Research Article

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Lethal and sublethal effects of chlorantraniliprole on *Spodoptera cosmioides* (Lepidoptera: Noctuidae)

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Abstract

BACKGROUND: The *Spodoptera cosmioides* (Walker, 1858) population has increased in *Bacillus thuringiensis* Berliner (Bt) soybean crops in Argentina. As there are no registered products for its control, the recommended insecticides for *S. frugiperda* are used. The aim of this study was therefore to determine the lethal concentration (LC) of chlorantraniliprole and its sublethal effects on the biological and reproductive functions of *S. cosmioides*, an emerging soybean pest in Argentina.

RESULTS: An ingestion toxicity bioassay showed that chlorantraniliprole was active against larvae of the second instar, and after 48 h of exposure LC_{50} was 0.054 µg mL^{-1} H_2O. In the study of sublethal effect, chlorantraniliprole induced changes in the life cycle of exposed *S. cosmioides*, which required more time to complete all stages of development (larval, pupal and adult stages). Pupal weight was also higher in larvae exposed to sublethal concentrations of chlorantraniliprole. Adult fecundity was decreased: the number of eggs laid by each adult female moth, as compared with control females, was two (LC_{15}) and eight (LC_{30}) times lower.

CONCLUSION: These results indicate that chlorantraniliprole has toxicity against *S. cosmioides* larvae. Sublethal effects on the biological and reproductive performance of this species can help optimize integrated pest management programs.

Keywords: chlorantraniliprole; insect control; sublethal effect; *Spodoptera cosmioides*; Bt soybean

1 INTRODUCTION

Argentina is the world's third largest soybean (*Glycine max* L. Merr.) producer, after the USA and Brazil.[1] Genetically engineered crops that express Cry proteins from *Bacillus thuringiensis* Berliner (Bt) are widely grown as a consequence of the crops' ability to effectively control lepidopteran pests. The first Bt soybean to be grown in Argentina on a commercial scale was developed by Monsanto and was first available in 2012. It has two gene constructs that are expressed in the same plant, one encoding glyphosate tolerance to allow broadly dispersive herbicide application, and one encoding the insecticidal Bt Cry1Ac protein, which is toxic to many insect pests that feed preferentially on soybean plants.[2]

Defoliating insects are important and frequent soybean pests. Most damage is caused by *Rachiplusia nu* (Guenée) and *Anticarsia gemmatalis* (Hubner). Other important defoliators are sporadic but can be economically devastating, such as *Spodoptera cosmioides* (Walker), *Spodoptera frugiperda* (J.E. Smith), *Achira bifidalis* (F.), *Spilosoma virginica* (F.), *Helicoverpa gelotopoeon* (Dyar), and *Pseudoplusia includens* (Walker).[3] Bt soybean does not control some pest species, including the polyphagous *Spodoptera* moth genus, which in its larval form – known by its common name the 'armyworm' – may cause considerable damage to both ornamental plants and agricultural crops.[3,4] The *Spodoptera* moth genus is relatively widely distributed across South America, for example in Brazil, Paraguay, and Argentina.[5]

In recent years, *S. cosmioides* ('black armyworm') populations have increased in Bt soybean crops grown in Argentina, reaching high densities, because the species is much less susceptible than other lepidopteran pests to the toxic Cry1Ac protein.[6–8] Alternative control methods, such as the use of biocontrol agents, have been proposed, but synthetic insecticides remain the most effective and reliable control methods.[5]

The group of insecticides known as anthranilic diamides provide effective control for many lepidopteran pests.[9] They have a unique mode of action on target ryanodine receptors, which play a critical role in insect muscle function. Under diamide action, calcium channels remain open and calcium deposits are depleted, inducing a gradual decrease in muscle contraction, which causes paralysis. A few minutes after exposure, insects stop feeding, and show food regurgitation, lethargy, muscular paralysis, and, finally, death in 1–3 days.[10,11] Chlorantraniliprole, one such anthranilic diamide, is a novel insecticide used in Argentina for lepidopteran pest control in several crops, including soybeans.[12]

