieved across all application times over the years (Figure 1d). Notably, the application conducted at 8:00 AM demonstrated slightly higher efficiency, underscoring the importance of environmental conditions during the application process.

Figure 1 illustrates the mortality rates over the three-year experiment, categorized by application times and their averages, revealing consistent patterns across different years (Figure 1a–c). For treatments where leaves were sprayed and immediately infested with caterpillars, higher mortality rates (%) were observed at all application times. However, effectiveness declined sharply over time, resulting in a steep downward curve. In 2023 (Figure 1c), the 8-h application showed a high percentage of dead caterpillars (%) across various collection times, likely due to favorable application conditions. This year was marked by El Niño conditions, which brought unusually rainy weather to Rio Grande do Sul, resulting in high cloud cover. These factors may have contributed to higher pest mortality rates up to the 72-h collection point compared to other years.

The 8:00 AM application results in a higher mortality rate within the first 24 h (Figure 1d). This improved effectiveness is attributed to favorable application conditions, which enhance the deposition of the product on the leaf, potentially increasing the number of viable spores and boosting Bt effectiveness.

The persistence of Dipel under field conditions significantly impacts its control of *C. includens*. Ultraviolet light exposure is a major factor contributing to the reduced residual effect of Bt-formulated products. Additionally, other abiotic factors such as rainfall and air humidity also play crucial roles in influencing the bioinsecticide's efficacy.

DISCUSSION

Biological control using *Bacillus thuringiensis* (Bt) represents an effective strategy within integrated pest management (IPM) programs for controlling various caterpillar species, including *C. includens*. A deeper understanding of the persistence dynamics of biological products and advancements in application technology is crucial for enhancing pest control efforts and optimizing the use of bioinsecticides.

This study indicates that Bt biopesticides are less effective in managing *C. includens* species, considering the criterion of 80% control for pesticide effectiveness.[31] It is important to point out that biological pesticides are expected to be less efficient than chemical pesticides because of their lower sensitivity to climate conditions.[32] To enhance the efficacy and persistence of biopesticides in the field, incorporating nanotechnology to develop nanobiopesticides could improve the stability of the active ingredients.[23]

Several laboratory and field studies have demonstrated the effectiveness of Bt technology in pest control, particularly in the form of Bt crops, where climatic conditions have minimal impact.[32,33] Bt biopesticides played an essential role in managing outbreaks of *C. includens* and *H. armigera* in Brazilian crops during the 2013/2014 growing season, when Bt soybean crops were not yet accessible to farmers.[35] An increasing adoption of biopesticides for pest control in Brazil was observed in response to pest outbreaks, which is attributed to their high efficacy and reduced environmental impact.[19]

Better product efficacy was anticipated for applications at 06 PM due to more favorable climatic conditions over an extended period. This includes lower UV ray incidence shortly after application, continuing through the night without solar radiation, and potentially enhancing pest insect control efficiency. A recent study has highlighted that susceptibility to UV radiation (290–400 nm) limits the persistence and efficacy of biopesticides.[36]

Applications conducted at 8 AM could also be significant, as historically, this time offers application conditions closer to the recommended parameters, with higher relative humidity and lower temperature compared to afternoon hours. In this study, the different temperatures did not show a pattern of behavior

