Management of Indian meal moth and maize weevil in stored popcorn using approved grain protectants

by

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Abstract

Spinosad, methoprene, deltamethrin, and deltamethrin plus methoprene, are approved in the United States for treating popcorn. The Indian meal moth, *Plodia interpunctella* (Hübner), and maize weevil, *Sitophilus zeamais* (Motschulsky) are two stored-product insects found in popcorn. The efficacy of spinosad and methoprene against *P. interpunctella* in popcorn were determined in laboratory and field studies. In the laboratory study, eggs (to represent first instars), third, and fifth instars of the laboratory strain of *P. interpunctella* were exposed to 0.7, 1.4, 2.8 ppm methoprene and 1 ppm spinosad treated popcorn, respectively, to assess larval or adult emergence. In the field study, untreated and treated popcorn samples were placed in vinyl mesh pouches with two mesh-opening sizes and were buried 5 cm below popcorn surface. Pouches with large mesh-opening were used to monitor natural insect infestation between May to October, 2017. Pouches with small mesh-opening were used to conduct laboratory bioassays to evaluate adult emergence of *P. interpunctella* from eggs after exposed to treated popcorn. Probe traps, food- and pheromone-baited traps, and sticky traps were used to monitor insects in storage bins and cleaning processing facility.

The laboratory study showed that there was no *P. interpunctella* adults emerged from eggs, third, and fifth instars in methoprene treated popcorn during 6 month storage. However, methoprene did not reduce egg-to-larval survival. Larval and adult emergence in the spinosad treated popcorn was significantly lower than controls. Field study showed that there was no adult emergence in methoprene treated popcorn in most cases, and significantly lower adult emergence in spinosad treated popcorn compare to control. *P. interpunctella* larva was the major insect found in large pouches. The red flour beetle, *Tribolium castaneum* (Herbst), and *P. interpunctella* were primary insect species captured by probe traps, food- and pheromone-baited

traps and sticky traps over the six months' study. These results suggested that methoprene could reduce *P. interpunctella* adult emergence. Spinosad also effectively suppressed the infestation of *P. interpunctella.*

The field strain of *S. zeamais* was exposed to spinosad (1 ppm), methoprene (0.7, 1.4, 2.8 ppm), deltamethrin (0.5, 1.0 ppm), and deltamethrin plus methoprene (0.5+1.25, 1.0+2.5 ppm) treated popcorn, respectively, for 1 to 336 h exposure time. Mortality was assessed at 0, 7, 14, and 21 d after transferring to clean popcorn except for methoprene treatments which only counted mortality at 0 d. Progeny and adult emergence were counted after 42 d incubation in clean or original popcorn, respectively. All insecticides showed no delay toxicity against *S. zeamais* adults. Spinosad caused 100% mortality of *S. zeamais* after 336-h exposure. Complete progeny reduction and highest adult emergence reduction at 168 h exposure. The highest mortality of *S. zeamais* was 67.1 and 70.5% in deltamethrin and deltamethrin plus methoprene treated popcorn, respectively. Methoprene showed limited efficacy against *S. zeamais* including low mortality, progeny reduction and high adult emergence. These results indicated that spinosad was effective against *S. zeamais.* It is necessary to combine other insecticides with deltamethrin and methoprene to control field strain of *S. zeamais* in stored popcorn.

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Chapter 1 - Efficacy of methoprene and spinosad applied to popcorn against immature stages of *Plodia interpunctella*

Abstract

Eggs, third instars, and fifth instars of a laboratory strain of the Indian meal moth, *Plodia interpunctella* (Hübner), were exposed to methoprene treated popcorn at labeled rates of 0.7, 1.4, and 2.8 ppm. The same stages were exposed to popcorn treated with spinosad at the labeled rate of 1 ppm. Eggs were used to represent first instars. Untreated and treated popcorn were evaluated against the immature stages at 0, 1, 3, and 6 months posttreatment. One hundred *P. interpunctella* eggs (< 24 h old), 50 third instars, and 50 fifth instars were added to 100 g of untreated or treated popcorn in containers and held at 28 °C and 65% r.h. Containers infested with eggs were examined after 21 d to determine number of live larvae. Adults emerging from eggs, third instars, and fifth instars were examined 49, 21, and 12 d after infestation, respectively. Damaged kernels were counted following the assessment of live larvae or adults that emerged. The number of live larvae from eggs at 21 d were not significantly different among the four storage times. No *P. interpunctella* adults that emerged from eggs, third instars, and fifth instars on methoprene treated popcorn among the four storage times. The number of live larvae and adults that emerged from eggs, third instars, and fifth instars on spinosad treated popcorn was significantly lower than in the corresponding controls. These results suggested that methoprene was effective in controlling *P. interpunctella* adult emergence from immature stages, but had little impact on live larvae found at 21 d from eggs exposed to methoprene. Spinosad significantly suppressed adult emergence from eggs and third instars but was less effective

against fifth instars. The insecticidal effect of methoprene and spinosad lasted six months on popcorn under laboratory conditions.

Introduction

The Indian meal moth, *Plodia interpunctella* (Hübner), is an external feeder, and a cosmopolitan insect pest of stored products. *P. interpunctella* has been found in various commodities including wheat, maize, millet, rye, barley, rice, and oats (Hagstrum and Subramanyam, 2006). *P. interpunctella* has five instars (Allotey and Goswami, 1990; Perez-Mendoza and Aguilera-Peña, 2004). Grain quality loss by *P. interpunctella* is mainly due to larval feeding, webbing, fecal contamination, and cast skins (Kaliyan et al., 2005). Large number of *P. interpunctella* larvae have been found in stored corn (Arbogast, 2007; Kaliyan et al., 2005; Mbata, 1990; Williams, 1964). The average number of *P. interpunctella* adults that emerged from 100 eggs when incubated at 28 ± 1 ºC, 60 ± 10% r.h., and 14:10 photoperiod for 37 to 42 d were 1.8, 4.3, and 2.3 in whole kernel of dent corn, semi flint corn, and flint corn, respectively (Predojević et al., 2017). Popcorn, is one specialty maize variety, and has become a popular snack, with its production steadily increasing since the 1950s (Soni and Khanorkar, 2014). Vincent et al. (1980) conducted methyl bromide fumigation to kill eggs, larvae, pupae, and adults of *P. interpunctella* in popcorn. All stages of *P. interpunctella* were completely killed after a 4-h exposure to a methyl bromide concentration of 32 g/m^3 at 15.6 ºC.

Methoprene, a juvenile hormone analog, disrupts the development of immature stages by preventing their metamorphosis to adults, and decreases the fecundity of adults that successfully emerge after the treatment (Arthur, 2001; Arthur and Hoernemann, 2004), but does not normally cause adult mortality (Oberlander and Silhacek, 2000). Egg hatchability and adult fecundity of the lesser grain borer, *R.h.yzopertha dominica* (Fabricius), significantly decreased after exposure to methoprene (Chanbang et al., 2008). Chanbang et al. (2008) reported that egg hatchability was 67.5% in untreated rice, and all eggs that hatched developed to adults. However, egg hatchability

was only 40% in rice treated with at 1 ppm, and no adults that emerged from eggs. Similarly, Jenson et al. (2010) showed that *P. interpunctella* adult emergence was 10.8% when eggs in 100-mm Petri dishes were exposed to methoprene aerosol at the labeled rates of 1.07 mg/m^3 for 2 h, compared to an egg hatchability of 72.1% in the control samples. Adult emergence of *P. interpunctella* from larvae was 7.1% in methoprene treated samples and 87.7% in the control treatment.

Spinosad is a commercial fermentation product of the soil actinomycete and the active ingredients are spinosyns A and D (Hertlein et al., 2011). Spinosad binds to nicotinic acetylcholine and gamma amino butyric acid (GABA) receptors in the insect nervous system, causing hyperexcitation and death to insects (Salgado and Sparks, 2005). Spinosad was shown to be effective in controlling *P. interpunctella* immature stages in wheat and corn at 1 ppm (Fang et al., 2002a; Huang et al., 2004; Huang and Subramanyam, 2007). Reduction of *P. interpunctella* adult emergence from eggs was 93 to 100% in four wheat classes, namely hard red winter, hard red spring, soft red winter and durum wheats after exposure to spinosad (Fang et al. 2002a). No *P. interpunctella* larvae that emerged from eggs after exposure for 21 d on spinosad treated corn (Huang and Subramanyam, 2007). Spinosad is currently registered as grain protectant for application to wheat, corn, barley, millets, oats, rice, and sorghum by the United States Environmental Protection Agency (EPA) in 2005 (EPA Reg. No. 264-995).