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Under field conditions, all insecticides can have lethal and sublethal effects on pests. Sublethal effects are defined as physiological, biological and/or behavioral changes in individual insects, or populations, exposed to low insecticide concentrations, which do not necessarily culminate in death, but can affect the life cycle, population growth, reproduction, feeding behavior and longevity in the exposed individuals.[13–16] Under field conditions, pest species may be exposed to sublethal concentrations because insecticides can be degraded by rain, high temperatures, and sunlight; it is also possible for insects to be wing-transported outside the treatment target area, or to receive a sublethal concentration for other reasons.[17]

Several studies have reported that chlorantraniliprole causes sublethal effects in lepidopteran pests: *Helicoverpa armigera* (Hübner, 1809),[18,19] *Plutella xylostella* (Linnaeus),[20,21] *Tuta absoluta* (Meyrick),[22] and *Spodoptera exigua* (Hübner)[23]; however, there is no report as yet on *S. cosmioides*.

In Argentina, however, there are no registered products available for *S. cosmioides* control, so the insecticides recommended for *S. frugiperda* control are used at higher concentrations. Even at higher concentrations, however, insecticides may cause only sublethal effects in the 'black armyworm' because the environmental conditions are not ideal for the application to be effective (high temperatures and low relative humidity, typical characteristics of the summer in the Pampean region of Argentina). The aim of the current work, therefore, was to determine the lethal and sublethal effects of chlorantraniliprole, an insecticide with a novel mode of action, on the biological and reproductive functions of this insect pest under high-temperature and low-humidity conditions.

2 MATERIALS AND METHODS

2.1 Insect rearing

Different larval instars of *S. cosmioides* were collected from soybean fields in Santa Fe Province (Argentina), and were transferred to growth chambers (Entomology Laboratory, Agronomy College, Litoral University, Santa Fe, Argentina), where the current research was performed. The larvae were reared on artificial diet cubes as per the methodology described by Poitout and Bues,[24] and insects remained *in situ* until they emerged from the pupal stage. Emerged adults were subsequently transferred to oviposition cages equipped with paper sheets for oviposition, and were fed with a honey-based solution (10%). Insects were reared for several generations in an environmental chamber under controlled conditions [25 ± 2 °C and 60 ± 5% relative humidity (RH), with a 14:10 h light:dark (L:D) photoperiod]. One half of the resultant population was kept for replacements, and the other half was used in bioassays. These procedures formed the basis for all subsequent experiments performed in the current research.

2.2 Insecticide dilutions

The product tested was chlorantraniliprole [Coragen®; 20% suspension concentrate (SC); DuPont™, Wilmington, DE, USA]. To determine lethal and sublethal concentrations, the Cámara de Sanidad Agropecuaria y Fertilizantes (CASAFE)-recommended field concentration for *S. frugiperda* control was used as a reference,[12] and seven dilutions (0.010, 0.021, 0.042, 0.083, 0.166, 0.333, and 0.666 μg mL^{-1} H_2O) were prepared based on the recommendation. Solutions were prepared by diluting the commercial product in distilled water, and by adding a surfactant (Tween® 80; 0.01%; Merck and Co., Kenilworth, NJ, USA) to decrease surface tension and to ensure maximum coverage. The resultant dilutions of insecticide were applied to artificial diet cubes (0.5 cm^3) with a Discovery Comfort micropipette (HTL Lab Solutions, Corning, Inc., Corning, NY, USA) and a 10-μL tip. A total of 5 μL per diet cube was applied, and a control set of diet cubes was left untreated.