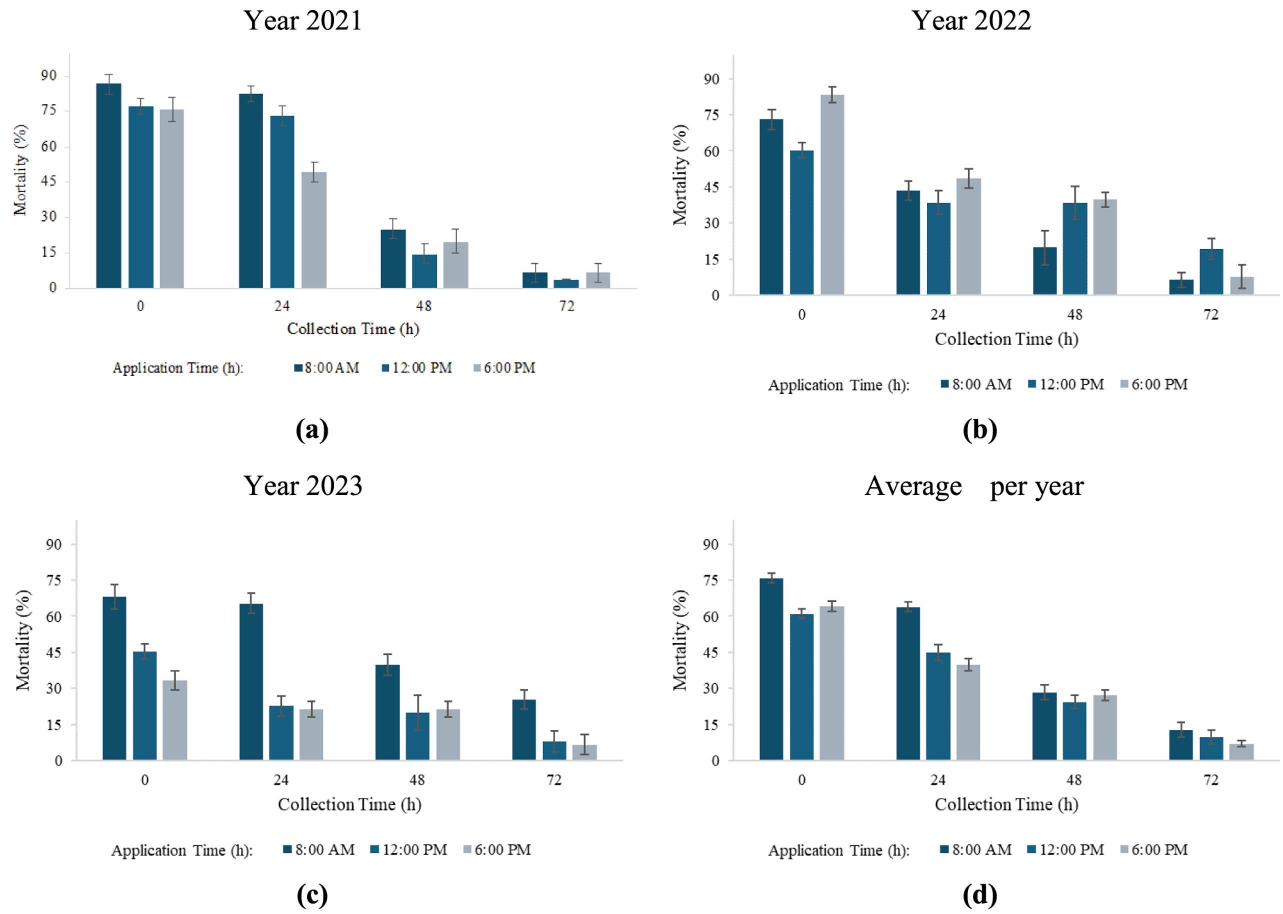

Figure 1. Correlation between application and collection times on estimated mortality using Abbott's equation for 2021, 2022, 2023, and the 3-year average.

in controlling caterpillars. In 2021, the highest temperature (27 °C) at the time of application occurred at 6 PM, which resulted in less control when compared to the application carried out at 8 AM with a temperature of 18 °C, in the first hours of leaf collection (0−24 h). This was different from what occurred in 2022, where the application at 6 PM with a temperature of 32 °C presented greater control compared to the application at 12 PM, which had 23 °C at the time of application. Mastore et al.[37] studied the influence of daily temperature fluctuations on the efficacy of different bio-insecticides and observed that the bioinsecticide based on *B. thuringiensis* var. *Kurstaki* was not affected by temperature fluctuations in the high (17−33 °C) and low (11−22 °C) ranges. This may indicate that other environmental conditions may have influenced the response to control, such as rainfall recorded in the first 24 h after application in 2022, which led to a sharper drop in mortality between leaf collection times 0 and 24 h.

