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CROP PROTECTION

# Influence of adjuvants added to teflubenzuron spray on resistant and susceptible strains of the soybean looper Chrysodeixis includens (Lepidoptera: Noctuidae)

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**ABSTRACT**: *The soybean looper (SBL), Chrysodeixis includens (Walker, [1858]) (Lepidoptera: Noctuidae), is a soybean and cotton pest in South America countries. Field-evolved resistance of SBL to inhibitors of chitin biosynthesis has been reported in Brazil; however, this mode of action is still widely used against SBL. On this basis, we conducted laboratory bioassays to investigate if adjuvants (Nimbus®, TA 35®, Break-Thru® S 240, and Rizospray Extremo®) added to the teflubenzuron spray increase the mortality of SBL strains (resistant, heterozygous, and susceptible to chitin biosynthesis inhibitors). Using chromatography analysis, we also evaluated the amount of teflubenzuron on soybean leaves when applied alone or in combination with adjuvants. In laboratory bioassays, the biological activity of teflubenzuron increased against the susceptible SBL strain when adjuvants were added. In contrast, no relevant effects of adjuvants added to the teflubenzuron spray against heterozygous and resistant SBL larvae were detected. In leaf bioassays, even leaves from the upper third part of the plants containing a significantly higher amount of teflubenzuron (3.4 mg/kg vs 1.7 and 0.6 mg/kg); the mortality of SBL strains was similar when teflubenzuron was applied alone or in mixture with adjuvants. Our findings indicated that adjuvants added to teflubenzuron spray do not provide a substantial increase in the mortality of SBL strains resistant to chitin biosynthesis inhibitors. Therefore, it is necessary to reduce the use of this mode-of-action insecticide against SBL and to give preference to other insecticides or control tactic.*
**Key words**: *benzoylphenylureas, soybean pest, insect resistance management, tank mixture.*

# Influência da adição de adjuvantes à calda de pulverização de teflubenzuron em linhagens resistentes e suscetíveis da lagarta falsa-medideira Chrysodeixis includens em soja (Lepidoptera: Noctuidae)

**RESUMO**: *A lagarta falsa-medideira, Chrysodeixis includens (Walker, [1858]) (Lepidoptera: Noctuidae), é uma praga da soja e do algodão nos países da América do Sul. A resistência de C. includens a inibidores da biossíntese de quitina tem sido relatada no Brasil. Entretanto, esse modo de ação ainda é amplamente utilizado para controle de C. includens. Com base nisso, conduzimos bioensaios em laboratório para investigar se adjuvantes (Nimbus®, TA 35®, Break-Thru® S 240 e Rizospray Extremo®) adicionados à calda inseticida de teflubenzuron aumentam a mortalidade de linhagens de C. includens (resistentes, heterozigotos e suscetíveis a inibidores da biossíntese de quitina). Usando análise cromatográfica, também avaliamos a quantidade de teflubenzuron em folhas de soja quando aplicado isolado ou em combinação com adjuvantes. Em bioensaios de laboratório, a atividade biológica do teflubenzuron aumentou para a linhagem suscetível quando os adjuvantes foram adicionados à calda inseticida. Em contraste, nenhum efeito relevante de adjuvantes adicionados ao teflubenzuron foi detectado para os heterozigotos e resistentes. Em bioensaios de folhas, mesmo naquelas do terço superior das plantas, as quais apresentaram uma maior deposição de teflubenzuron (3,4 mg/kg vs 1,7 e 0,6 mg/kg); a mortalidade das linhagens de C. includens foi semelhante quando o teflubenzuron foi aplicado isolado ou com adjuvantes. Nossos resultados indicam que os adjuvantes adicionados ao teflubenzuron não fornecem um aumento substancial na mortalidade de linhagens de C. includens resistentes aos inibidores da biossíntese de quitina. Portanto, é necessário reduzir o uso desse modo de ação para o manejo de C. includens e dar preferência a outros inseticidas ou tática de controle.*
**Palavras-chave**: *benzoilfeniluréias, pragas da soja, manejo da resistência de insetos, mistura de tanque.*

