Toxicicity and Histological Changes Caused by Insecticides in Spodoptera frugiperda (Lepidoptera: Noctuidae) Eggs

Authors: Soares, Walyson Silva, Davi Junior, Salmo De Melo, De Sena Fernandes, Maria Elisa, De Souza, Edmilson Amaral, Serrão, José Eduardo, et al.

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Toxicicity and histological changes caused by insecticides in *Spodoptera frugiperda* (Lepidoptera: Noctuidae) eggs

Walyson Silva Soares[1], Salmo de Melo Davi Junior[1], Maria Elisa de Sena Fernandes[2], Edmilson Amaral de Souza[3], José Eduardo Serrão[4], Angélica Plata-Rueda[2], Luis Carlos Martínez[4], and Flávio Lemes Fernandes[2,]*

Abstract

Insecticides typically are used to control *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) larvae in corn crops; however, both eggs and larvae are affected by these applications. The purpose of this study was to evaluate the effects of 9 insecticides commonly used on corn crops in Brazil on ovicide and embryonic development of *S. frugiperda*. The insecticides were applied with an airbrush to the outer surface of eggs at 72, 96, 120, 144, and 168 h after oviposition. Larval emergence rates then were calculated. Eggs in the control and the alpha-cypermethrin and methomyl + novaluron treatment were evaluated by light microscopy to investigate possible histological changes in the embryos. The insecticides methomyl + novaluron, chlorantraniliprole + lambda-cyhalothrin, and deltamethrin reduced the emergence rate of *S. frugiperda* larvae. A mixture of alpha-cypermethrin and methomyl + novaluron did not affect the embryonic development of *S. frugiperda*; however, methomyl + novaluron-treated larvae did not emerge. Therefore, the insecticides methomyl + novaluron, chlorantraniliprole + lambda-cyhalothrin, and deltamethrin have an ovicidal effect and may be recommended for managing *S. frugiperda*.

Key Words: fall armyworm; neurotoxic insecticide; growth regulator; ovicidal effect; *Zea mays*

Resumo

Na cultura do milho, inseticidas são usados no controle de larvas de *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae), embora, os ovos e larvas são expostos à ação química. O objetivo de este estudo foi avaliar o efeito ovicida e o desenvolvimento embrionário de *S. frugiperda* de nove inseticidas usados na cultura de milho. Inseticidas foram aplicados com um aerógrafo sobre a superfície externa dos ovos desse inseto com idades de 72, 96, 120, 144, e 168 horas após a oviposiçao. Posteriormente, a taxa de emergência de larvas foi calculada. Ovos do controle e tratados com inseticida alfa-cipermetrina e metomil + novaluron foram analizados por microscopia de luz para observar as possíveis mudanças histológicas sobre os embriões. Os inseticidas metomil + novaluron, clorantraniliprole + lambda-cialotrina, e deltametrina reduziram a taxa de emergencia de larvas de *S. frugiperda*. A mistura da alfa-cipermetrina e metomil + novaluron não afetaram o desenvolvimento embrionário de *S. frugiperda*, no entanto, larvas tratadas por metomil + novalurom não emergiram. Portanto, os inseticidas metomil + novaluron, clorantraniliprole + lambda-cialotrina, e deltametrina tem efeito ovicida e podem se recomendados no manejo das populações de *S. frugiperda*.

Palavras Chaves: efeito ovicida; inseticida neurotóxico; lagarta-do-cartucho; regulador de crescimento; *Zea mays*

The fall armyworm, *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) is a polyphagous pest in the Americas. This species damages and destroys numerous crops such as corn (*Zea mays* L.; Poaceae), cotton (*Gossypium hirsitum* L.; Malvaceae), pearl millet (*Pennisetum glaucum* L.; Poaceae), potato (*Solanum tuberosum* L.; Solanaceae), rice (*Oryza sativa* L.; Poaceae), sorghum (*Sorghum bicolor* [L.] Moench; Poaceae), and soybean (*Glycine max* [L.] Merr.; Fabaceae) (Farias et al. 2001; Barros et al. 2010; Juarez et al. 2014; Iita 2016). In Brazil, the damage to corn crops by *S. frugiperda* is severe, leading to $400 million in annual losses (Iita 2016). In a single life cycle, a *S. frugiperda* female lays 1,500 eggs that overlap on the adaxial face of a corn leaf. Four days after oviposition, the first instar larvae emerge and scrape the leaf whereas subsequent instars completely consume the leaf causing severe damage and plant death. The last larval instar typically is found within a leaf sheath (Cruz 1995; Galo et al. 2002; Capinera 2008; Valicente & Tuelher 2009) where it is protected from insecticide applications (Gassen 1996).