2.3 Larval toxicity

To assess the lethal concentrations of chlorantraniliprole for *S. cosmioides* larvae, a single artificial diet cube was placed in each Petri dish (standard 9 cm diameter). After 1 h, five second-instar larvae of *S. cosmioides* were added. Ten replicates were thus performed for each of the seven dilution treatments (50 larvae per dilution treatment). Petri dishes were kept in an environmental chamber under controlled conditions. After 48 h of exposure, larval mortality was recorded, and the 50% lethal concentration (LC_{50}) was determined.

2.4 Sublethal effects on life cycle

To assess the effect of sublethal chlorantraniliprole concentrations on the life cycle of *S. cosmioides*, second-instar larvae were treated with two different sublethal insecticide concentrations, 0.010 and 0.021 μg mL^{-1} H_2O, to obtain the two partial kills necessary for probit analysis. The two dilutions tested were chosen based on the LC_{50} value. Larvae were randomly selected, and five were placed in each of 10 Petri dishes per treatment 1 h after the insecticide had been applied to the artificial diet cube. The control group was introduced to artificial diet cubes treated with distilled water. Ten replicates were performed for each of the two treatments (50 larvae per treatment). Petri dishes were kept in an environmental chamber under controlled conditions.

After 120 h of exposure to the sublethal concentrations, dead larvae were removed and survivors were placed individually in Petri dishes (9 cm diameter), where they were fed on artificial diet cubes without pesticide until pupation. The food cubes were replaced daily to prevent contamination and to ensure normal larval growth. Data recorded included the length each individual spent in the larval and pupal stages of the life cycle, the percentage of malformed pupae, and pupal weight, taking into account only viable pupae. An Ohaus Pioneer™ precision scale (0.0001 g sensitivity; Ohaus Corp., Parsippany, NJ, USA) was used to weigh pupae. Adult longevity was also recorded. Pupae were considered dead if adult moths had not emerged after 12 days.

2.5 Sublethal effects on reproduction

Once the pupae had formed, they were individually transferred to plastic boxes (15 cm diameter) and were placed on top of moistened filter paper in the boxes. Sex was determined by observing the terminal portion of the pupae.[25] The emerging adult moths were paired, and each pair was placed on filter paper in its own plastic oviposition container (17 cm high, 11 cm upper diameter, and 7 cm lower diameter). Oviposition containers were secured with an elastic band to allow some air circulation but to prevent adult moths from escaping.

Moth pairs were randomly assigned to treatments. The moths were fed daily with a 10% honey:water solution administered by cotton bud. To determine *S. cosmioides* fecundity, eggs masses deposited by the female after mating were collected daily, and the number of egg masses was recorded by counting with an Olympus SZ40 stereomicroscope (Olympus Corporation, Tokyo, Japan) set at 40× magnification. The egg masses were individually placed in Petri dishes (9 cm diameter) with absorbent paper and enough diet for emerging neonates. To estimate pair fertility (viable eggs),

Table 1. Toxicity of chlorantraniliprole in second-instar *Spodoptera cosmioides* larvae

n	LC_{50} (95% CL)	LC_{30} (95% CL)	LC_{15} (95% CL)	χ^2	df	Regression equation
350	0.054 ± (0.031–0.069)	0.021 ± (0.006–0.035)	0.01 ± (0.001–0.018)	966 471	68	$y = 1.632 + 1.284x'$

The total number of larvae (n) tested was 350 individuals. All lethal concentration (LC) values are based on exposure of larvae to chlorantraniliprole insecticide at dilutions measured in μg mL^{-1} H_2O. Degrees of freedom (df) and chi-square (χ^2) values were calculated by probit analysis (SPSS version 23.0). In the regression equation used, x' = logarithm in base 10 of the covariate (X). The confidence limit (CL) for lethal concentration (LC) values was 95%.

the number of viable larvae that hatched from each egg mass was recorded. Eggs that did not hatch within 5 days of oviposition were considered non-viable. Moth pairs that did not oviposit were not factored into the analysis.