Currently, there is no published research evaluating the efficacy of methoprene and spinosad against immature stages of *P. interpunctella* in popcorn. Objectives of this study were to determine the efficacy of methoprene and spinosad to control eggs, third instars, and fifth instars of *P. interpunctella* in popcorn, and to evaluate the residual effects of these two chemicals during six months of storage.

Materials and Methods

Insecticides. The liquid formulation of *S*-methoprene (Diacon®-IGR) containing 33.6% active ingredient (a.i.) was obtained from Central Life Sciences (Schaumburg, Illinois, USA). To obtain final methoprene solutions with concentrations of 0.7, 1.4, and 2.8 mg (a.i.)/ml, distilled water was mixed with 1.04, 2.08, and 4.17 ml of Diacon®-IGR liquid, respectively, in a 500-ml volumetric flask. The liquid formulation of spinosad (Sensat™) containing 8.66% active ingredient was obtained from Bayer CropScience (Durham, North Carolina, USA). Sensat™ liquid of 5.77 ml was diluted with distilled water in a 500 ml volumetric flask to obtain the spinosad concentration of 1 mg (a.i.)/ml.

Insect rearing. Cultures of *P. interpunctella* were reared in 0.95-L glass jars with 250 g poultry-mash per jar based diet containing 1 kg poultry-mash, 150 ml glyceryl, 150 ml honey, and 75 ml water (Subramanyam and Cutkomp, 1987). Jars were kept in growth chambers set at 28˚C, 65% r.h., and a photoperiod of 14: 10 (L:D) (Percival Scientific, Inc., Model I-36VL, Perry, Iowa, USA). Eggs laid within 24 h were used in bioassays. To collect third and fifth instars, one hundred eggs were placed in 250 g rearing diet, and third and fifth instars were collected from jars after 12 and 17 d of incubation, respectively.

Hatchability of *P. interpuntella* eggs. One hundred eggs were placed in glass Petri dishes (25 mm diameter × 10 mm height) and kept at 28˚C, 65% r.h., and a photoperiod of 14: 10 (L:D). Number of eggs that hatched was evaluated after 5 d. Egg hatchability was calculated as the percentage of eggs that hatched out of 100 eggs. There were fifteen replications of this test. The mean ± SE hatchability of *P. interpunctella* eggs was 95.1 ± 0.5%.

Popcorn. Yellow Tenderflake popcorn was purchased from Popcorn County, USA (North Loup, Nebraska, USA). Popcorn was frozen for two weeks at -13ºC to kill any live

insects prior to bioassays. Popcorn was tempered to a moisture content of 13.5% (wet basis). Each kilogram of popcorn was treated with 1 ml of methoprene or spinosad solution to obtain the labeled rates of 0.7, 1.4, and 2.8 ppm of methoprene or 1 ppm of spinosad. Popcorn treated with distilled water served as the control treatment. Popcorn was mechanically tumbled for 15 minutes in tempering drums after addition of insecticides or distilled water. Treated and untreated (control) popcorn were placed in clean plastic containers and stored in an environmental growth chamber at 28˚C and 65% r.h. for 1, 3, and 6 months.

Bioassay. One hundred grams of popcorn were placed in separate 0.45-L jars along with 100 *P. interpunctella* eggs (to represent first instars), 50 third instars, or 50 fifth instars. Jars were held at 28˚C 65% r.h. All stages were exposed to popcorn which was treated with methoprene at three concentrations (0.7, 1.4 and 2.8 ppm) and stored for four storage times (0, 1, 3, and 6 months posttreatment). Similarly, they were also exposed to spinosad treated popcorn (1 ppm) at each of the four storage times. Larvae and adults that emerged from eggs were counted after 21 and 49 d of infestation, respectively. In tests with eggs, third instars, and fifth instars, each concentration-storage time combination was replicated three times. Adults that emerged from third and fifth instars were counted after 21 and 12 d of infestation, respectively. Popcorn kernels without the germ portion or with holes in endosperm due to larval feeding were considered damaged. Kernel damage in tests with eggs was assessed after 21 and 49 d, and kernel damage in tests with third instars and fifth instars assessed after 21 and 12 d, respectively.

Data analysis. The number of *P. interpunctella* larvae at 21 d and number of adults emerging from eggs, third instars, and fifth instars are shown in bar graphs created by SigmaPlot 12.5 (Systat Software, San Jose, CA, U.S.). Data on number of live larvae and adults was transformed to log (x+1) scale and subjected to two-way analysis of variance (ANOVA) to

determine the significance of main effects (concentration and storage time) and interactive effect of concentration and storage time at $\alpha = 0.05$ (SAS Institute, 2013).

Damaged kernels in methoprene treatments were calculated as a percentage: (number of damaged kernels ÷ number of total kernels) × 100. The reduction of damaged kernels in spinosad treatments relative to control was calculated as: (1- percentage of damaged kernels in treatment ÷ percentage of damaged kernels in the control treatment) ×100. Data on the reduction of damaged kernels was transformed to angular values to normalize treatment variances prior to analysis (Zar, 1984). Data on the percentage of damaged kernels due to larval development at 21 d and after adult emergence from eggs, third instars, or fifth instars were subjected to two-way ANOVA to determine the significance of main effects (concentration and storage time) and interactive effect of concentration and storage time. One-way ANOVA was used to determine significant differences in damaged kernels among insecticide concentrations for each storage time or among storage times at each concentration. Means were separated by Bonferroni *t* tests at $\alpha = 0.05$ level (SAS Institute, 2013).

Results

***Plodia interpunctella* larvae at 21 d and adults at 49 d in untreated and methoprene treated popcorn.** Two-way ANOVA showed no significant differences in the number of live larvae at 21 d in tests with eggs among concentrations of methoprene, storage times, and the interaction of concentration and storage time (*F*, range = 1.20 – 1.50; df, concentration and storage time = 3, 32; df, interaction = 9, 32; *P*, range = 0.2324 – 0.3296). The mean ± SE of *P. interpunctella* larvae in tests with eggs at 21 d in untreated popcorn ranged from 7.7 ± 2.9 to 17.3 ± 1.8 among four storage times. Correspondingly, the mean ± SE of *P. interpunctella* larvae in

treated popcorn at concentrations of 0.7, 1.4, and 2.8 ppm ranged from 5.3 ± 1.9 to 12.7 ± 4.7, 7.7 ± 2.6 to 21.0 ± 2.1, and 4.3 ± 1.3 to 19.0 ± 4.5, respectively, among the four storage times (Fig. 1.1A).

Two-way ANOVA showed that the number of adults that emerged from eggs was significantly different among concentrations (F = 66.23; df = 3, 32; P < 0.0001), but not among storage times (F = 0.25; df =3, 32; P = 0.8626). The interaction of concentration and storage time also was not significant (F = 0.25; df = 9, 32; P = 0.9839). The mean ± SE of *P. interpunctella* adults that emerged from eggs ranged from 0.7 ± 0.3 to 4.3 ± 0.3 in untreated popcorn, while no adults that emerged in methoprene treated popcorn at each of the four storage times (Fig. 1.1B). Two-way ANOVA on the number of adults that emerged from third instars was significant among concentrations of methoprene (F = 198.33; df = 3, 32; P < 0.0001), but not among storage times (F = 0.13; df = 3, 32; P = 0.9431). The interaction of concentration and storage time was not significant (F = 0.13; df = 9, 32; P = 0.9986). The mean ± SE of *P. interpunctella* adults that emerged from third instars was 7.3 ± 1.2 to 15.7 ± 4.3 in untreated popcorn among the four storage times. No adults that emerged in methoprene treated popcorn (Fig. 1.1C). Similar results were found for fifth instars after exposure to methoprene. Two-way ANOVA showed a significant effect among concentrations (F = 1999.41; df = 3, 32; P < 0.0001), but not among storage times (F = 0.18; df = 3, 32; P = 0.9112). The interaction of concentration and storage time also was not significant (F = 0.18; df = 9, 32; P = 0.9952). No adults that emerged from fifth instars on methoprene treated popcorn, but a mean ± SE of 46.7 ± 1.9 to 49.7 ± 0.3 adults that emerged in untreated popcorn among the four storage times. (Fig. 1.1D).