Neurotoxic insecticides such as alpha-cypermethrin (Fazolin et al. 2016), chlorantraniliprole + lambda-cyhalothrin, chlorantraniliprole (Cessa 2013; Guerreiro 2013), spinosad (Martins et al. 2006), deltamethrin, methomyl, and chlorfenapyr (Viana & Costa 1998) are used to control *S. frugiperda* larvae. However, these insecticides also may be

[1]Universidade Federal de Uberlândia, Instituto de Ciências Agrárias, Uberlândia, Minas Gerais, Brazil; E-mail: walysonagronomia@gmail.com (W. S. S.); salmo.junior@outlook.com (S. M. D. J.)
[2]Universidade Federal de Viçosa, Instituto de Ciências Agrárias, Rio Paranaíba, Minas Gerais, Brazil; E-mail: maria.sena@ufv.br (M. E. S. F.); flaviofernandes@ufv.br (F. L. F.); angelicaplata@yahoo.com.mx (A. P.-R.)
[3]Universidade Federal de Viçosa, Instituto de Ciências Biológicas e da Saúde, Rio Paranaíba, Minas Gerais, Brazil; E-mail: edmilson.souza@ufv.br (E. A. S.)
[4]Universidade Federal de Viçosa, Departamento de Biologia Geral, Viçosa, Minas Gerais, Brazil; E-mail: jeserrao@ufv.br (J. E. S.); lc.martinez@outlook.com (L. C. M.)
*Corresponding author; E-mail: flaviofernandes@ufv.br

used at other stages of insect development such as the egg (Tavares et al. 2011) and adult (Pratissoli et al. 2004) stages. Applying insecticides at the egg stage of the life cycle of *S. frugiperda* may increase control efficiency. The eggs of this insect exist as an immobile mass that favors exposure to insecticide applications. For example, insecticides such as azadiractin, lufenuron, and deltamethrin can penetrate the chorion of *S. frugiperda* eggs, disrupting embryonic development and preventing larval emergence and consequent pest-host infestation (Rodrigues et al. 2002; Bortoli 2013; Correia et al. 2013).

Given the importance of reducing populations of *S. frugiperda* at early developmental stages before crop damage occurs, the purpose of this study was to evaluate the histological changes and biocidal effects of using insecticides on *S. frugiperda* eggs.

Materials and Methods

INSECTS

Spodoptera frugiperda eggs (48 h old) were purchased from the Farroupilha Lallemand Bio Control Laboratory in Patos de Minas, Brazil, and subsequent experiments were performed at the Integrated Pest Management laboratory at the Federal University of Viçosa in Rio Paranaíba, Brazil.

INSECTICIDES

The chemical insecticides were selected from the Agrofit database (MAPA 2017) in order to represent different chemical groups, modes of action, and commercial doses recommended for corn crops (Table 1). In addition, molecular weight and solubility data for insecticides were considered (FAO 2002; FAO 2008a, b; FAO 2012a, b; FAO 2013). For the bioassays, the insecticides were diluted in distilled water to an aliquot of 1 mL or 1 g of each active ingredient and then used to prepare effective field applications.

OVICIDE

A card with 80 eggs was attached with tape to the back of a plastic box (48 × 69 mm) for the insecticide applications. Distilled water served as a control. An airbrush (Comp1 Wimpel, São Paulo, Brazil) was used to spray 1 mL of each treatment at 50 psi on the external surface of the *S. frugiperda* eggs. To avoid damaging the eggs, the spray tip was held at a distance of 15 cm. The treated eggs then were placed in a climate-controlled chamber (25 ± 1 °C, 70 ± 1% RH, and photoperiod of 12:12 h [L:D]) and the numbers of emerged larvae were counted at 72, 96, 120, 144, and 168 h. The bioassay was conducted in a completely randomized design in quadruplicate. The treated eggs then were checked daily until all larvae hatched or the eggs died. The larval emergence data were evaluated by analysis of variance (Sisvar software; Ferreira 2011) and the treatment averages were compared by the Scott-Knott mean test at $P < 0.05$.