2.6 Data analysis

To calculate the LC_{50} and sublethal concentrations, mortality data from the larval toxicity experiment were subjected to probit regression analysis against the log insecticide concentration, using SPSS software (version 23.0; IBM Corp., Armonk, NY, USA). For the effects analysis of sublethal concentrations of chlorantraniliprole on larval, pupal, and adult longevity and pupal weight, number of days spent in each life stage, pupal weights and number of deformed pupae were subjected to analysis of variance (ANOVA), and the means were compared by Tukey's test ($P \leq 0.050$). For the comparison of fecundity and fertility parameters, Kruskal–Wallis's non-parametric test was applied, with a significance level of 5%. Analyses were performed using the statistical program InfoStat.[26]

3 RESULTS AND DISCUSSION

3.1 Larval toxicity

Based on larval mortality after 48 h of exposure to the seven chlorantraniliprole treatments, it was calculated that the LC_{50} value for second-instar larvae of *S. cosmioides* was 0.054 μg mL^{-1} H_2O, and the LC_{15} and LC_{30} values were determined to be 0.010 and 0.021 μg mL^{-1} H_2O, respectively (Table 1). These two dilutions were used in sublethal effect trials. The LC_{50} was lower than that found by Hardke *et al.* for *S. frugiperda* (0.068 μg mL^{-1}),[27] but higher than the LC_{50} for *P. xylostella* (0.230 μg mL^{-1}) determined by Han *et al.* and the LC_{50} for *Agrotis ipsilon* (Hufnagel) (0.354 μg g^{-1}) derived by Xu *et al.*[21,28] Because chlorantraniliprole has a unique mode of action, high insecticidal activity and low mammalian toxicity, it is recommended for lepidopteran pest control in commercial agricultural applications.

3.2 Sublethal effects on life cycle

Exposure to sublethal concentrations of chlorantraniliprole affected the life cycle and development of *S. cosmioides*. Larval development time increased significantly with insecticide treatments [56.08 and 46.14 days for 0.021 (LC_{30}) and 0.01 μg g^{-1} (LC_{15}) dilutions, respectively], in comparison with the control (20.45 days) (Table 2). Larval development time increased by 174.23 and 125.62% for LC_{30} and LC_{15} treatments, respectively. Zhang *et al.* and Lai and Su obtained similar results in *H. armigera* (Hübner) and *S. exigua*,[19,23] respectively, where larvae were exposed to low insecticide concentrations: not only was development time prolonged, but the exposed insects also showed additional instars during the larval stage of development. According to Desneux *et al.*,[14] chlorantraniliprole can cause disturbances to normal neuronal tissue development, which might explain why development times were altered.

Table 2. Sublethal effects of chlorantraniliprole on the development of *Spodoptera cosmioides*

	Mean (± SD) development time (days)					
Treatment	n	Larva	n	Pupa	n	Adult
Control	38	20.45 ± 0.86	38	8.68 ± 0.44	38	6.11 ± 0.82
LC_{15}	21	46.14 ± 1.16	15	17.47 ± 0.71	15	14.13 ± 1.26
LC_{30}	12	56.08 ± 1.53	9	19.56 ± 0.91	9	11.44 ± 1.68

The LC_{15} and LC_{30} were determined for larval, pupal, and adult development times as measured in days [mean ± standard deviation (SD)]. Means for LC_{15} and LC_{30} treatments in the pupal and adult stages did not differ significantly according to Tukey's test ($P \leq 0.050$), but were significantly different from those of the control group. Means for LC_{15}, $LC_{30,}$ and controls were significantly different in the larval stage. n = number of specimens whose development was followed.