***Plodia interpunctella* larvae at 21 d and adults at 49 d in untreated and spinosad treated popcorn.** Two-way ANOVA indicated that the number of live larvae in tests with eggs

at 21 d, and adults that emerged from eggs, third, and fifth instars at 49, 21 and 12 d, respectively, were significant between 0 and 1 ppm of spinosad (F, range = 69.18 – 259.88; df = 1, 16; $P < 0.0001$). However, differences were not significant among storage times (F, range = 0.37 – 1.26; df = 3, 16; P, range = 0.0551 – 0.9063), except for in the number of adult that emerged from eggs ($F = 6.39$; df = 3, 16; $P = 0.0047$). The interactions were all not significant in the number of live larvae in tests with eggs at 21 d, and number of adults that emerged from eggs, third instars, and fifth instars (F, range = 0.34 – 3.13; df = 3, 16; P, range = 0.0551 – 0.7940). The mean ± SE of *P. interpunctella* larvae after 21 d in untreated and spinosad treated popcorn ranged from 8.3 ± 0.8 to 19.3 ± 4.4, and 0.0 ± 0.0 to 1.7 ± 0.9, respectively, among the four popcorn storage times (Fig. 1.2A). The mean ± SE of *P. interpunctella* adults that emerged from eggs, third instars, and fifth instars in the untreated popcorn ranged from 3.7 ± 0.3 to 5.0 ± 1.0, 7.3 ± 1.5 to 14.0 ± 1.5, and 43.3 ± 0.7to 48.7 ± 0.7, respectively, among the four popcorn storage times. The mean ± SE of *P. interpunctella* adults that emerged from eggs, third instars, and fifth instars in spinosad-treated popcorn ranged from 0.0 ± 0.0 to 1.3 ± 0.3, 1.0 ± 0.58 to 3.7 ± 0.9 and 29.7 ± 3.5 to 36.7 ± 3.3, respectively (Fig. 1.2B, Fig. 1.2C, Fig. 1.2D).

Damaged kernels in untreated and methoprene treated popcorn. The percentage of damaged kernels produced by live larvae in tests with eggs at 21 d and adults at 49 d in methoprene treated popcorn is shown in Table 1.1. Two-way ANOVA indicated no significant differences in percentage of damaged kernels among methoprene concentrations (F, range = 0.34 – 1.28; df =3, 32; P, range = 0.2973 – 0.7971), storage times (F_{range} = 1.19 – 1.39; df =3, 32; P, range = 0.2636 – 0.3307), and the interaction of concentration and storage time (F, range = 0.84 – 1.88; df =9, 32; P, range = 0.0913 – 0.5889). The mean ± SE percentage of damage caused by larvae at 21 d among the four storage times ranged from 2.7 ± 0.7 to 5.2 ± 0.5%, 1.7 ± 0.2 to 4.4

± 1.1%, 2.4 ± 0.5 to 5.7 ± 0.6%, and 1.8 ± 0.2 to 6.0 ± 1.3%, at methoprene concentrations of 0, 0.7, 1.4, and 2.8, respectively. The percentage of damaged kernels at 49 d in tests with eggs after adult emergence among the four storage times ranged from 3.9 ± 0.2 to 6.6 ± 0.4%, 3.9 ± 0.5 to 7.2 ± 1.1%, 3.2 ± 0.3 to 7.3 ± 0.2%, and 3.5 ± 3.2 to 7.3 ± 0.9% at methoprene concentration of 0, 0.7, 1.4 and 2.8 ppm, respectively (Table 1.1).

The percentage of damaged kernels after emergence of *P. interpunctella* adults from third and fifth instars in methoprene treated popcorn after 21 d and 12 d of exposure are shown in Tables 1.2 and 1.3, respectively. One-way ANOVA indicated that the percentage of damaged kernels after emergence of adults from third instars was significantly different among the four storage times in methoprene treated popcorn at all concentrations except the control treatment. Damaged kernels slightly increased as popcorn storage time increased. There were no significant differences among four concentrations at each storage time (F, range = 0.87 – 3.95; df =3, 8; P, range = 0.0535 – 0.4944).

One-way ANOVA showed that the percentage of damaged kernels after emergence of adults from fifth instars was significantly different among the four concentrations at 3 and 6 months of storage (Table 1.3). Methoprene treated popcorn had similar percentage of damaged kernels in 0 and 1 month popcorn samples among the four concentrations. Damaged kernels in methoprene treated popcorn stored for 3 and 6 months was lower than in damage in 0 and 1 month aged samples. At 0.7 and 2.8 ppm, damaged kernels were significantly different among storage times at each concentration. The lowest percentage of damaged kernels occurred in 3 month-old popcorn at 0.7 and 3.8 ppm. Generally, there were slightly less damaged kernels in treated popcorn compared to controls.

Damaged kernels in untreated and spinosad treated popcorn. The percentage of damaged kernels at 21 d caused by *P. interpunctella* larvae and at 49 d after adult emergence from eggs in untreated and spinosad treated popcorn are shown in Table 1.4. Results of two-way ANOVA of damaged kernels at 21 and 49 d were similar. Results indicated a significant effect of concentration (F, range = 124.55– 145.44; df = 1, 16; $P < 0.0001$), but no significant effect among storage times (F, range = 0.29 – 0.77; df = 3, 16; P, range = 0.5265 - 0.8316), or the interaction of concentration and storage time (F, range = 0.22 – 2.18; df = 3, 16; P, range = 0.1301 – 0.8805). The mean ± SE percentage of damaged kernels at 21 d due to larval development in spinosad treated popcorn ranged from 0.1 ± 0.1 to 1.1 ± 0.4% among the four storage times, and in untreated popcorn mean ± SE percentage of damaged kernels ranged from 2.1 ± 0.2 to 4.2 ± 1.0%. The reduction in damaged kernels in spinosad-treated popcorn relative to that in the control treatment was > 70% across all storage times, and the highest reduction observed was 97.3% in 6-month old popcorn ($F = 4.94$; df = 3, 8; $P = 0.0315$) (Table 1.4). The mean ± SE percentage of damaged kernels at 49 d after emergence of adults from eggs among the four storage times ranged from 0.3 ± 0.1 to 0.8 ± 0.1% and 3.6 ± 0.4 to 6.3 ± 0.9% in treated and untreated popcorn, respectively. The reduction of damaged kernels at 49 d in spinosad treatments relative to damage in the controls, among the four storage times was 83.7 ± 4.4% to 94.6 ± 1.6% (Table 1.4). The reduction in damaged, however, was not significant among the four storage times ($F = 3.95$; df = 3, 8; $P = 0.0538$).

The percentage of damaged kernels after emergence of *P. interpunctella* adults from third and fifth instars in untreated and spinosad treated popcorn are shown in Table 5. Two-way ANOVA results showed no significant effect between 0 and 1 ppm of spinosad (F, range = 3.27 – 3.54; df = 1, 16; P, range = 0.0784 – 0.0893). Similarly, the percentage of damaged kernels

were not significantly different among the four storage times (*F*, range = 0.65 – 1.48; df = 3, 16; *P*, range = 0.2567 – 0.5976). The interaction of concentration and storage time also was not significant (*F*, range = 1.01 – 2.81; df = 3, 16; *P* = 0.0730 – 0.4134). The reduction of damaged kernels after emergence of adults from third instars and fifth instars ranged from -15.3 ± 11.5 to 22.2 ± 12.6%, and 1.6 ± 0.8 to 22.1 ± 6.5%, respectively, but differences were not significant among storage times (*F*, range = 1.12 – 3.74; df = 3, 8; *P*, range = 0.0602 – 0.3954) (Table 1.5). The negative values suggest that there were more damaged kernels in spinosad treated popcorn compared to untreated popcorn.