EGG HISTOLOGY

Spodoptera frugiperda eggs at 72, 96, 120, 144, and 168 h were exposed to α-cypermethrin and methomyl + novaluron insecticides. Distilled water was used as a control. Afterwards, the eggs were transferred to Zamboni's fixative solution (Stefanini et al. 1967) for 24 h and placed in a vacuum chamber. The samples then were dehydrated in a grade ethanol series (70°, 80°, 90°, and 95°) and embedded in historesin (Leica Biosystem Nussloch GmbH, Wetzlar, Germany) for 24 h at 5 °C. Sections (3 µm thick) were obtained, stained with toluidine blue,

Table 1. Chemical group, commercial application rate, active ingredient concentration, molecular weight, and solubility of insecticides registered for control of *Spodoptera frugiperda* in corn crop.

Insecticide	Chemical group	Commercial application rate ($mL\ ha^{-1}$ or $g\ ha^{-1}$)	Concentration (mg i.a. ha^{-1})	molecular weight ($g\ mol^{-1}$)	Solubility ($mg\ L^{-1}$ or $g\ L^{-1}$)
Indoxacarb 150 CE	Oxadiazines	400	60	527.8	0.2
Alpha-cypermethrin 100 FS	Pyrethroid	50	5	416.3	2.06
Deltamethrin 25 CE		200	5	505.2	1.3×10^{-6}
Chlorantraniliprole 200 SC	Anthranilic	125	25	483.15	1.023
Methomyl + novaluron 440 + 35 CE	Oxime methyl carbamate + benzoylurea	500	220 + 17.5	162.20 + 492.7	54.7
Novaluron 100 CE	Benzoylurea	400	40	492.7	3×10^{-6}
Lambda-cyhalothrin + chlorantraniliprole 50 + 100 SC	Pyrethroid + anthranilamide	150	7.5 + 15	449.9	6.3×10^{-6}
Chlorfenapyr 240 SC	Pyrazole analogue	750	180	407.6	5.28
Spinosad 480 SC	Spinosyns	100	48	732.0 (Spinosyn A) + 746.0 (Spinosyn D)	235 + 0.332