## INTRODUCTION

The soybean looper (SBL), Chrysodeixis includens (Walker [1858]) (Lepidoptera: Noctuidae) is an important defoliator pest of soybean [Glycine max L. (Merr.)] and cotton (Gossypium hirsutum L.) crops in South America (SANTOS et al., 2017; SILVA et al., 2020). For decades, SBL management on soybean and cotton has been performed with chemical insecticides (PANIZZI, 2013). However, the development and deployment of transgenic soybean and cotton plants, expressing insecticidal proteins

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from Bacillus thuringiensis Berliner (Bt), has enabled other control tactics against SBL (BERNARDI et al., 2012; SORGATTO et al., 2015; MARQUES et al., 2016). These Bt crops are planted on areas of nearly 1.1 (cotton) and 36 (soybean) million hectares, representing 82 and 85% of the total area cultivated with these crops in Brazil during the 2020/21 crop seasons (BROOKES & BARFOOT, 2018; COUNCIL BIOTECHNOLOGY INFORMATION, 2018; CONAB, 2021).

Currently, the management of SBL in soybean and cotton crops is mainly performed by chemical and biological insecticides (on non-Bt areas) or by Bt plants. Among the chemical insecticides, inhibitors of chitin biosynthesis (benzoylphenylureas) have been used since the 1970s against lepidopteran pests, including SBL (BEEMAN, 1982). Chemical control of SBL is difficult because larvae are less exposed to insecticide sprays due to their habit of remaining sheltered under the plant canopy (PAPA & CELOTO, 2007; FUNICHELLO et al., 2019). Long-time use of benzoylphenylureas against SBL has contributed to field resistance to the chitin synthesis inhibitors teflubenzuron, novaluron, and lufenuron in Brazil (STACKE et al. 2019, 2020). Such resistance has also been reported in Plutella xylostella (L., 1758) (Lepidoptera: Plutellidae) (SANTOS et al., 2011) and Spodoptera frugiperda (J. E. Smith, 1797) (Lepidoptera: Noctuidae) (NASCIMENTO et al., 2016).

In the insecticide application technology context, some adjuvants are used in tank mixtures of insecticides to increase its effectiveness (MELO et al., 2019). Adjuvants have the function of modifying the physicochemical characteristics, increasing the efficacy, and protecting phytosanitary products in the mixture (ABDELGALEIL et al., 2018; MELO et al., 2015; SANTOS et al., 2019). Previous studies indicated that adjuvants added to diamides increased the mortality of adults of *A*myelois transitella (Walker, 1863) (Lepidoptera: Pyralidae) (DEMKOVICH et al., 2018). This was also verified when adjuvants were added to indoxacarb and cartap to control Neoleucinodes elegantalis (Guenée, 1854) (Lepidoptera: Pyralidae) (DE BORTOLI et al., 2013), to thiamethoxam + lambda-cyhalothrin against Enneothrips flavens Moulton, 1941 (Thysanoptera: Thripidae) (CALORE et al. 2015), and dimethoate and spinetoram to control Thrips tabaci Lind., 1888 (Thysanoptera: Thripidae) (NEGASH et al., 2020).

According to these studies, adjuvants allowing a better deposition of the active ingredient on the foliage and the rate of penetration or ingestion by the insects, improving control effectiveness. However, the effects of adjuvants added to insecticide sprays against insects resistant to insecticides remain unknown. Understanding if adjuvants increase the mortality of resistant and mainly heterozygous insects due to a better deposition on foliage or rate of ingestion of the active ingredient by the insects is important to support resistance management plans; heterozygous are mainly responsible for the dispersion of resistance alleles in field populations (CAPRIO & SUMERFORD, 2018).

To fill this knowledge gap, we conducted laboratory studies to investigate whether adjuvants added to teflubenzuron spray increased the mortality of SBL strains (resistant, heterozygous, and susceptible to chitin biosynthesis inhibitors). We hypothesized that the response of SBL strains exposed to teflubenzuron + adjuvants or teflubenzuron alone varies based on the SBL genotype. We conducted these evaluations using diet-overlay and soybean-leaf bioassays. The amount of teflubenzuron on soybean leaves was also quantified using chromatographic analysis.