Discussion

No *P. interpunctella* adults emerged from eggs, third, or fifth instars in methoprene treated popcorn. However, the number of live larvae found at 21 d in tests with eggs in methoprene treated popcorn was not significantly different than in control among the four storage times. Methoprene has been tested on several stored-product insects (McGregor and Kramer, 1975; Athanassiou et al., 2010; Wijayaratne et al., 2012a, b). Methoprene disrupts the development of *P. interpunctella* immature stages in wheat and corn (McGregor and Kramer, 1975). McGregor and Kramer (1975) applied methoprene in wheat or corn at the concentration of 2 ppm to control *P. interpunctella* immature stages at 27 ± 2ºC and 60 ± 5% r.h. They found that the progeny of adults that emerged from second or third instar ranged from 185 to 496 in control samples, and only extra-large larvae were observed in treatments. Athanassiou et al. (2010) showed that methoprene reduced adult progeny of *Liposcelis bostrychophila* Badonnel, *Liposcelis decolor* (Pearman), *Liposcelis entomophila* (Enderlein), *Liposcelis paeta* Pearman (Psocoptera: Liposcelididae), and *Lepinotus reticulatus* Enderlein (Trogiidae) when applied at

concentrations of 5 and 10 ppm in wheat, rice, and corn. Progeny of these five species of psocids were 53.7 to 155.3, 3.9 to 8.3, and 3.7 to 7.9 in 0, 5 and 10 ppm methoprene treated wheat, respectively. In another study, adult emergence of the red flour beetle, *Tribolium castaneum* (Herbst), from late-instars in 0.033 ppm methoprene treated wheat was 4.6% whereas in the control treatment it was 94.1% (Wijayaratne et al., 2012a).

In our study, methoprene residues on popcorn persisted at all three concentrations for 6 months and provided control of *P. interpunctella* in popcorn at all tested concentrations. The residue of methoprene can last for 2 years at ambient conditions in a grain bin (Arthur, 2016). Arthur (2016) reported that there was no progeny of *R. dominica*, and significantly lower progeny of the Angoumois grain moth, *Sitotroga cerealella* (Oliver), in methoprene treated (1.25 and 2.5 ppm) brown rice that was sampled every two months from the grain bin for 24 months. In the control samples, progeny production of *R. dominica* and *S. cerealella* were 154.2 to 1229.6 and 65.4 to 260.5 adults over 24 months, respectively. Daglish et al. (1995) found that chlorpyrifos-methyl plus methoprene residues caused 100% mortality, and over 99% progeny reduction of the maize weevil, *Sitophilus zeamais* Motschulsky in corn which was stored in concrete silos for 29 weeks. Similarly, the current study indicated that methoprene residue in popcorn lasted for 6 months, since no *P. interpunctella* adults that emerged in methoprene treated popcorn.

Our results demonstrated that spinosad reduced *P. interpunctella* egg-to-larval survival, and was more effective in controlling egg-to-adult emergence in popcorn compared to methoprene. However, spinosad did not completely prevent adults emerging from third or fifth instars. A previous study reported that reduction of egg-to-larval survival and egg-to-adult emergence of *P. interpunctella* in spinosad treated pistachio nuts (1 ppm) was greater than 90%,

but less than 100% (Mollaie et al., 2011). Subramanyam et al. (1999) reported that number of adults that emerged from 100 *P. interpunctella* eggs were 34.0 and 0.2 in untreated wheat and wheat treated with 1 ppm, respectively.

Our study indicated that third instars were more susceptible to spinosad than fifth instars in popcorn. Subramanyam (1984) studied that efficacy of *Bacillus thuringiensis*, malathion, and pirimiphos-methyl against *P. interpunctella* larvae, and found that the insecticidal effect was related to body weight of instars. He concluded that the LD_{50} values of *P. interpunctella* fifth instars were 7-11 fold higher than that of third instars. Correspondingly, the body weight of fifth instars was 5 fold higher than third instars. Our results are consistent with this inference.

Spinosad residues were stable in popcorn for 6 months. Reduction of larval and adult emergence was significant in spinosad-treated popcorn after 6 months of storage compared to control. Other studies reported that the residues of spinosad on wheat and corn can last for 6 months to 2 years (Fang et al., 2002b; Szabela, 2005; Subramanyam et al., 2007). Subramanyam et al. (2007) applied spinosad at the concentration of 1 ppm to 60-120 metric tons of hard red winter wheat held in round metal bins on three Kansas farms between July 2002 and January 2003. There were no live adults of *R. dominica* or the rusty grain beetle, *Cryptolestes ferrugineus* (Stephens) in spinosad treated wheat between August 2002 and January 2003 compared to 0.04 to 4.6 live adults per kilogram of untreated wheat.

Damaged popcorn kernels were mainly caused by *P. interpunctella* larvae, and especially fifth instars caused the most damage to kernels. Spinosad treated popcorn had significantly lower damaged kernels due to egg-to-larval survival and egg-to-adult emergence. More damaged kernels were observed when higher number of larvae or adults were present. Huang and Subramanyam (2007) showed that number of damaged kernels after emergence of *P.*

interpunctella adults from eggs in spinosad treated corn (0.1 – 2.0 ppm) was 0 to 0.2 per 100 g corn, while controls had 73.8 per 100 g corn. Huang et al. (2007) reported that number of damaged kernels at 21 d in spinosad treated wheat (0.1 – 1.0 ppm) was 0, while controls showed 3.3 per 100g of wheat. In the same study, the number of damaged kernels after emergence of *P. interpunctella* adults from eggs in spinosad treated wheat (0.1 – 1.0 ppm) was 0 to 2.7, while in the control it was 35.7.

Our study revealed that methoprene reduced adult emergence of *P. interpunctella.* However, it did not affect larval development in popcorn. Thus, damaged kernels in methoprene treated popcorn were present even when adult emergence from immature stages was zero.

In conclusion, methoprene achieved complete control of *P. interpunctella* adult emergence from eggs, third, and fifth instars in stored popcorn. However, methoprene had limited effect on *P. interpunctella* larval development from eggs at 21 d. Damaged kernels in methoprene treated popcorn was not significantly different compared to untreated popcorn among the four storage times. Spinosad can cause a significant reduction of *P. interpunctella* larval survival from eggs, and adult emergence from eggs and third instars. Spinosad was less effective against fifth instars, and more adults emerged from fifth instars compared to adults that emerged from eggs and third instars in treated popcorn. Reduction of damaged kernels in spinosad treated popcorn was significant at 21 d in tests with eggs and at 49 d after emergence of adults from eggs. Reduction in damaged kernels after emergence of adults from third and fifth instars was not significant. Methoprene and spinosad residues were persistent for 6 months in popcorn. Our results suggest that methoprene and spinosad are effective grain protectants to control *P. interpunctella* immature stages in popcorn.

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Table 1.1. Mean ± SE percentage of damaged kernels at 21 d and 49 d in tests with *P. interpunctella* eggs exposed to untreated and methoprene treated popcorn[a].

Storage time (month)	21 d (live larvae)				49 d (after adult emergence)			
	0 ppm	0.7 ppm	1.4 ppm	2.8 ppm	0 ppm	0.7 ppm	1.4 ppm	2.8 ppm
0	2.7 ± 0.7	1.7 ± 0.2	2.4 ± 0.5	1.8 ± 0.2	3.9 ± 0.2	3.9 ± 0.5	5.0 ± 1.0	3.5 ± 3.2
1	5.2 ± 0.5	4.4 ± 1.1	5.7 ± 0.6	6.0 ± 1.3	5.3 ± 1.0	7.2 ± 0.4	6.5 ± 0.3	7.3 ± 0.9
3	3.9 ± 0.6	3.6 ± 1.0	3.2 ± 0.4	3.6 ± 0.9	6.6 ± 0.4	7.2 ± 1.1	7.3 ± 0.2	7.1 ± 0.4
6	3.2 ± 0.5	3.6 ± 0.2	3.0 ± 0.4	2.2 ± 0.5	4.1 ± 0.5	5.7 ± 1.0	3.2 ± 0.3	4.8 ± 0.9

[a]Each mean is based on $n = 3$.

Table 1.2. Mean ± SE percentage of damaged kernels after adult emergence of adults in tests with *P. interpunctella* third instars exposed to untreated and methoprene treated popcorn[a].

Storage time (months)	0 ppm	0.7 ppm	1.4 ppm	2.8 ppm	*F*-value (df)	*P*-value[b]
0	8.3 ± 1.9	4.7 ± 0.2a	4.1 ± 0.7a	5.3 ± 0.3a	3.95 (3,8)	0.0535
1	8.3 ± 1.2	6.8 ± 0.2ab	6.5 ± 0.7ab	6.2 ± 0.5ab	1.64 (3,8)	0.2548
3	5.5 ± 0.3	6.0 ± 1.2bc	5.2 ± 0.4b	6.5 ± 0.3c	1.04 (3,8)	0.4257
6	5.9 ± 1.0	6.1 ± 0.4c	5.3 ± 0.3ab	6.0 ± 0.7bc	0.87 (3,8)	0.4944
F-value (df)	0.07 (3,8)	24.13 (3,8)	6.26 (3,8)	11.30 (3,8)		
P-value	0.9755	0.0002*	0.0171*	0.0030*		

[a]Each mean is based on $n = 3$.