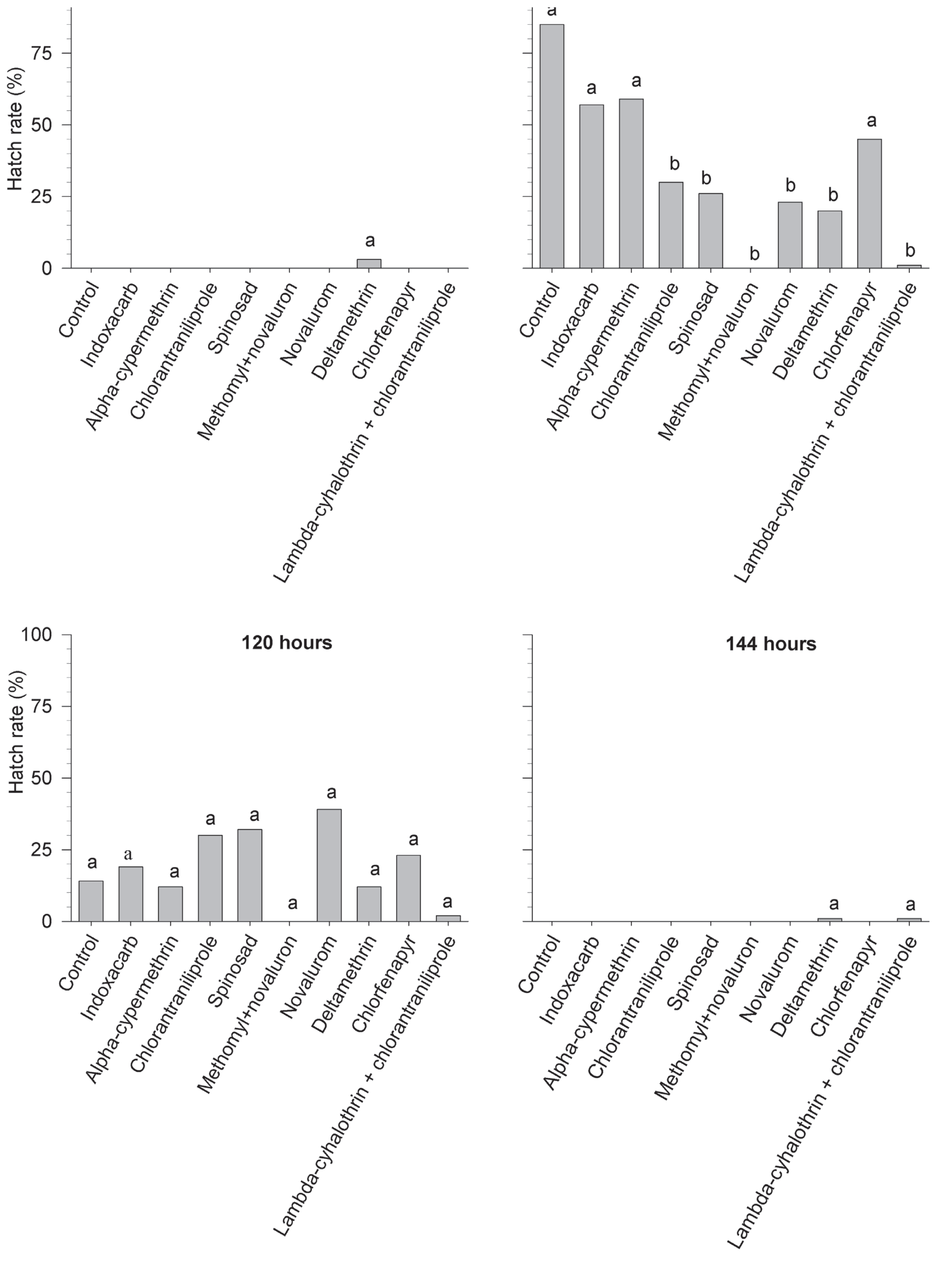

Fig. 1. Emergence (%) of *Spodoptera frugiperda* larvae from 72, 96, 120, and 144 h-old eggs after insecticide exposure. Different letters within a column indicate significant differences by the Skott-Knott test ($P < 0.05$).

Table 2. Cumulative emergence (%) of *Spodoptera frugiperda* larvae.

Treatments	Hatch rate (%)*
Methomyl + novaluron	17.50 b
Chlorantraniliprole + lambda-cyhalothrin	20.25 b
Deltamethrin	34.50 b
Novaluron	60.75 a
Chlorantraniliprole	66.00 a
Spinosad	69.75 a
Chlorfenapyr	74.75 a
Alpha-cypermethrin	76.75 a
Indoxacarb	79.00 a
Control	100.00 a

*Different letters within a column indicate significant differences by the Skott-Knott test ($P < 0.05$).

mounted with Permount, and analyzed under an Olympus CX-41 light microscope (Olympus Corporation, Tokyo, Japan) coupled to a Nikon D3100 camera (Nikon Inc., New York, USA).

Results

OVICIDE

Larval emergence at 72 h was 10% in the deltamethrin treatment and 0% in all other treatments (Fig. 1). At 96 h, emergence differed among the treatments ($F = 3.11$; df 9, 30; $P < 0.001$) with 85% of the emerged larvae in the control group. Emergence also differed among the treatments at 120 h. Here, novaluron was the most notable treatment at 38% (Fig. 1). At 144 h, larval emergence was 5% with deltame-