## MATERIALS AND METHODS

### *Insects*

A teflubenzuron-resistant SBL colony (Teflu-R) was isolated from field populations as described in detail by STACKE et al. 2020. The Teflu-R strain presented a high resistance ratio to teflubenzuron >36,300-fold. We also used a strain of SBL that has been maintained in the laboratory since 2015 without exposure to insecticides and Bt toxins, referring to this colony as a susceptible strain (Sus). To evaluate heterozygotes, reciprocal crosses between resistant ♀ × susceptible ♂ were performed. We only used this heterozygote strain because inheritance of resistance is autosomally inherited (STACKE et al., 2020).

### *Adjuvants*

The following adjuvants were added to the teflubenzuron spray: Nimbus® (Syngenta Proteção de Cultivos Ltda, São Paulo SP, Brazil), TA 35® (Inquima Ltda, Cambé PR, Brazil), Break-Thru® S 240 (Evonik Degussa Brazil Ltda, São Paulo SP, Brazil) and Rizospray Extremo® (Rizobacter do Brasil, Londrina PR, Brazil). The dose of adjuvants Nimbus® and Rizospray Extremo® was 0.5% v/v, whereas for TA 35® and Break-Thru® S 240 the dose was 0.05% v/v.

### *Diet-overlay bioassays*

The bioassays were conducted in 24-well acrylic plates (Costar®, São Paulo SP, Brazil)

to evaluate the susceptibility of SBL strains to teflubenzuron (Nomolt®, 150 g teflubenzuron/L, BASF SA, São Paulo SP, Brazil) alone or applied with adjuvants. Each well received 1 mL of artificial diet based on white bean, wheat germ, and yeast, commonly used for rearing SBL (adapted from GREENE et al., 1976). After a drying period, five to seven concentrations of teflubenzuron alone or teflubenzuron + adjuvants were prepared with distilled water. The control treatment was only distillated water. In preliminary bioassays, all adjuvants alone were tested against SBL larvae, but they did not cause any mortality. A volume of 30 µL of each concentration was applied to the diet surface in each well (surface area of 1.88 $cm^2$) and allowed to dry. Subsequently, a single early L3 larva was added to each well. Plates were sealed with their covers and placed in a room at 27 ± 2 °C, 60 ± 10% RH and a photoperiod of 14:10 h. The bioassays were repeated twice for each SBL strain on distinct days, with each concentration being repeated twice per bioassay (two replications of 48 larvae per concentration). Mortality was assessed after 5 days. Larvae without movement were considered dead. Concentration-mortality data were subjected to Probit analysis to estimate the $LC_{50}$ and $LC_{90}$ lethal concentrations and 95% confidence intervals (95% CIs), using the Polo-PC program (LeOra Software, 2002). A likelihood ratio test was performed to test the hypothesis that the $LC_{50}$ and $LC_{90}$ values are equal. If rejected, pairwise comparisons were performed, and significance was declared if 95% of CIs did not overlap (SAVIN et al., 1977).

