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Mixture compatibility of *Chin*NPV baculovirus with herbicides and fungicides used in soybean

Compatibilidade de mistura do baculovírus *Chin*NPV com herbicidas e fungicidas utilizados na soja

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Highlights

*Chin*NPV is compatible in a mixture with tested herbicides.
*Chin*NPV is compatible in a mixture with tested fungicides used in agriculture.
*Chin*NPV has insecticide potential with herbicides and fungicides after two hours.

Abstract

Chrysodeixis includens Walker, 1858 (Lepidoptera: Noctuidae), commonly known as soybean looper, is one of the main pests that reduces soybean yield due to its defoliation capacity. Population outbreaks of this pest can occur in the vegetative stage of the crop, together with the occurrence of weeds or in the reproductive stage, at the same time as that of diseases. This often requires the use of pesticides against pests and weeds, or fungi at the same time. Thus, the objective of this study was to evaluate the compatibility of baculovirus *Chin*NPV with different synthetic chemical herbicides and fungicides used in soybean. Four bioassays were carried out, with or without the addition of *Chin*NPV to different herbicides and fungicides. The artificial diets were immersed in the solutions of the pesticides and their mixture and supplied to the caterpillars of *C. includens*, immediately and after one and two hours of mixing. The evaluation was performed by quantifying the number of dead caterpillars. The results showed that the *Chin*NPV baculovirus is compatible for mixing with all studied herbicides and fungicides, even after two hours of mixing. In all the studied scenarios, baculovirus mixed with chemical pesticides triggered the same mortality as sprayed with baculovirus alone, and values of over 80% mortality of *C. includens* have always been recorded.

Key words: Biological control. Entomopathogen. Integrated pest management. Soybean looper.

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Resumo

Chrysodeixis includens Walker, 1858 (Lepidoptera: Noctuidae) falsa-medideira é uma das principais pragas que reduz o rendimento da soja devido à sua capacidade de desfolha. Surtos populacionais desta praga podem ocorrer no estágio vegetativo da cultura, juntamente com a ocorrência de ervas daninhas ou no estágio reprodutivo, ao mesmo tempo em que ocorrem doenças. Muitas vezes, isso pode exigir o uso de pesticidas contra pragas e ervas daninhas ou fungos ao mesmo tempo. Assim, o objetivo deste trabalho foi avaliar a compatibilidade do baculovírus *Chin*NPV com diferentes herbicidas e fungicidas químicos sintéticos utilizados na soja. Foram realizados quatro bioensaios, com ou sem adição de *Chin*NPV em diferentes herbicidas e fungicidas. As dietas artificiais foram imersas nas soluções dos agrotóxicos e sua mistura e fornecidas às lagartas de *C. includens,* imediatamente e após uma e duas horas de mistura. A avaliação foi realizada quantificando o número de lagartas mortas. Os resultados mostraram que o baculovírus *Chin*NPV é compatível para mistura com todos os herbicidas e fungicidas estudados mesmo após duas horas de mistura. Em todos os cenários estudados, o baculovírus misturado com pesticidas químicos desencadeou a mesma mortalidade que o baculovírus pulverizado sozinho, e valores de mais de 80% da mortalidade de *C. includens* sempre foram registrados.
Palavras-chave: Controle biológico. Entomopatógeno. Manejo integrado de pragas. Falsa-medideira.

Introduction

Soybean, *Glycine max* [(L.) Merrill], is one of the major worldwide crops and Brazil is one of its largest producers (Bueno, Raetano, Dorneles, & Carvalho, 2017; Hartman, West, & Herman, 2011). However, numerous diseases, weeds, and arthropods reduce its yield (Oerke, 2006). The simultaneous incidence of pest organisms causes the overuse of chemicals to control them (Pignati et al., 2017). Among the groups of pest organisms that feed on soy, the defoliating caterpillars of the Noctuidae family are unique for their injury capacity (Bortolotto et al., 2015).