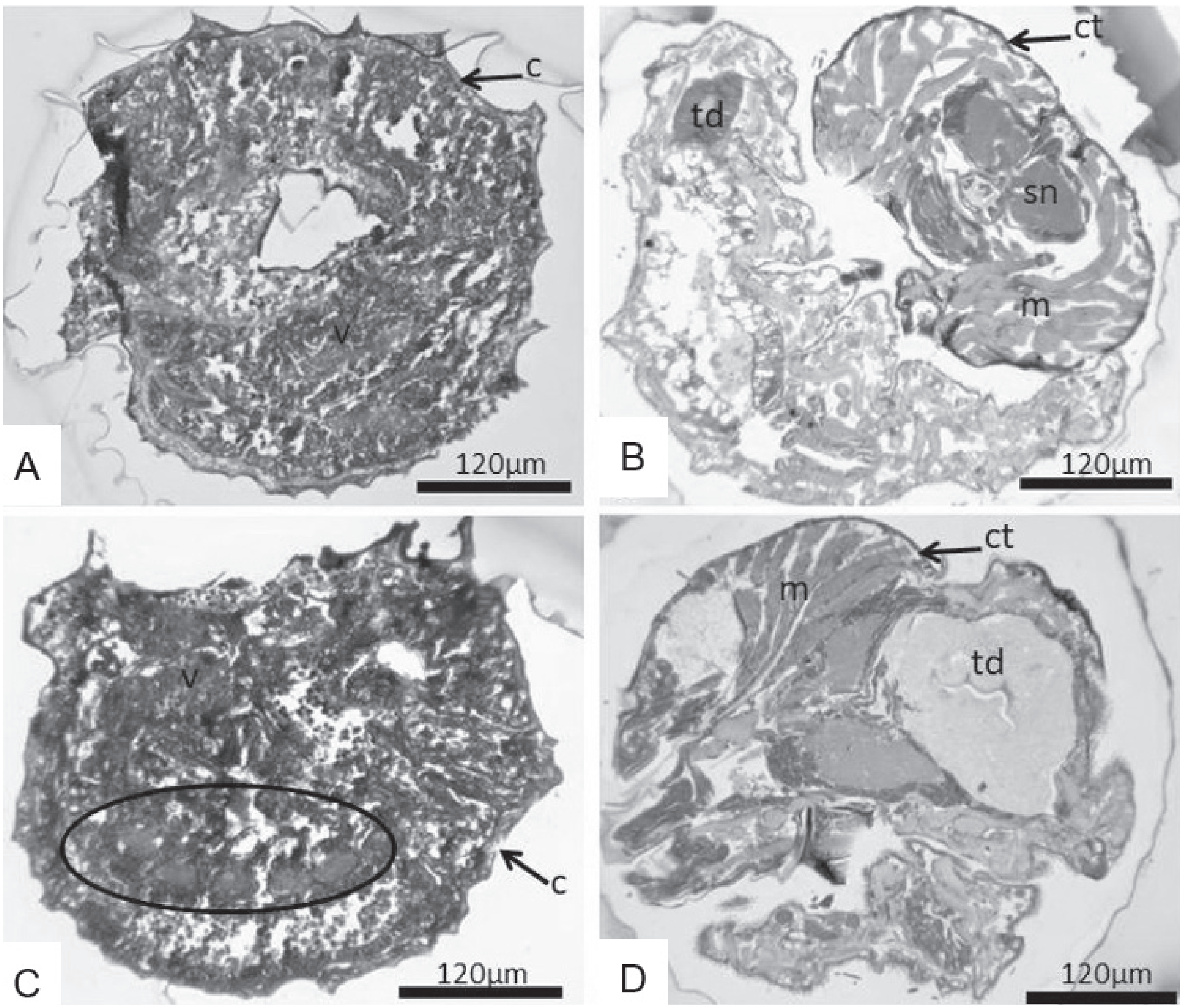

Fig. 2. *Spodoptera frugiperda* eggs from the control group at 72 and 96 h (A, B). Eggs treated with α-cypermethrin at 72 and 96 h (C, D). (A) Eggs from the control group at 72 h showing vitellum (v), cuticle (arrow), chorion (circle), and muscle (m) formation. (B) Eggs at 96 h showing embryo with developed striated muscle (m) cuticle (arrow), complete digestive (td) and central nervous systems (supraesophageal ganglion) (sn). (C) Differentiated embryo (circle) at 72 h. (D) Differentiated embryo at 96 h occupying the internal space of the egg, showing normal midgut (td) cells, cuticle (arrow) and advanced stage of muscle development (m).