*Leaf-bioassays*

Leaf-bioassays were performed to evaluate the survival of SBL strains on soybean leaves sprayed with teflubenzuron alone (Nomolt®: 150 g teflubenzuron/L, BASF SA, São Paulo SP, Brazil) or mixed with adjuvants. Soybean seeds (ICS 1032 RR, Sementes Ponteio, Cruz Alta RS, Brazil) were sown in field conditions during the crop season of 2019–2020. Planting was performed on 27 November 2019 in Santa Maria, RS, Brazil (29º71'54" S e 53º73'56" W), at a density of 280,000 plants/ha. At sowing, 200 kg/ha of Nitrogen–Phosphorus–Potassium (NPK; 5–20–20) were applied. Soybean were sown in four identical blocks arranged in a randomized design with treatments were distributed in 12-$m^2$ plots (each plot was comprised of 6 soybean rows of 4 m in length and with a spacing of 0.50 m between rows). At the $R_1$ growth stage (FEHR & CAVINESS 1981), soybean plots were sprayed with teflubenzuron (22.5 g a.i./ha), and respective adjuvants diluted in 150 L of water using a pressurized-$CO_2$ backpack sprayer with a 3-m bar and 0.5-m nozzle spacing (MGA 90º hollow cone nozzle, MagnoJet, Ibaiti PR, Brazil). The dose of teflubenzuron sprayed correspond to the field recommendation against SBL on soybean. Unsprayed leaves were used as a control treatment. After 30 min of application, leaflets of the lower (0 to 0.6 m height), middle (0.6 to 0.8 m height) and upper (0.8 to 1 m height) parts of the soybean plants were removed and transported to the laboratory. Subsequently, leaves were placed over a gelled mixture of 2.5% agar-water in 100-mL plastic pots (one leaves/pot). Each pot was infested with a single L3 larva of the resistant, heterozygous, or susceptible strains (4 repetitions of 10 larvae, totalizing 40 larvae/strain/treatment). Pots were sealed and placed in a room at 25 ± 2°C, 60 ± 5% relative humidity, and a photoperiod of 12:12 h. Survival was evaluated after 5 days. The numbers of larvae tested and dead in each treatment were used to estimate the 95% confidence intervals (95% CIs) for the probability of mortality, according to a binomial distribution (DORAI-RAJ, 2009). For this analysis, the function *binom.probit* from the package *binom* in R 3.6.1 (R DEVELOPMENT CORE TEAM, 2019) was used. Percent mortality was corrected using the Abbott's formula (ABBOTT, 1925) and considered significantly different when the 95% CIs did not overlap.

*Chromatography to quantify teflubenzuron on soybean leaves*

To perform the chromatographic analysis, soybean leaflets were collected in the same plots and at the same time as the leaves used in leaf bioassays. In total, 12 leaflets of each plant part were sampled from the lower (0 to 0.6 m height), middle (0.6 to 0.8 m height), and upper (0.8 to 1 m height) parts of the plants. Leaflets were stored in plastic bags and transported to the laboratory. The sample preparation method was based on a previous method described by VIERA et al. (2017). Leaflets were homogenized using a food processor, and the original QuEChERS procedure was performed as follow: 3 g of the sample were weighed in a 50/mL polypropylene (PP) tube, 10 mL of acetonitrile were added, and the tube was vortexed for 1 min. A mixture of 1.5 g of NaCl and 4.0 g of MgSO4 was used to promote the partitioning step. The tube was vigorously shaken for 1 min and centrifuged for 8 min at 2,600 g. The clean-up step was performed in a 15/mL polypropylene tube with 2 mL of the supernatant and 300 mg of MgSO4, 50 mg C18, and 10 mg GCB. The tube was vortexed for 1

min, followed by centrifugation at 2,600 g for 8 min. Finally, the extract was filtered (0.2/µm nylon syringe filter) and diluted five times with ultrapure water prior to analysis by UHPLC–MS/MS. The amount of teflubenzuron on leaves from each soybean part (lower, middle, and upper) were compared by PROC ANOVA, using the Tukey test ($P < 0.05$), in the SAS® software (SAS INSTITUTE, 2002).

## RESULTS

*Diet-overlay bioassays*

The low mortality response of the Teflu-R strain to concentration increases of teflubenzuron did not allow the estimation of LC values. The Teflu-R strains exposed to the maximum concentration (15,000 µg a.i./cm²) of teflubenzuron alone or in mixture with adjuvants presented a similar mortality (39.0 to 48.3%) ($F = 1.65$; $df = 4, 25$; $P = 0.1931$) (Figure 1). When the $F_1$ progeny from Teflu-res♀ × Sus♂ (heterozygote) was exposed to teflubenzuron + Nimbus® (0.5%), the $LC_{50}$ value was significantly lower (0.69 a.i./cm²) than that of teflubenzuron in mixture with other adjuvants or teflubenzuron alone ($LC_{50}$ from 1.18 to 1.35 a.i./cm²) (Table 1). However, estimated $LC_{90}$ values of teflubenzuron alone or associated with adjuvants were similar for the heterozygote strain. The Sus strain exposed to teflubenzuron applied with adjuvants presented $LC_{50}$ (0.36 to 0.53 a.i./cm²) and $LC_{90}$ (1.55 to 1.76 a.i./cm²) values significantly lower than when teflubenzuron was used alone (0.73 and 2.52 a.i./cm², respectively), indicating an increase in the biological activity of teflubenzuron against susceptible larvae when applied with adjuvants (Table 1).