[b]At each storage time, there was no significant difference among the concentrations ($P > 0.05$).

*Means followed by different lower-case letters are significantly different among four storage times at the same concentration ($P < 0.05$; Bonferroni *t* test).

Table 1.3. Mean ± SE percentage of damaged kernels after emergence of adults in tests with *P. interpunctella* fifth instars exposed to untreated and methoprene treated popcorn[a].

Storage time (month)	0 ppm	0.7 ppm	1.4 ppm	2.8 ppm	*F*-value (df)	*P*-value
0	14.7 ± 1.2	16.4 ± 2.5a	15.0 ± 3.2	13.8 ± 0.3ab	0.37 (3,8)	0.7796
1	15.5 ± 0.7	17.2 ± 2.1a	14.8 ± 1.8	17.4 ± 0.4a	0.45 (3,8)	0.7256
3[b]	8.2 ± 0.6A	4.8 ± 0.6bB	6.1 ± 0.2AB	6.5 ± 0.7bAB	5.98 (3,8)	0.0193
6[b]	11.2 ± 0.6A	8.7 ± 0.7abAB	6.8 ± 0.3B	10.3 ± 0.4abA	10.23 (3,8)	0.0041
F-value (df)	0.02 (3,8)	5.34 (3,8)	3.32 (3,8)	6.28 (3,8)		
P-value	0.9962	0.0259*	0.0778	0.0169*		

[a]Each mean is based on $n = 3$.

[b]Means followed by different upper-case are significantly different among four concentrations at the same storage time ($P < 0.05$; Bonferroni *t* test).

*Means followed by different lower-case letters are significantly different among four storage times at the same concentration ($P < 0.05$; Bonferroni *t* test).

Table 1.4. Mean ± SE percentage of damaged kernels at 21 d and 49 d in tests with *P. interpunctella* eggs exposed to untreated and spinosad treated popcorn[a].

Storage time (month)	21 d (live larvae)			49 d (after adult emergence)		
	0 ppm	1 ppm	Reduction of damaged kernels (%)*	0 ppm	1 ppm	Reduction of damaged kernels (%)
0	2.7 ± 0.1	0.2 ± 0.1	90.9 ± 2.3ab	3.8 ± 0.6	0.6 ± 0.2	83.7 ± 4.4
1	4.2 ± 1.0	1.1 ± 0.4	74.5 ± 8.7b	5.7 ± 0.7	0.8 ± 0.1	85.7 ± 1.1
3	2.7 ± 0.8	0.5 ± 0.1	83.0 ± 4.2ab	6.3 ± 0.9	0.3 ± 0.1	94.6 ± 1.6
6	2.1 ± 0.2	0.1 ± 0.1	97.3 ± 2.7a	3.6 ± 0.4	0.3 ± 0.1	91.3 ± 1.7

[a]Each mean is based on $n = 3$.

*Means followed by different lower-case letters are significantly different among storage times ($F = 4.94$; df= 3,8; $P = 0.0315$; one-way ANOVA and Bonferroni t test).

Table 1.5. Mean ± SE percentage of damaged kernels after emergence of adult sin tests with *P. interpunctella* third and fifth instars exposed to untreated and spinosad treated popcorn[a].

	Third instars to adults			Fifth instars to adults		
Storage time (m)	0 ppm	1 ppm	Reduction of damaged kernels (%)	0 ppm	1 ppm	Reduction of damaged kernels (%)
0	3.9 ± 0.3	3.4 ± 0.5	13.6 ± 12.6	8.2 ± 0.2	7.6 ± 0.3	7.4 ± 4.4
1	4.6 ± 0.3	5.3 ± 0.5	–15.3 ± 11.5[b]	8.1 ± 0.2	6.3 ± 0.5	22.1 ± 6.5
3	4.3 ± 0.1	3.4 ± 0.6	22.2 ± 12.6	7.7 ± 0.9	7.6 ± 0.1	1.6 ± 0.8
6	6.1 ± 0.7	5.1 ± 0.4	17.0 ± 6.6	10.4 ± 0.5	10.2 ± 0.4	3.7 ± 2.7

[a]Each mean is based on $n = 3$.

[b]Negative value is a result of having more damaged kernels in spinosad treated popcorn compared to untreated popcorn.

Figure 1.1. Mean ± SE number of live larvae from eggs at 21 d (A), adults that emerged from eggs (B), third instars (C) and fifth instars (D) after 49 d, 21 d, and 12 d respectively, in untreated and methoprene treated popcorn.