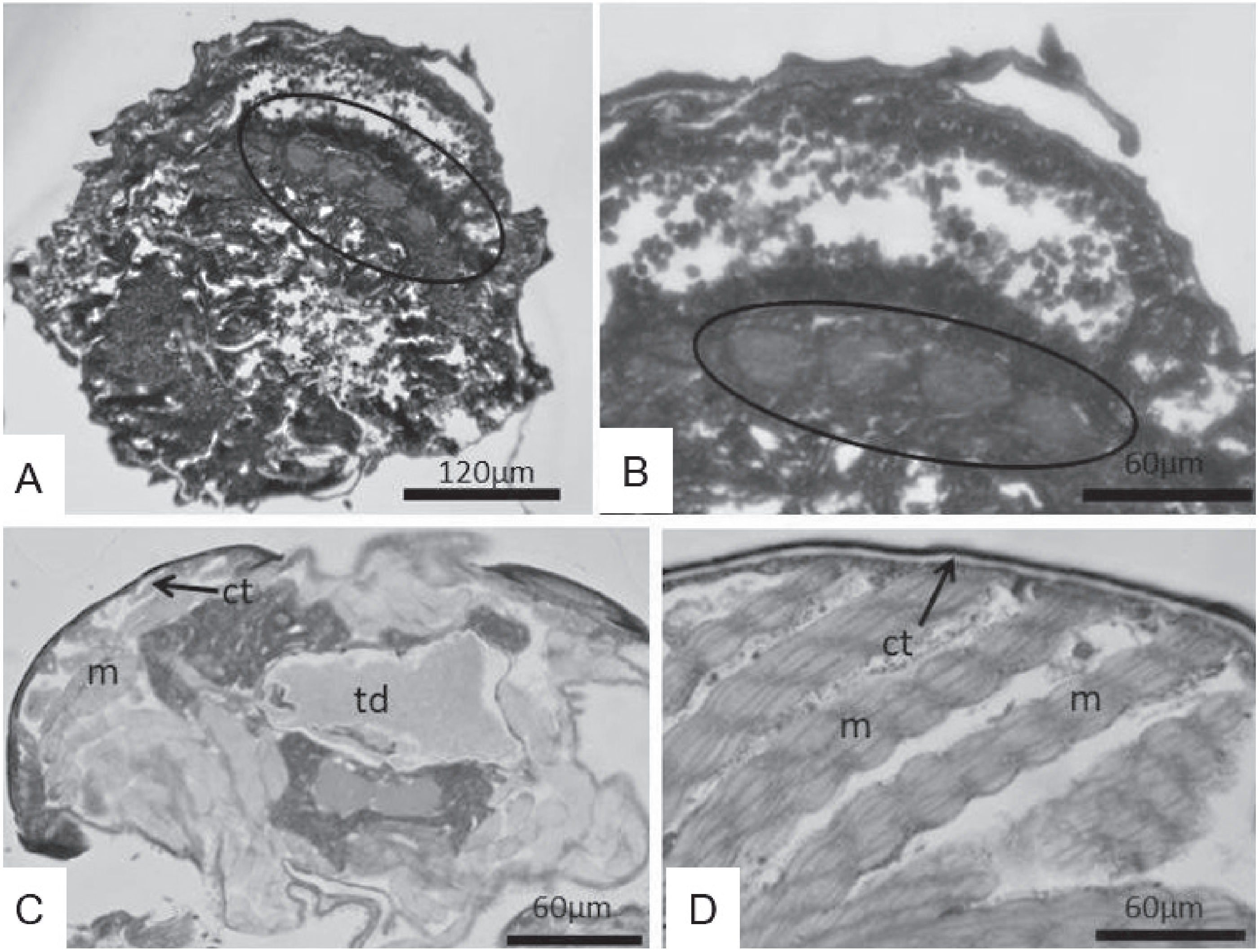

Fig. 3. *Spodoptera frugiperda* eggs treated with methomyl novaluron at 72, 96, 120, and 144 h. (A, B) Embryo showing differentiated regions at 72 and 96 h (circle). (C) Embryo at 120 h showing cuticle (ct), midgut (td), and muscle (m) formation. (D) Embryo at 144 h showing muscle (m) and cuticle (ct) formation.

thrin and chlorantraniliprole + lambda-cyhalothrin. Larval emergence was not observed at 168 h (Fig. 1).

Differences between treatments were found (F = 715.79; df 9, 30; P < 0.001) for insecticides with greater potential to reduce the emergence rate of *S. frugiperda* larvae (Table 2). *Spodoptera frugiperda* emergence was lowest with methomyl + novaluron, chlorantraniliprole + lambda-cyhalothrin, and deltamethrin (Table 2). Among the indoxacarb insecticides, alpha-cypermethrin, novaluron, chlorantranilirprole, chlorfenapyr, and spinosad did not have an ovicidal effect on *S. frugiperda* (Table 2).