*Leaf-bioassays*

There were no significant differences in the mortality of the Teflu-R strain fed on leaves from upper (from 12.5 to 20%), middle (from 7.7 to 12.8%), and lower (from 2.5 to 7.0%) thirds of the soybean plants sprayed with teflubenzuron alone or in mixture with adjuvants (Table 2). No significant differences were also detected when the $F_1$ progeny from Teflu-res♀ × Sus♂ and Sus strains were fed on leaves from the three parts of the plant treated with teflubenzuron alone or in combination with adjuvants (Table 2). The mortality of heterozygous and Sus strains on leaves of each part of the soybean plants sprayed with teflubenzuron alone or in combination with adjuvants was higher when exposed to leaves from upper (77 to 100%), middle (64.1 to 92.3%), and lower (55.2 to 84.6%) parts than the mortality of the Teflu-R strain exposed to leaves of any part (mortality < 20%) (Table 2). In contrast, the mortality of all SBL strains on untreated leaves was < 2.5%.

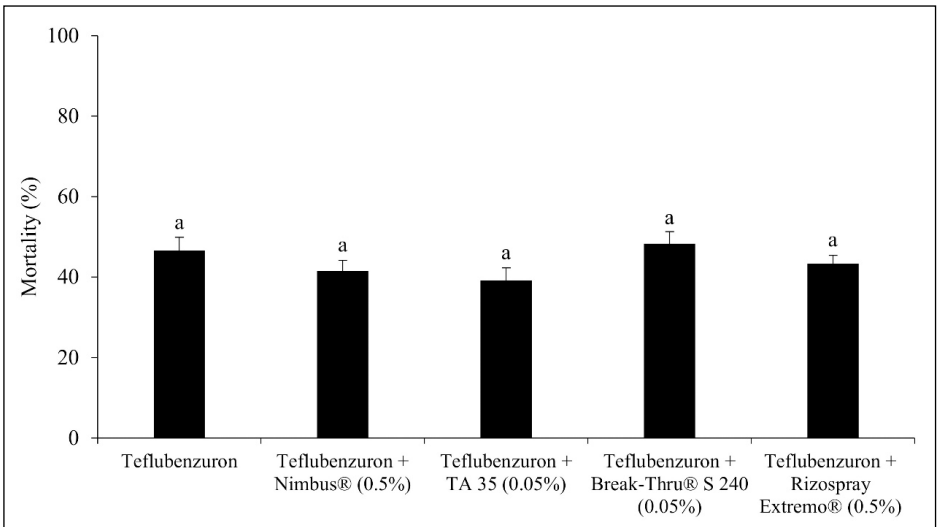


Figure 1 - Mortality (± SE) of Teflu-R strain at the concentration of 15,000 µg teflubenzuron/cm² of diet applied alone or in combination with adjuvants. Bars with the same letter are not significantly different (Tukey test at $P \leq 0.05$).

Table 1 - Concentration-mortality (LC; µg a.i./cm$^2$) response of SBL strains exposed to teflubenzuron alone or in combination with adjuvants.