Chrysodeixis includens Walker, 1858 (Lepidoptera: Noctuidae) is a key pest in soybean, against which control is carried out predominantly with synthetic chemical insecticides (Stacke et al., 2020). This caterpillar species often occurs in the same plant developmental stages (Zulin, Ávila, & Schlick-Souza, 2018) in which crops are also injured by weeds or plant pathogens (Hartman et al., 2011). Therefore, it is a common practice in the field that pesticides are sprayed simultaneously to manage pests and weeds early in the crop season or to manage pests and diseases late in the crop season. While weed and disease management is exclusively done with the use of chemical herbicides and fungicides, *C. includens* can be alternatively managed by spraying baculovirus with efficacy (Godoy et al., 2019; Moscardi, 1999).

There is an increasing demand worldwide for pest management tools that are less harmful to the environment, which requires the use of more environmentally friendly pest management strategies and fewer synthetic chemicals. One of the most important tools for sustainable pest management is the use of augmentative biological control, among which baculovirus is distinct, particularly for the control of caterpillars, due to its high virulence and specificity, causing efficient epizootics in the target insect population (Moscardi, 1999; Muraro et al., 2018). However, to succeed in integrated pest management, it is crucial to harmoniously use different control tools, particularly the use of harmless pesticides with natural biological control agents. Thus, it is of theoretical and practical interest to study the mixture compatibility of *Chin*NPV with different herbicides and fungicides. Moreover, not only is it important to evaluate the efficacy of pesticides immediately after mixing but also at different times following mixing because in field conditions, it might take a few hours for growers to spray the whole crop after preparing the sprayer with pesticides. Therefore, this study aimed at verifying the mixture compatibility of *Chin*NPV with herbicides and fungicides commonly used in soybean crops with immediate application or one or two hours after mixing.

Material and Methods

Four independent bioassays were carried out evaluating *C. includens* mortality: pesticides (fungicides and herbicides) (bioassay 1), *Chin*NPV mixture with herbicides and fungicides immediately (bioassay 2), after one hour (bioassay 3), and after two hours (bioassay 4) (Table 1). The bioassays were carried out under controlled conditions of temperature (25 ± 2°C), relative humidity (70 ± 10%), and photoperiod (14:10 h light/dark) at the Laboratory of Entomology from Embrapa Soja, Londrina, Paraná, Brazil.

Laboratory rearing of C. includens

Chrysodeixis includens caterpillars that were used in the bioassays originated from insects reared at Embrapa Soybean (one of the units of the Brazilian Agricultural Research Corporation), Londrina, State of Paraná, Brazil. The insects were kept under controlled environmental conditions inside Biochemical Oxygen Demand (BOD) climate chambers (ELETROLab®, model EL 212, São Paulo, SP, Brazil) set at 70 ± 10% humidity, temperature of 25 ± 2°C, and a 14:10 h (L:D) photoperiod according to methodologies previously described in literature (Andrade et al., 2016) and briefly summarized as follows.

Chrysodeixis includens individuals were originally collected from soybean fields in Embrapa Soybean Experimental Farm, Londrina, State of Paraná, Brazil (23° 11′ 11.7′′ S and 51° 10′ 46.1′′ W). These populations were kept in the laboratory for approximately 3 years and new field insects were introduced each year to maintain colony quality.

Caterpillars were individually kept in 50-mL plastic cups sealed with cardboard caps, containing an artificial diet developed by Greene, Leppla and Dickerson (1976). Adults were placed in cages (PVC tubes of 10 cm diameter × 21.5 cm height) and fed with solution (10 g of honey, 60 g of sugar, 1 g of sorbic acid, 1 g of methylparaben / 1 liter of distilled water) and 1 mL of beer. Cage walls were covered with A4 paper for moth oviposition. Eggs were removed, and the cages were cleaned daily. Eggs were placed into 200-mL plastic cups containing 20 mL of artificial diet (Greene et al., 1976). The caterpillars were kept in these containers until they reached the 3rd instar when they were used for the trials.