EGG HISTOLOGY

The outer egg layer (chorion), which delimits the entire internal content in the control group, was observed in the control at 72 h. The external content showed the development of musculature surrounded by a thick cuticle layer and vitellus (Fig. 2A). At 96 h, *S. frugiperda* embryos showed ganglion masses, indicating the formation of a central nervous system (supraesophageal ganglion), and other tissues in advanced stages of development (Fig. 2B).

The α-cypermethrin treatment showed tissue differentiation within the eggs at 72 h (Fig. 2C). Midgut, cuticle, and musculature formation were observed at 96 h (Fig. 2D) and larval emergence occurred at the same time as in the control group.

Embryos treated with methomyl + novaluron developed a thick cuticle with associated striated muscles and a midgut, and some cuticular sensillae were identified (Fig. 3). Although embryonic development occurred normally, the larvae did not hatch.

Discussion

In the present study, a mixture of methomyl + novaluron, chlorantraniliprole + lambda-cyhalothrin, and deltamethrin (used singly) reduced the emergence of *S. frugiperda* larvae. The results for deltamethrin were similar to those reported by Correia et al. (2013) at 0.002 mL mL^{-1}. The results from the combination of insecticides suggests that interactions among the active ingredients had a stronger effect on *S. frugiperda*, which reduced larval emergence. The synergistic and antagonistic effects of insecticide mixtures may result from several mechanisms. In one such mechanism, the active ingredient in 1 compound may facilitate the penetration of another compound. In another mechanism, 1 active ingredient may affect the active transport of a second ingredient to the target, promoting biotransformation through interaction with monoxygenase and esterase enzymes of cytochrome P450 (Woznica et al. 2001; Cedergreen et al. 2007; Walker 2009; Demkovich et al. 2015). Finally, in the current study, reductions in larval ermergence may have resulted from the low water solubility of methomyl + novaluron, chlorantraniliprole + lambda-cyhalotrin. Therefore, the lipophilic properties of an insecticide may influence penetration of outer egg layers, which are more than 90% protein and coated with wax, and translocation to the site of action (Campbel et al. 2015). Insecticides with higher lipophilicity penetrate the chorion and translocate to the site of action more easily (Moscardini et al. 2013). For example, methomyl mixed with vegetable oil reduced the emergence rate of *Neoleucinodes elegantalis* (Guenée) (Lepidoptera: Crambidae) (Bortoli et al. 2013).

The relationship between egg age and susceptibility differs by insecticide chemical group or target insect (Salkeld & Potter 1953). In the current study, novaluron, chlorantraniliprole, spinosad, chlorfenapyr, alpha-cypermethrin, and indoxacarb probably were less successful at penetrating the egg chorion at 48 h. For example, chlorantraniliprole, spinosad, and chlorfenapyr caused egg mortality in *Spodoptera litura* (Fabricius) (Lepidoptera: Noctuidae) of 24, 19, and 29%, respectively, with higher rates (83, 69, and 66%, respectively) at 24 h (Natikar & Balikai 2015). In addition, indoxacarb had a limited lethal effect on *S. litura* eggs at 24 and 48 hours (Natikar & Balikai 2015). These insecticides, which act on younger eggs, interfered with cuticle formation in embryonic cells and prevented larval emergence (Hamadah & Ghoeim 2017).

Before insecticide exposure, the *S. frugiperda* eggs were 48 h old. Figueiredo et al. (2006) showed that *S. frugiperda* eggs typically hatch with 3 d after oviposition. These data help explain the low penetration rates achieved by the insecticides in *S. frugiperda* eggs at 48 h. Nevertheless, histological changes were observed in embryos treated with α-cypermethrin. Although the methomyl + novaluron treatment inhibited the emergence of *S. frugiperda* larvae, the effects on embryonic development caused by these insecticides still are debatable (Campbel et al. 2016) and need to be clarified.

The toxicity of insecticides with different modes of action may provide efficient control of *S. frugiperda* eggs. Methomyl + novaluron, chlorantraniliprole + lambda-cyhalothrin, and deltamethrin disrupted embryonic development and were lethal for this insect. The results show that these insecticides cause high mortality rates and may be used to effectively manage *S. frugiperda* populations.

A mixture of active ingredients (methomyl + novaluron, chlorantraniliprole + lambda-cyhalothrin) and deltamethrin (used alone) prevented the emergence of *S. frugiperda* larvae and provided effective control of eggs before 72 h.

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