| Treatment | *n* | Slope ± SE | $LC_{50}$ (95% CI)[a,b] | $LC_{90}$ (95% CI)[a,b] | $\chi^2$ (*df*)[c] |
|---|---|---|---|---|---|
| ----------Teflu-res♀ × Sus♂---------- | | | | | |
| Teflubenzuron | 419 | 2.27 ± 0.36 | 1.35 (1.11–1.59) b | 4.95 (3.73–7.80) a | 3.10 (4) |
| Teflubenzuron + Nimbus® | 418 | 1.57 ± 0.18 | 0.69 (0.49–0.94) a | 4.53 (2.74–11.16) a | 6.28 (4) |
| Teflubenzuron + TA 35 | 477 | 1.68 ± 0.20 | 1.18 (0.97–1.44) b | 6.79 (4.77–11.26) a | 2.23 (5) |
| Teflubenzuron + Break-Thru® S 240 | 420 | 1.96 ± 0.21 | 1.41 (1.20–1.69) b | 6.37 (4.67–9.81) a | 2.78 (4) |
| Teflubenzuron + Rizospray Extremo® | 380 | 1.67 ± 0.20 | 1.20 (0.99–1.47) b | 7.06 (4.93–11.81) a | 2.42 (5) |
| ----------Sus---------- | | | | | |
| Teflubenzuron | 420 | 2.38 ± 0.24 | 0.73 (0.63–0.84) c | 2.52 (2.06–3.27) b | 2.06 (4) |
| Teflubenzuron + Nimbus® | 478 | 2.20 ± 0.19 | 0.46 (0.40–0.53) ab | 1.76 (1.45–2.25) ab | 1.75 (5) |
| Teflubenzuron + TA 35 | 420 | 2.58 ± 0.29 | 0.42 (0.36–0.48) ab | 1.33 (1.08–1.74) a | 3.84 (4) |
| Teflubenzuron + Break-Thru® S 240 | 420 | 2.22 ± 0.22 | 0.36 (0.31–0.41) a | 1.36 (1.10–1.78) a | 2.13 (4) |
| Teflubenzuron + Rizospray Extremo® | 473 | 2.75 ± 0.25 | 0.53 (0.45–0.62) b | 1.55 (1.27–2.02) a | 5.08 (5) |

[a]L *C50*: concentration of teflubenzuron (µg a.i./cm$^2$) required to kill 50% of insects in the observation period of 5 days. $LC_{90}$ is the concentration of teflubenzuron required to kill 90% of larvae tested. [b]$LC_{50}$ and $LC_{90}$ values designated by different letters in each SBL strain are significantly different due to non-overlap of 95% Cis [c]$P > 0.05$ in the goodness-of-fit test.

*Quantification of the amount of teflubenzuron on soybean leaves*

Adjuvants added to the teflubenzuron spray did not increase the amount of active ingredient on leaves of soybean located in the upper ($F = 0.85$, $df = 4, 10$, $P = 0.5228$), middle ($F = 0.48$; $df = 4, 10$; $P = 0.7518$), and lower ($F = 0.30$; $df = 4, 10$; $P = 0.8698$) parts of the plants when compared to teflubenzuron alone (Table 3). However, on leaves from the upper third part of soybean plants the amount of teflubenzuron (3.4 ± 0.4 mg/kg) was significantly higher than on leaves from other parts (middle = 1.7 ± 0.2 mg/kg and lower = 0.6 ± 0.2 mg/kg) ($F = 28.66$; $df = 2, 42$; $P < 0.0001$). Therefore, deposition of teflubenzuron alone or in combination with adjuvants was greater in the upper third leaves followed by middle and lower leaves of the soybean plants.

## DISCUSSION

The addition of adjuvants to teflubenzuron spray increased the mortality of the teflubenzuron-susceptible SBL strain in diet-overlay bioassays but did not affect the mortality of resistant and heterozygous strains. This can be explained by a better spread of the insecticide on diet surface when adjuvants were used, allowing that susceptible larvae ingest a greater amount of teflubenzuron, increasing its mortality. In contrast, adjuvants added to teflubenzuron did not cause high mortality of resistant and heterozygotes larvae, because the amount of teflubenzuron is still not enough to change the mortality rates.

In leaf bioassays, no differences in mortality for teflubenzuron alone or in mixture with adjuvants on SBL strains were detected. Contrary to this, adjuvants added to the insecticide spray of other biosynthesis chitin inhibitors (flufenoxuron, triflumuron, novaluron, and lufenuron) increased the mortality of N. elegantalis (DE BORTOLI et al., 2013). In laboratory and field applications of chlorantraniliprole and flubendiamide, the addition of adjuvants increased the mortality of A. transitella (DEMKOVICH et al., 2018). Adjuvants added to fipronil, lambda-cyhalothnin, and dimethoate sprays also increased the control efficacy of T. tabaci (GANGWAR et al., 2016; NEGASH et al., 2020) and the biological activity of chlorantranilprole against Anticarsia gemmatalis (HÜBNER, 1818) (Lepidoptera: Erebidae) (ARRUÉ et al., 2014).