Treatment with chlorantraniliprole caused longer mean pupal development times, compared with controls: 225.34% for the LC_{30} (0.021 μg mL^{-1} H_2O), and 201.26% for the LC_{15} (0.010 μg mL^{-1} H_2O). Significant differences were found in mean pupal development times for both the 0.010 μg mL^{-1} H_2O (LC_{15}) and 0.021 μg mL^{-1} H_2O (LC_{30}) groups – 17.47 and 19.56 days, respectively – compared with the control group, which only took 8.68 days (Table 2). Larvae that have consumed insecticide should use more resources in detoxification, and should thus take more time to complete immature stages of development.[29] Indeed, the results of the current research were replicated by Song *et al.* with *Ostrinia furnacalis* (Guenée) and Han *et al.* with *P. xylostella*, where sublethal concentrations of the same insecticide were used.[21,30] Taken together, these research results could have implications for population dynamics, because a longer development time would allow greater exposure of pest larval stages to predators and parasitoids.[31]

Insects treated with LC_{15} and LC_{30} of chlorantraniliprole spent far more time in the adult phase of the life cycle (14.13 and 11.44 days, respectively), compared with the control group (6.11 days) (Table 2). Mean pupal weights [0.480 g (LC_{15}) and 0.490 g (LC_{30})] significantly different from those of the control group (0.390 g) were also obtained after the two sublethal insecticide treatments (Table 3). In fact, larvae exposed to sublethal concentrations of chlorantraniliprole in the current study exhibited 23.07 and 25.63% higher mean pupal weights after the LC_{15} and LC_{30} treatments, respectively, compared with the control group (Table 3), results that agree with those of Lai and Su,[23] Sang *et al.*,[32] Xu *et al.*,[28] and Yu *et al.*,[33] who worked with *S. exigua, A. ipsilon* (Hufnagel), and *Spodoptera litura* (Fabricius), respectively. According to Luttrell *et al.*,[7] higher or lower weights could be attributable to alterations of the enzymes involved in nutrient absorption. Lai and Su also argued that higher pupal weights in *S. exigua* could be attributed to supernumerary instars,[23]

Table 3. Sublethal effects of chlorantraniliprole on *Spodoptera cosmioides* pupae

Treatment group	n	Mean pupal weight (g)	Deformed pupae (%)
Control	38	0.39 ± 0.01	0.00
LC_{15}	21	0.48 ± 0.01	28.50
LC_{30}	12	0.49 ± 0.02	25.00

Pupal weights (g) (mean ± standard deviation) and the percentage of deformed pupae for both insecticide treatments did not differ significantly according to Tukey's test ($P \leq 0.050$), but there were significant differences between them and the control treatment (without insecticide). n = number of specimens whose development was followed.

Table 4. Sublethal effects of chlorantraniliprole on the fecundity and fertility of *Spodoptera cosmioides*

Treatment group	N	Eggs/female	Eggs hatched (%)
Control	10	3057.10 ± 1903.67	46
LC_{15}	4	1560.00 ± 2082.45	38
LC_{30}	4	361.50 ± 723.00	58

Number of eggs laid per female was not significantly different between any of the groups. Eggs hatched successfully (mean ± standard deviation) differed significantly between the control and LC_{30} groups, but results for the LC_{15} group were not significantly different from those for either the control or the LC_{30} group. Significance was measured by Kruskal–Wallis's non-parametric test ($P \leq 0.050$). n = number of specimens whose reproduction was followed.

allowing a longer feeding period, while Xu *et al.* noted that insecticide treatment killed the weaker larvae and left strong, robust individuals, which could also account for higher weights.[28]

In the current research, mean life cycle duration was 77.74 days (LC_{15}) and 87.08 days (LC_{30}), while individuals exposed to the control treatment (artificial diet) completed the life cycle in 35.24 days (2.2 and 2.5 times faster than those exposed to the LC_{15} and LC_{30} treatments, respectively).