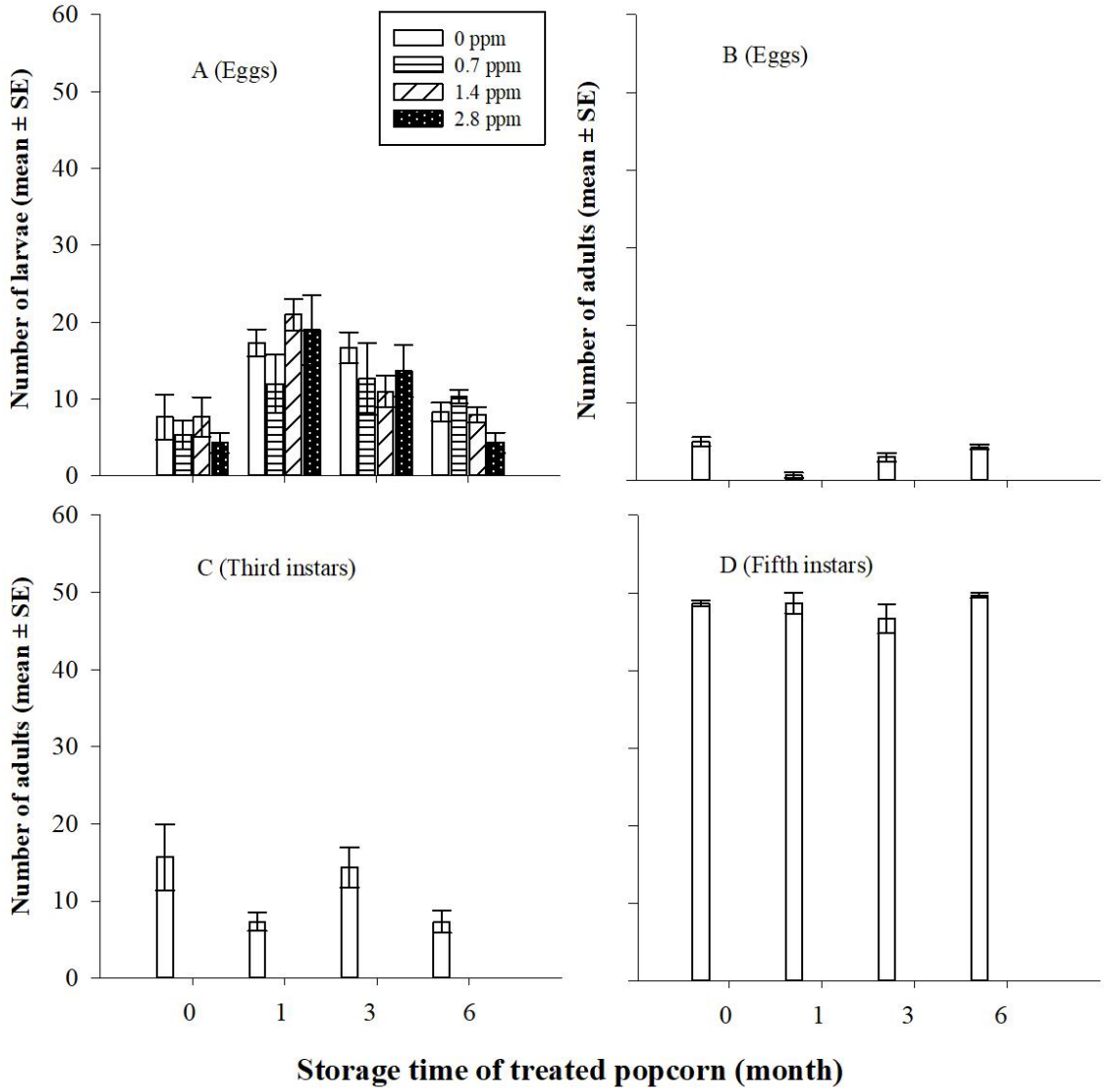
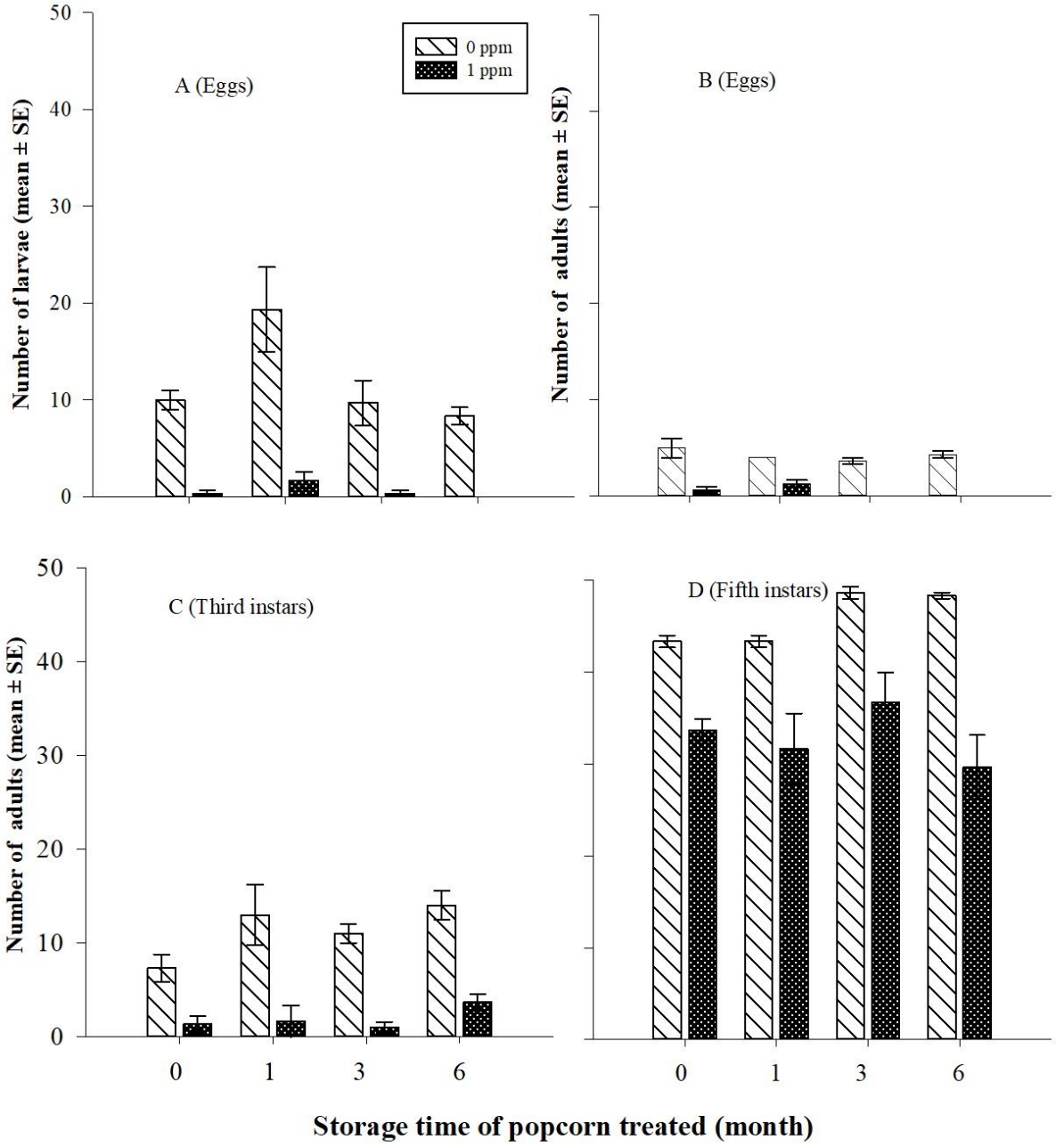


Figure 1.2. Mean ± SE number of live larvae from eggs at 21 d (A), adults that emerged from eggs (B), third instars (C) and fifth instars (D) after 49 d, 21 d, and 12 d respectively, in untreated and spinosad treated popcorn.



Chapter 2 - Persistence and efficacy of methoprene and spinosad residues against on *Plodia interpunctella* in stored popcorn

Abstract

The efficacy and persistence of methoprene and spinosad applied to popcorn was evaluated in commercial popcorn storage bins. Popcorn was treated and bagged in the laboratory and placed in fully filled popcorn storage bin to evaluate the efficacy and persistence over a six month period. Methoprene was applied at the concentrations of 0.7, 1.4 and 2.8 ppm, and spinosad was applied at 1 ppm. Untreated and treated popcorn were placed in vinyl mesh pouches (250g each) with two opening sizes and buried 5 cm below popcorn surface. Pouches with large mesh-openings were used to monitor natural infestations. Pouches with small mesh-opening were used in laboratory bioassays to evaluate adult emergence of *Plodia interpunctella* (Hübner) from eggs at 28 °C and 65% r.h. Probe traps, food- and pheromone-baited traps, and sticky traps were placed in storage bins and processing facility to captured insects. Temperature and relative humidity during six months of storage ranged from 4.6 to 29.9°C and 62.1 to 73.5%, respectively. Moisture content of popcorn was 12.6 to 13.5%. Adults of *P. interpunctella* did not emerge in methoprene treated popcorn over the 6 month study except in September and October only in methoprene treatment of 0.7 ppm. Percentage of damaged kernels in methoprene treated popcorn were similar among control and three tested concentrations for each month. In spinosad treated popcorn, adult emergence and percentage of damaged kernels were significantly less compared to control for each month. In large mesh-opening pouches, *P. interpunctella* larvae were the predominant insect. The red flour beetle, *Tribolium castaneum* (Herbst), and *P. interpunctella* were the dominant species captured by probe traps, food- and pheromone-baited

traps and sticky traps. These results indicated that the persistence and efficacy of methoprene and spinosad lasted for least six months under field conditions.

Introduction

Popcorn is distinguishably different from other corn types due to its unique kernel shape and size, and popping ability when heated (Ziegler, 2003). Popcorn was one of the earliest cereal snacks (Rooney and Serna-Saldivar, 1987) and had become a commercial commodity in the United States since 1890s (Brunson and Richardson, 1958). Moisture content, storage temperature, and insect pests are major concerns for popcorn storage in bins (Ziegler, 2003). *Plodia interpunctella* (Hübner), the Indian meal moth, is an external feeder, and one wide-spread stored-product insect pest. *P. interpunctella* had been found in various commodities including wheat, corn, millet, rye, barley, rice, and oats (Hagstrum and Subramanyam, 2006.) The quality loss of commodities is primarily due to *P. interpunctella* larval feeding and webbing (Kaliyan et al., 2005). Large number of *P. interpunctella* larvae were found in stored corn (Williams, 1964; Mbata, 1990; Kaliyan et al., 2005; Arbogast, 2007). The mean fecundity of *P. interpunctella* was 227.7 at 25ºC when they were reared in cracked corn (Arbogast, 2007).

Methoprene, a juvenile hormone analog, disrupts the development of insects by preventing their metamorphosis to adults, and reduces the fecundity of surviving adults (Arthur, 2001; Arthur and Hoernemann, 2004), but does not cause adult mortality (Oberlander and Silhacek, 2000). Mian and Mulla (1982) reported that the mortality of the lesser grain borer, *Rhyzopertha dominica* (Fabricius) was over 98% after exposure to methoprene (1 to 10 ppm) treated wheat, barley and corn for more than 12 months. Another study from Manzelli (1982) proved that methoprene residues in tobacco caused zero adult emergence of the cigarette beetle, *Lasioderma serricorne* (Fabricius) for 48 months. Arthur (2004) found no progeny production of *R. dominica* in methoprene treated wheat after the 24-week incubation at 22ºC.