*Mortality of C. includens due to the application of different pesticides alone or in combination with Chin*NPV

Different suspensions containing herbicides and fungicides (Table 1) used alone or in association with the entomopathogenic baculovirus *Chin*NPV (Baculovirus Simbiose WP® 50 g.150 L H2O^{-1}) were evaluated in the dosage recommended by the manufacturers. Sterile distilled water and sterile distilled water containing *Chin*NPV were used as control treatments. The artificial diet (Greene et al., 1976) was cut into cubes (1 cm^3) and immersed in the treatments for 5 s, and then placed in plastic containers with a volume of 50 mL, containing two caterpillars of the 3rd instar. The cubes of diet with the treatments were offered to the caterpillars for 24 h, guaranteeing the consumption of the treatments and later exchanged for cubes of a diet free of any pesticides (Morgado et al., 2020).

Each bioassay was conducted in a completely randomized design with four repetitions, which consisted of a set of 20 caterpillars (10 plastic pots with two caterpillars each). A total of 80 caterpillars were subjected to each suspension. The evaluation was performed daily for ten days to quantify the number of dead caterpillars. The specimen was considered dead when it was immobile and insensitive to mechanical touch using forceps and was considered alive when the specimen was moving and feeding.

Table 1
Herbicides and fungicides used alone or in a mixture with *Chin*NPV (Baculovirus Simbiose WP® 50 g.150 L H_2O^{-1}) under controlled laboratory conditions (temperature of 25 ± 2°C, relative humidity of 70 ± 10%, and photoperiod of 14:10 h light/dark) and commercial dose

Pesticide	Active ingredient (a.i.)	Commercial product (c.p.)	Chemical group	Concentration of a.i.	Dosage (ha) c. p.
Herbicide	Cletodim	Poquer EC®	Oximacyclohexanedione	240g L^{-1}	0.45 L.200 L H_2O^{-1}
	Chlorimurom-ethyl	Clorimuron Master Nortox WG®	Sulfonylurea	250g kg^{-1}	80 g.200 L H_2O^{-1}
	Flumioxazin	Flumyzin 500 WP®	Cyclohexenodicarboximide	500g kg^{-1}	200 g.200 L H_2O^{-1}
	Imazapique + imazapir	Soyvance Pre WG®	Imidazolinone + imidazolinone	525g kg^{-1} + 175g kg^{-1}	100 g.200 L H_2O^{-1}
	Imazapique + imazapir	Soyvance WG®	Imidazolinone + imidazolinone	175g kg^{-1}+ 525g kg^{-1}	100 g.200 L H_2O^{-1}
	Bentazona	Basagran 600 SL®	Benzothiadiazinone	600 g L^{-1}	1.6 L.200 L H_2O^{-1}
	Cletodim	Select 240 EC®	Oximacyclohexanedione	240g L^{-1}	0.45 L.200 L H_2O^{-1}
	Glyphosate-isopropylamine salt	Trop SL®	Substituted glycine	480g L^{-1}	2 L.200 L H_2O^{-1}

continue...

contuation...

Fungicide	Azoxystrobin + benzovindiflupyr	Elatus WG®	Strobilurin + pyrazolcarboxamide	300g kg^{-1}+ 150g kg^{-1}	300 g.200 L H_2O^{-1}
	Azoxystrobin + cyproconazole	Priori Xtra SC®	Strobilurin + triazole	200g L^{-1} + 80g L^{-1}	0.3 L.200 L H_2O^{-1}
	Cyproconazole + trifloxystrobin	Sphere Max SC®	Strobilurin + triazole	160g L^{-1} + 375g L^{-1}	0.2 L.200 L H_2O^{-1}
	Metconazole + pyraclostrobin	Opera Ultra EC®	Strobilurin + triazole	80g L^{-1} + 130g L^{-1}	0.6 L.200 L H_2O^{-1}

Statistical analysis

The data obtained in the bioassays were analyzed for normality (Shapiro & Wilk, 1965) and homogeneity of variance for each treatment (Burr & Foster, 1972), and if necessary, were transformed to perform ANOVA. The treatment means were then compared using Tukey's test at the 5% probability level (Statistical Analysis System Institute [SAS Institute], 2001).