In our study, adjuvants added to teflubenzuron spray did not increase the amount of active ingredient on soybean leaves. We detected a high amount of teflubenzuron on leaves of the upper part of soybean plants, albeit without an increased mortality of SBL strains. The low effects of adjuvants added to the teflubenzuron spray against SBL strains can be explained by the capacity of this species to detoxify and eliminate insecticides prior to ingestion (MARTIN & BROWN 1984). This can also be influenced by the mechanisms of resistance to chitin

Table 2 - Percentage of mortality of SBL strains fed on soybean leaves treated with teflubenzuron alone or in combination with adjuvants obtained from different parts of the plant canopy.

| Treatment | % mortality (95% CI)[a] | | |
|---|---|---|---|
| | Teflu-R | Teflu-R♀ × Sus♂ | Sus |
| Upper | | | |
| Teflubenzuron | 12.5 (4.9–25.7) aA | 77.5 (62.7–88.2) aB | 97.5 (87.1–99.7) aB |
| Teflubenzuron + Nimbus® (0.5%) | 12.5 (4.9–25.7) aA | 80.0 (65.4–90.0) aB | 100.0 (91.1–100.0) aC |
| Teflubenzuron + TA 35 (0.05%) | 12.5 (4.9–25.7) aA | 80.0 (65.4–90.0) aB | 100.0 (91.1–100.0) aC |
| Teflubenzuron + Break-Thru® S 240 (0.05%) | 17.5 (8.2–31.63) aA | 82.5 (68.4–91.7) aB | 100.0 (91.1–100.0) aB |
| Teflubenzuron + Rizospray Extremo® (0.5%) | 20.0 (9.9–34.5) aA | 77.5 (62.7–88.2) aB | 97.5 (87.1–99.7) aB |
| Middle | | | |
| Teflubenzuron | 7.7 (3.3–11.2) aA | 66.7 (51.9–77.5) aB | 87.2 (74.2–94.3) aB |
| Teflubenzuron + Nimbus® (0.5%) | 7.7 (3.3–11.2) aA | 74.4 (59.8–84.4) aB | 92.3 (80.5–97.5) aB |
| Teflubenzuron + TA 35 (0.05%) | 7.7 (3.3–11.2) aA | 74.4 (59.8–84.4) aB | 89.7 (77.3–95.9) aB |
| Teflubenzuron + Break-Thru® S 240 (0.05%) | 12.8 (6.3–18.2) aA | 66.7 (51.9–77.5) aB | 92.3 (80.5–97.5) aC |
| Teflubenzuron + Rizospray Extremo® (0.5%) | 9.7 (4.7–14.8) aA | 64.1 (49.3–75.1) aB | 84.6 (71.1–92.4) aB |
| Lower | | | |
| Teflubenzuron | 5.0 (0.1–16.1) aA | 52.5 (37.2–67.4) aB | 79.5 (65.3–88.5) aB |
| Teflubenzuron + Nimbus® (0.5%) | 2.5 (0.2–12.8) aA | 65.0 (49.4–78.3) aB | 84.6 (71.1–92.4) aB |
| Teflubenzuron + TA 35 (0.05%) | 5.0 (0.1–16.1) aA | 70.0 (54.6–82.4) aB | 84.6 (71.1–92.4) aB |
| Teflubenzuron + Break-Thru® S 240 (0.05%) | 5.0 (0.1–16.1) aA | 65.0 (49.4–78.3) aB | 76.9 (62.6–86.5) aB |
| Teflubenzuron + Rizospray Extremo® (0.5%) | 7.5 (2.1–19.4) aA | 55.0 (39.5–69.6) aB | 79.5 (65.3–88.5) aB |

[a]Percentage values (95% CI) in each soybean leaf location followed by the same lowercase letter within a column or capital letter in a line are not significantly different due to nonoverlap their 95% CIs.