Spinosad (1 ppm) was another commercial insecticide approved as grain protectant for application to wheat, corn, barley, millets, oats, rice and sorghum (EPA Reg. No. 264-995). Spinosad residues were highly stable over several months on grains stored in bins (Daglish and Nayak, 2006; Vayias et al., 2010). Mortality of *R. dominica* was 100% in treated wheat, corn and barely after 6 months. Daglish and Nayak (2006) reported that the concentration of spinosad in wheat decreased by 30% after a 9-month storage at 30°C at relative humidity levels of 55 and 75% in laboratory tests. They observed 100% mortality and zero progeny of *R. dominica* after 9 months. Subramanyam et al. (2007) evaluated the persistence of spinosad (1 ppm) in wheat stored bins on three Kansas farms. There were less than 3 live adults/kg of the red flour beetle, *Tribolium castaneum* (Herbst), rusty grain beetle, *Cryptolestes ferrugineus* (Stephens), and sawtoothed grain beetle, *Oryzaephilus surinamensis* (L.), in six months. Vayias et al. (2010) reported the efficacy of spinosad (1 ppm) against the rice weevil, *Sitophilus oryzae* (Linnaeus) and *R. dominica* in wheat, corn and barley for 6 months at 25°C and 65% r.h. Mortality of *S. oryzae* was 90–100% during the first four months of storage, and 80% by the end of six months.

Methoprene and spinosad are commercially available, and were tested on various grains and raw commodities. However, there is lack of data on the efficacy of these chemicals on popcorn. The objectives of this study were to determine the stability of methoprene and spinosad residues in popcorn stored in steel bins for six months through laboratory bioassays, and to monitor insect infestation in popcorn storage bins.

Materials and Methods

Preparation of insecticides. Diacon®-IGR liquid formulation containing 33.6% of *S*-methoprene was obtained from Central Life Sciences (Schaumburg, IL, USA). To obtain final

methoprene solutions with methoprene concentrations of 0.7, 1.4, and 2.8 mg/ml, distilled water was mixed with 1.04, 2.08, and 4.17 ml IGR liquid, respectively, in a 500 ml volumetric flask. Sensat^{TM} liquid formulation containing 8.66% of spinosad was obtained from Bayer CropScience (Durham County, NC, USA). Sensat^{TM} liquid of 5.77 ml was mixed with diluted by water in a 500 ml volumetric flask to obtain the final solution with a spinosad concentration of 1 mg/ml.

***P. interpunctella* rearing and hatchability.** *P. interpunctella* has been reared in the Department of Grain Science and Industry, Kansas State University for more than 10 years. Cultures of *P. interpunctella* were kept in 0.95-L glass jars containing 250 g of poultry mash based diet consisting of 1 kg poultry mash, 150 ml glyceryl, 150 ml honey, and 75 ml water (Subramanyam and Cutkomp, 1987). Jars were placed in growth chambers set at 28˚C, 65% r.h., and a photoperiod cycle of 14: 10 (L:D). Eggs used in bioassays were collected within 24 h prior to the tests. The average hatchability of *P. interpunctella* eggs was 95.1 ± 0.47% (n = 15) under laboratory conditions.

Popcorn treatment and handling. Yellow Tenderflake popcorn was purchased from Popcorn County, USA (North Loup, NE, USA). Popcorn was frozen for two weeks at -13ºC prior to bioassays to kill any live insects present. Popcorn were tempered to obtain 13.5% moisture content. One kilogram popcorn was treated with 1 ml of final solutions of methoprene (0.7, 1.4, and 2.8 mg/ml), spinosad (1 mg/ml) or distilled water (control), respectively. Popcorn was mechanically tumbled for 15 minutes in tempering drums after addition of insecticide solutions. Treated popcorn of 250 g was placed in 15 cm × 15 cm vinyl mesh pouches of two mesh-openings sizes, respectively. The mesh-opening sizes were 2 mm × 2 mm (large) and 0.6 mm × 0.6 mm (small). Pouches with large mesh openings were used to monitor natural insect infestations in popcorn stored in steel bins since most stored insect species can go through large-

mesh openings. Pouches with small mesh-opening were used to conduct laboratory bioassays, since adults cannot go through 0.6 mm × 0.6 mm openings.

Three steel bins at North Loup, Nebraska, USA were selected for the field study. The size of one bin was 5.6 m (diameter) × 4.9 m (height), with the capacity of 95 metric tons. The other two bins were 9.4 m (diameter) × 6.5 m (height) with 360 metric tons capacity. Bins were made from galvanized ribbed steel and their surfaces were leveled at the eaves. Each bin was divided into six locations for placement of pouches. At each location, one pouch with large mesh-opening and one pouch with small mesh-opening of each treatment, were placed horizontally 5 cm below the popcorn surface. Each location had a total 12 pouches. HOBO® data loggers (Onset Computer Corporation, Bourne, MA), were placed in small mesh-opening pouches containing 0.7 ppm methoprene treated popcorn at each location per bin. Pouches were placed in bins in May 2017 and retrieved monthly from May to October 2017.

Efficacy of methoprene and spinosad residues. Popcorn recovered from pouches with big mesh-opening were sifted through a 2.1-mm round-holed sieve to separate insects. Insects were identified and counted. Kernels without germs and endosperm with feeding holes were considered as damaged. Number of damaged kernels per sample was counted in 250g popcorn.

One hundred grams popcorn from each small mesh-opening pouch were transferred to one 0.45-L glass jar. Each jar was inoculated with *P. interpunctella* 100 eggs (< 24 h) and was capped with wire mesh and filter paper lids. Jars were held in the environmental growth chamber at 28°C, and 65% r.h., with a photoperiod of 14: 10 (L:D). Number of adults that emerged from eggs were counted after 49 d of incubation. Damaged kernels were counted after adult emergence.

Probe traps, food and pheromone baited traps and sticky traps handling. Insect species from the surface layer of popcorn in each bin were monitored by inserting ten perforated plastic probe traps (Storgard WB II traps, Trécé, Salinas, CA) 5 cm below popcorn surface. Two probe traps placed in the center of each bin, one trap near the periphery in each cardinal (N, S, W, and E) direction, and one trap at the halfway of each cardinal direction. Traps were emptied biweekly from May to October 2017. One sticky trap with Indian meal moth pheromone lure (Storgard II traps, Trécé, Salinas, CA) was hanged 0.9 m (3 ft) above the grain surface and at the center of each bin. Traps were checked biweekly from May to October 2017.

Insects in popcorn cleaning facility were monitored by ten food- and pheromone-baited traps with red flour beetle/ confused flour beetle, and khapra beetle/warehouse beetle lures (DOME^{TM} traps, Trécé, Salinas, CA) and ten sticky traps with Indian meal moth pheromone lure (Storgard II traps, Trécé, Salinas, CA). Ten food- and pheromone-baited traps and ten sticky traps were placed at ten selected places including 3 locations in clean storage/bagging room, 2 locations in head house, 2 locations in control room, and 1 location each in cleaning room, tool room, and office. Food- and pheromone-baited traps were placed on the floor or equipment and sticky traps were hung at eye level above the food- and pheromone-baited traps. Traps were checked biweekly from June to October 2017. All captured adults of insect species in traps were identified and counted.

Popping quality evaluation. Popcorn popping quality was measured by metric weight volume tester (Wood Dale, IL, USA). Two hundred and fifty grams popcorn from small and large mesh-opening pouches were heated with one hundred grams of popping oil at 485°F. Popcorn were allowed to pop until popping slows to 1 or less for every five seconds. All popped popcorn was transferred to expansion tube. Popped and unpopped kernels were counted. Popped

and unpopped kernels were counted. Popping quality were expressed as a percentage: (number of unpopped kernels ÷ total number of kernels) × 100.

Data analysis. Temperatures and relative humidity were averaged every 12 h for each bin. The average temperatures and relative humidity for each bin were averaged again among the three bins, and the final means of temperature and humidity were plotted against time by using SigmaPlot 12.5 (Systat software Inc., 2011). The moisture contents of popcorn were analyzed by one-way analysis of variance (ANOVA) to determine significant differences ($P < 0.05$) among six months of storage.