No deformed pupae were found in the control treatment group (Table 3), but in the LC_{15} and LC_{30} treatments, the number of defects was significant, with defects being found in six individuals (28.5%) and three individuals (25.0%), respectively. Han *et al.* also found significant differences in the number of deformed pupae of *P. xylostella* as the concentration of chlorantraniliprole increased.[21] They also reported that the insecticide produced sublethal effects in immature stages of *S. cosmioides*, and could cause somewhat surprising alterations in pest biology, such as enhanced population growth.[21] In the present research, although the differences were not significant between treatments, we also found that the number of emerged adult insects decreased in the second generation, when the insecticide was applied, with negative consequences for population dynamics. With these somewhat conflicting results in mind, it is worth noting that chlorantraniliprole is still the most effective compound for use against defoliating caterpillars, while having low lethal and sublethal effects on beneficial arthropods.[34]

3.3 Sublethal effects on reproduction

In the current study, significant effects on the reproductivity of *S. cosmioides* were observed; offspring were fewer when larvae were exposed to chlorantraniliprole at both concentrations and then were mated (Table 4). When larvae were exposed to the LC_{30}, the number of eggs deposited per female (mean 361.50 eggs) was eight times lower compared with eggs deposited (mean 3057.10 eggs) by *S. cosmioides* subjected to the control treatment, a significant difference. The number of eggs deposited per female for those subjected to the LC_{15} treatment was two times lower than the controls, a non-significant difference ($P \leq 0.050$). Thus, it seemed that low concentrations of chlorantraniliprole tended to decrease *S. cosmioides* fecundity.

Fertility was not affected by chlorantraniliprole treatment in the current research. There were no significant differences in the number of viable larvae between treatments. Although Lai and Su found no decrease in fertility in *S exigua* treated with sublethal concentrations of chlorantraniliprole during the larval stage,[23] Ioriatti *et al.* and Knight and Flexner[35,36] verified the disruptive behavior of anthranilic diamides on intercourse and ovaries in several lepidopteran pests. Other researchers found that the number of eggs per female and the number of viable larvae hatched decreased in *H. armigera* and *A. ipsilon* when reproducing adults were treated with chlorantraniliprole during the larval stage.[19,28] Reproductive dysfunctions after insecticide treatment could be either physiological or behavioral alterations,[14] but with the end result being a lower probability that males will locate females via sex pheromones.[37]

Low concentrations of chlorantraniliprole can negatively affect various life cycle traits of both *S. cosmioides* and *P. xylostella* populations; in addition to direct sublethal effects, in particular slower larval development and decreased adult fecundity, transgenerational effects in F1 individuals that were never exposed to the insecticide have been reported.[20] Thus, population declines could be the result, a key demographic parameter used to measure insecticide efficacy. Sublethal effects also negatively impact fecundity in both parental and offspring generations,[21,23,30,33] because insecticides alter insects' chemical communication systems, decrease reproduction opportunities and affect insect reproduction in other ways.[13] In addition, available technical information for chlorantraniliprole assumes ovicidal action when eggs are directly exposed, and effects on reproduction are not ruled out when contact is indirect.[12]

The assessment of sublethal and lethal effects of insecticides on pests yields insights on their efficacy.[16] In the current work, we verified that the LC_{50} for second-instar larvae of *S. cosmioides* exposed to chlorantraniliprole was 0.054 $\mu g\, mL^{-1}$ H_2O. Sublethal effects caused larval, pupal and adult stages in the life cycle to lengthen, pupal weights to increase, and fecundity to decrease. Thus, the present research will help agroindustry practitioners understand the primary and secondary effects of chlorantraniliprole on the life cycle and reproduction of a key Bt soybean pest in Argentina, *S. cosmioides*. Because experiments in the present work were conducted under controlled conditions, they are an accurate preliminary prediction of sublethal effects on this new Bt soybean pest in the field. However, it will be necessary to evaluate concentration efficacy in the field, because environmental factors in uncontrolled conditions may modify effects.[17] In conclusion, lethal and sublethal effects discovered herein have direct implications for pest management, and can help to optimize integrated pest management programs.

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DISCLOSURES

The authors report no conflicts of interest in this work.

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