Despite the findings of this study, the literature highlights that time should be considered a crucial factor in the application of biological products.[19] Applications during the hottest parts of the day should be avoided due to the potential ″magnifying glass effect,″ where sunlight concentrates in spray droplets, potentially reducing the persistence of Bt.

A decrease in mortality over time (1 to 7 days) following the application when examining the residual activity of the bioinsecticide Dipel, tested with various spray volumes, was reported.[38] No larval mortality of *Agoma trimenii* (Lepidoptera: Agaristidae) was recorded after the sixth day. The residual effects of these products are characterized by a rapid reduction in spores under field conditions, with up to 95% to 99% of the initial spores being eliminated within 3 days of application.[39,40]

These findings align with those reported elsewhere, which reported that the loss of active ingredients increased proportionally with direct sunlight exposure,[41] and the Bt formulations' activity decreased within days of application, with mortality rates dropping below 40% after 3 days of sun exposure and reaching complete inactivity after 10 days.[42] The Bt *kurstaki* sensitivity evaluation of UV−B radiation revealed that this variety has a substantial coating of Cry insecticidal proteins compared with Bt *israelensis*.[43] However, this excess coating paradoxically increases the sensitivity to ultraviolet rays due to a deficiency in protective polypeptides.

In this study, persistence was analyzed about *C. includens* mortality over time. Leaf samples were collected for up to 72 h after spraying and infestation. The control efficiencies at 0 h were 76%, 61%, and 64% at 08:00 AM, 12:00 PM, and 06:00 PM, respectively. This efficiency decreased to 12%, 10%, and 7% at 72 h for the same application times. The most effective control was observed within 24 h of application, with a significant decline in mortality at 48 and 72 h. The warmer conditions typical of tropical climates, such as those in Brazil, accelerate spore loss and reduce product persistence.

Recent studies in the field of application technology have focused on the encapsulation of biopesticides.[22−44] This strategy involves coating the product to enhance its field persistence, enabling it to better withstand biotic and abiotic stresses.[45] One of the primary goals of these formulations is the shelf life of the product, both in the field and during storage.[22] An alternative approach involves manipulating cry genes, taking advantage of the diverse proteins produced by bacteria and their various modes of action. Genetic engineering techniques can be employed to improve the effectiveness of proteins and environmental persistence while eliminating unwanted traits.[46,47]

Our data strongly suggest the necessity of adopting strategies to increase the persistence of Bt biopesticides. The applications in this study utilized only commercial products without adjuvants, which could have improved the application technology in terms of spread and persistence. However, not all adjuvants are compatible with the Bt formulations. A recent study involving the application of Dipel SC with various adjuvants found that only those based on propionic acid and lecithin were compatible with the product and effective in controlling *S. frugiperda*.[48] Moreover, Bt encapsulation is reported to provide increased persistence and slower release,[22] but no product has yet been registered with such characteristics in the Brazilian market. Encapsulation improvements seem to be underestimated by biopesticide companies because many of them seek for "microbial silver bullets," delivering agents of insecticidal toxins in a chemical pesticide model,[49] to provide novelties for the market share, instead of improving the well-known microorganisms and/or strains.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsagscitech.5c00303.

A summary of the analysis of variance (ANOVA) for the three years of the experiment, with detailed statistical support for the results (PDF)

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Funding

The Article Processing Charge for the publication of this research was funded by the Coordenacao de Aperfeicoamento de Pessoal de Nivel Superior (CAPES), Brazil (ROR identifier: 00x0ma614).

Notes

The authors declare no competing financial interest.

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