Results and Discussion

Caterpillars of *C. includens* that fed on treated diets with herbicides or fungicides without association with *Chin*NPV (Bioassay 1) had similar mortality (%) as control (sterile distilled water) (5.65%), except when the diet was treated with the herbicide Soyvance Pre, which resulted in higher caterpillar mortality (30.29%). This value was lower than the mortality recorded for *Chin*NPV (81.35%) (Table 2).

When the diets were treated with herbicides or fungicides mixed with *Chin*NPV (bioassay 2), the mortality of caterpillars was equivalent to the isolated use of *Chin*NPV (86.80%), with values varying from 79.67% (Flumyzin 500) to 90.98% mortality [imazapique + imazapir (Soyvance Pre)]. Lower *C. includens* mortality (%) was observed in the control (6.67%) (Table 2).

Table 2
Mortality of *Chysodeixis includens* (%) due to the ingestion of different pesticides alone (bioassay 1) or in a mixture with *Chin*NPV offered at different times of mixing (bioassays 2, 3, and 4)

	Pesticides	Without *Chin*NPV in mixture (bioassay 1)	Mixed with *Chin*NPV and offered at different times		
			Immediately (bioassay 2)	After 1 hour (bioassay 3)	After 2 hours (bioassay 4)
Herbicides	Control with virus (*Chin*NPV)	81.35 ± 2.98 a	86.80 ± 5.24 a	94.52 ± 2.27 a	93.24 ± 2.64 ab
	Poquer®	11.99 ± 2.50 c	85.72 ± 6.57 a	81.48 ± 1.82 a	84.12 ± 2.27 ab
	Clorimuron Master Nortox®	10.96 ± 3.99 c	79.72 ± 8.80 a	88.42 ± 4.32 a	97.22 ± 2.78 a
	Flumyzin 500®	10.33 ± 2.20 c	79.67 ± 7.44 a	92.17 ± 1.56 a	81.51 ± 3.37 b
	Soyvance Pre®	30.29 ± 3.81 b	90.98 ± 2.35 a	92.43 ± 1.41 a	92.37 ± 3.26 ab
	Soyvance®	17.40 ± 4.59 bc	88.75 ± 8.26 a	78.52 ± 5.79 a	88.01 ± 1.27 ab
	Basagran 600®	16.15 ± 3.80 bc	86.84 ± 5.48 a	92.81 ± 4.36 a	88.08 ± 3.34 ab
	Select 240 EC®	18.38 ± 3.64 bc	89.60 ± 5.69 a	87.41 ± 2.97 a	88.14 ± 2.34 ab
Fungicides	Trop®	21.86 ± 3.59 bc	89.60 ± 1.93 a	81.96 ± 4.70 a	85.66 ± 2.59 ab
	Priori Xtra®	12.97 ± 3.14 bc	85.99 ± 5.65 a	84.26 ± 2.05 a	85.53 ± 5.42 ab
	Elatus®	15.67 ± 5.69 bc	80.08 ± 2.21 a	93.10 ± 1.63 a	89.80 ± 3.63 ab
	Opera Ultra®	19.18 ± 3.38 bc	89.40 ± 3.69 a	94.58 ± 3.93 a	94.24 ± 2.42 ab
	Sphere Max®	7.83 ± 1.56 c	87.24 ± 6.26 a	92.30 ± 3.25 a	92.37 ± 2.55 ab
Statistics	Control without virus (distilled water)	5.65 ± 2.41 c	6.67 ± 1.30 b	12.29 ± 4.38 b	9.02 ± 2.25 c
	CV (%)	35.26	13.84	8.29	7.21
	F	28.20	15.02	37.40	52.80
	p	<0.01	<0.01	<0.01	<0.01
	$df_{residue}$	42	42	42	42

Means ± EPM followed by the same letter in each column do not differ based on Tukey's test (5% probability).