biosynthesis inhibitors, which are associated with target site mutations (VAN LEEUWEN et al., 2012; DOURIS et al., 2016), detoxification mediated by monooxygenase P450 enzymes (SONODA & TSUMUKI 2005; NASCIMENTO et al., 2016), and reduced cuticular penetration (PIMPRIKAR & GEORGHIOU, 1979). Therefore, these mechanisms may prevent binding of the active ingredient or, alternatively, the insecticide was degraded, reducing the amount that reaches the target sites. Understanding the resistance mechanisms to chitin biosynthesis inhibitors in SBL should be a research topic for future studies.

The low mortality of resistant and some survival of heterozygous insects when exposed to

Table 3 - Deposition and penetration of teflubenzuron on soybean leaves applied alone or in combination with adjuvants in different parts of the plant canopy.

| Treatment[a] | Amount of teflubenzuron (mg/kg) ± SE | | |
|---|---|---|---|
| | Upper | Middle | Lower |
| Teflubenzuron | 2.5 ± 1.0 a | 1.2 ± 0.5 a | 0.5 ± 0.2 a |
| Teflubenzuron + Nimbus® (0.5%) | 4.6 ± 1.4 a | 2.0 ± 0.5 a | 0.7 ± 0.3 a |
| Teflubenzuron + TA 35 (0.05%) | 2.8 ± 0.4 a | 1.6 ± 0.7 a | 0.8 ± 0.3 a |
| Teflubenzuron + Break-Thru® S 240 (0.05%) | 3.7 ± 0.9 a | 1.9 ± 0.1 a | 0.5 ± 0.2 a |
| Teflubenzuron + Rizospray Extremo® (0.5%) | 3.2 ± 0.3 a | 1.8 ± 0.3 a | 0.7 ± 0.4 a |
| Mean | 3.4 ± 0.4 A | 1.7 ± 0.2 B | 0.6 ± 0.2 C |

[a]Means ± SE followed by lowercase letters in a column are not significantly different, while uppercase letters indicate that the amount of teflubenzuron varied according with the part of the plant (Tukey test at $P \leq 0.05$).

teflubenzuron alone or in mixture with adjuvants is due to their high resistance to inhibitors of chitin biosynthesis, which leads us to infer that under field conditions, the use of adjuvants did not contribute to reduce the resistance frequency to teflubenzuron in populations of SBL. In an insect resistance management (IRM) context, the survival of heterozygotes when exposed to teflubenzuron explain, in part, the generalized resistance of SBL populations to this insecticide in Brazil (STACKE et al., 2020). The survival of heterozygotes favors a rapid increase in resistance frequency under field conditions, as it is the main responsible for the dispersion of resistance alleles in natural populations (CAPRIO & SUMERFORD, 2018). Thus, the integration of chemical control with Bt soybean and Bt cotton technologies (GREENBERG et al., 2010; BERNARDI et al., 2012; SORGATTO et al., 2015), and biological control agents (MURARO et al., 2019), may delay the development of further resistance and can contribute to the reversion of the resistance of SBL to inhibitors of chitin biosynthesis. In addition, for successful IPM and IRM plans, the reduction in the use of inhibitor of chitin biosynthesis against SBL, as well as to give preference to insecticides with distinct modes of action, is essential to manage SBL in soybean and cotton crops in Brazil.

## CONCLUSION

In the present study, the adjuvants added to teflubenzuron spray did not cause a substantial increase in the mortality of SBL strains. The adjuvants tested also did not increase the deposition of teflubenzuron on soybean leaves, resulting in similar mortality for SBL strains with similar resistance level to chitin biosynthesis inhibitors.

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## DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

## AUTHORS' CONTRIBUTIONS

BLH conceptualization, methodology, formal analysis, investigation, writing, review, editing and data curation. DNG, MRH, MC, RPM and EFL methodology, validation, investigation. RZ methodology and writing. OB and AAM project administration, conceptualization, methodology, formal analysis, resources, writing, supervision, data curation, funding acquisition. All authors critically revised the manuscript and approved of the final version.

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