Regarding the mixture compatibility of *Chin*NPV with herbicides and fungicides for one h (bioassay 3) after mixing, both herbicides and fungicides did not reduce C. includens mortality (%) compared with the mortality using baculovirus alone (94.52%), ranging from 78.52% (Soyvance) to 94.58% mortality (Opera Ultra). These values were all higher than C. includens mortality (%) recorded in the control (sterile distilled water) (12.29%) (Table 2).

Similarly, in the evaluation of C. includens mortality (%) triggered by *Chin*NPV after mixing with herbicides or fungicides for two h (bioassay 4), all mixed treatments triggered caterpillar mortality (%) significantly higher than that of the control (9.02%). Although *Chin*NPV mixed with Flumyzin 500 had lower mortality (81.51%) than *Chin*NPV mixed with Clorimuron Master Nortox (97.22%), both had similar mortality to that by using *Chin*NPV alone (93.24%), and all of them triggered C. includens mortality higher than 80% (Table 2), which is considered acceptable for integrated pest management.

In the present study, it was proved that some of the most commonly used fungicides and herbicides in soybean crops can be used in a mixture with *Chin*NPV in a tank without reducing its pathogenicity to C. includens compared to the isolated spray of baculovirus. In soybean, a similar combination of tools to manage different pests has been previously reported in the literature with the simultaneous use of the herbicide Roundup Ready® and the parasitoid Palmistichus elaeisis Delvare & LaSalle, 1993 (Hymenoptera: Eulophidae), which controls Anticarsia gemmatalis Hubner, 1818 (Lepidoptera: Erebidae) (De La Cruz et al., 2017). The active ingredients of the fungicides myclobutanil, potassium bicarbonate, and cyprodinil + fludioxonill has been shown to be safe for Orius insidiosus Say, 1832 (Hemiptera: Anthocoridae), which is a generalist predator present in different major crops (Gradish, Scott-Dupree, Shipp, Harris, & Ferguson, 2011). In contrast, the mixture of herbicides with active ingredients oxyfluorfen, glufosinate-ammonium, metribuzin, and linuron, was not compatible with entomopathogenic nematodes belonging to the genera Steinernema and Heterorhabditis (Laznik & Trdan, 2017), demonstrating the importance of studying different combinations in order to offer growers the most precise information about what could and could not be done. These different outcomes, depending upon the studied combination with fungicides, have also been reported earlier. The entomopathogenic fungi Beauveria bassiana and Metarhizium anisopliae are not compatible with fungicides with the active ingredients azoxystrobin, chlorothalonil, and thi-ophanate-methyl, causing a decrease in the growth of the fungi (Fiedler & Sosnowska, 2017).

The compatibility of *Chin*NPV with herbicides and fungicides was shown in this study. We demonstrated yet another important factor. The effectiveness of the baculovirus in tank mixing conditions during the application period, even after two h of the mixing, was maintained the same with caterpillar mortality close to or higher than 80%. This is similar to the mortality triggered by different baculoviruses. The use of *Chin*NPV alone caused mortality rates greater than 95% for the soybean looper (Morgado et al., 2020; Muraro et al., 2018). This indicates that it is possible to use *Chin*NPV in a mixture in the application of herbicides and fungicides when necessary, without compromising the control of C. includens. The use of viruses to control soybean looper, in addition to achieving success in controlling the target insect, can be a resistance management tool (Godoy et al., 2019). The compatibility and pathogenicity studies of any biological control agent are very important; however, guaranteeing the stability of the control agent by simulating the conditions found at the time of application generates practical knowledge to strongly support their recommendation in the field.

Conclusion

In the present study, simulating the mixing conditions inside the application tank and the average application time in the field, demonstrated that these factors did not interfere with the pathogenicity of *Chin*NPV. These factors also demonstrate that *Chin*NPV, in addition to being compatible with different studied pesticides, has the necessary stability to maintain effectiveness under conditions of application in the field.

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