The mean of adult emergence from *P. interpunctella* eggs in six months popcorn samples were plotted using SigmaPlot 12.5. In laboratory bioassays, damaged kernels was calculated as a percentage: (number of damaged kernels ÷ total number of kernels) × 100. Percentage reduction of damaged kernels in treated samples compared to controls was calculated as: (1- percentage of damaged kernels in treatment ÷ percentage of damaged kernels in control) ×100. In the field study, the actual number of damaged kernels were counted. Number of adults that emerged from eggs and number of damaged kernels data were transformed to $\log_{10}(x+1)$ scale to normalize treatment variances prior to analysis of variance (ANOVA). Percentage reduction of damaged kernel was transformed to angular values prior to ANOVA (Zar, 1984). Adult emergence, damaged kernels, and popping quality data were subjected to two-way ANOVA to determine significant differences ($P < 0.05$) of main (concentrations, storage times, or pouch types) and interaction effects. For each insecticide, damaged kernel data were analyzed by one-way ANOVA ($P < 0.05$), and means were separated by Bonferroni *t* tests at $\alpha = 0.05$ (SAS Institute, 2013).

The total number of adults of each species captured by probe traps and food- and pheromone-baited traps were calculated for each month. The total number of *P. interpunctella* captured by sticky traps in the cleaning facility or storage bins was plotted using SigmaPlot 12.5.

Results

Popcorn temperature, relative humidity, and moisture content. Temperatures at 5 cm below popcorn surface ranged from 4.6 to 29.9 °C between May and October 2017. The average temperatures in May and October were 14.6 and 15.7 °C, respectively, which were lower than other months (Fig. 2.1a). Higher temperature were recorded between June and September ranging from 20.3 to 25.3 °C. The mean relative humidity during six months' ranged from 62.1 to 73.5% (Fig. 2.1b). The mean moisture contents of popcorn were from 12.6 to 13.2%, and did not change significantly (F = 2.06; df =1, 16; P = 0.1706) during the six months of storage (Fig. 2.2).

Efficacy of methoprene and spinosad against *P. interpunctella* in popcorn (laboratory bioassay). Two-way ANOVA of adult emergence from *P. interpunctella* eggs in methoprene treated popcorn indicated a significant effect of concentration (F = 268.39; df = 3, 48; P < 0.0001), but not storage times (F = 0.67; df = 3, 48; P = 0.6472). The interaction of concentration and storage time also was not significant (F = 0.40; df =15, 48; P = 0.9728). The average number of *P. interpunctella* adults that emerged from eggs in untreated popcorn ranged from 3.7 to 4.7 in samples stored from May to October (Fig. 2.3). However, no adults emerged in methoprene treated popcorn except for September and October at the concentration of 0.7 ppm.

Two-way ANOVA showed significant differences of adult emergence in spinosad-treated popcorn among concentrations (F = 121.23; df = 1, 28; P < 0.0001), storage times (F = 7.84; df

= 6, 28; $P < 0.0001$), and the interaction ($F = 3.69$; df = 6, 28; $P = 0.0080$). The average number of adults that emerged in untreated popcorn ranged from 2.7 to 5.7 across six months. By contrast, the average number of adults that emerged in spinosad treated popcorn was 0 to 0.3 (Fig. 2.4).

Damaged kernels caused by egg-to-adult development of *P. interpunctella* in methoprene treated popcorn after 49 d of incubation is shown in Table 2.1. Two-way ANOVA indicated that effect of storage times was significant ($F = 3.39$; df = 5, 48; $P = 0.0105$), but the effect of concentrations ($F = 1.05$; df = 3, 48; $P = 0.3795$), and the interaction ($F = 1.76$; df = 15, 48; $P = 0.0703$) were not significant. The percentage of damaged kernels can be separated by storage time only at 2.8 ppm, and the value (4.7%) was significantly lower in September. In September, the percentage of damaged kernels of control and 1.4 ppm methoprene samples was significantly higher than other treatments.

Damaged kernels in spinosad treated popcorn are shown in Table 2.2. Two-way ANOVA indicated a significant concentration effect ($F = 302.86$; df = 1, 24; $P < 0.0001$). There were no significant differences storage times ($F = 0.13$; df = 5, 24; $P = 0.9830$). The interaction of concentration and storage times also was not significant ($F = 0.23$; df = 5, 24; $P = 0.9472$). The percentage of damaged kernels in controls ranged from 5.0 to 6.8% among the six months, and by contrast only 0.2 to 0.6% of damaged kernels were observed in treated samples. Reduction of damaged kernels between control and spinosad treated samples was not significantly different among the six months of storage time, and the reduction in damage at 1 ppm spinosad relative to damage in controls ranged from 89.5 to 96.0%.

Insect infestation in storage bins. Adults of eight stored-product insect species were captured in probe traps with a total of 689 insects from June to October 2017 (Table 2.3). Red

flour beetle, *Tribolium castaneum* (Herbst); drugstore beetle, *Stegobium paniceum* (Linnaeus); rusty grain beetle, *Cryptolestes ferrugineus* (Stephens); and *P. interpunctella* accounted for 98.4% of the total insects captured in traps. The sawtoothed grain beetle, *Oryzaephilus surinamensis* (L.); swollen fungus beetle, *Cartodere nodifer* (Westwood); carpet beetle, *Anthrenus verbasci* (Linnaeus); and warehouse beetle, *Trogoderma variabile* (Ballion) constituted 1.6% of total insects captured in probe traps. Traps from September had the highest number of captured insects, and number of insects in June was the lowest among six months (Table 2.3). One insect species, *P. interpunctella,* was captured in sticky traps from May to October 2017 (Fig. 2.5). A total of 1285 of *P. interpunctella* adults were captured in three sticky traps in storage bins. Number of *P. interpunctella* adults increased from June to August, then decreased in September, and was the lowest in October (Fig. 2.5).

Insects and damaged kernels found in pouches with large mesh-openings. Unlike commercial traps, very few insects were found in untreated, methoprene treated, and spinosad treated popcorn samples in pouches with large mesh-openings from May to October 2017. *P. interpunctella* was the main species, and most of them were fourth or fifth instars (Table 2.4). In methoprene treatments, the total number among three bins of *P. interpunctella* was 0 – 8 among four concentrations during six months of storage times, and no insects were found in June. In spinosad treated samples, no insects were found in May and July. No insects were found in June control samples. The highest number of *P. interpunctella* found was 7 in treated July samples. Two *O. surinamensis* adults were found in control samples in July (data not shown in Table 2.4).

Two-way ANOVA of damaged kernels in methoprene treated popcorn showed a significant effect among storage times ($F = 8.36$; df = 5, 48; $P < 0.0001$). There were no differences among methoprene concentrations ($F = 2.23$; df = 3, 48; $P = 0.0968$); the intgeraction

of concentration and storage time also was not significant (F = 0.46; df =15, 48; P = 0.9503). The number of damaged kernels was significantly different among storage times only at concentrations of 0.7 and 2.8 ppm (Table 2.5). Generally, number of damaged kernels increased as storage time increased.

Two-way ANOVA indicated that concentration (F = 12.97; df = 1, 24; P = 0.0014) and storage times (F = 4.09; df = 5, 24; P = 0.0079) had significant effects on damaged kernels in spinosad treated popcorn, but interaction of concentration and storage time was not significant (F = 1.31; df = 5, 24; P = 0.2946). The number of damaged kernels in spinosad treated popcorn was significantly reduced only in September (t = -2.97; df = 4; P = 0.0410) (Table 2.6).

Insect infestation in popcorn cleaning facility. There were total of 3171 insects of eight species captured in food- and pheromone-baited traps (Table 2.8). *T. castaneum* was the predominant species (87.8% of total), followed by *S. paniceum* (5.6%), and the maize weevil, *Sitophilus zeamais* Motschulsky (5.3%). *P. interpunctella*, *C. ferrugineus*, and confused flour beetle, *Tribolium confusum* Jacquelin du Val, and *O. surinamensis* constituted 1.2% of total insects captured food- and pheromone-baited traps. Traps from August and September captured significantly more insects than other months (Table 2.8). Total number of *P. interpunctella* adults captured in sticky traps was 3077 (Fig. 2.6). The trends for number of *P. interpunctella* in sticky traps were similar between facility and popcorn storage bins (Fig 2.5 and 2.6). The highest number of *P. interpunctella* adults (770) was captured in July, and lowest number (4) was captured in October.

Popping quality. Based on two-way ANOVA analysis for popping quality of methoprene treated popcorn, effects of pouch types, concentrations, and the interaction were not significant (F, range = 0.48 – 1.72; df, pouch type =1, 16; df, concentration and interaction = 3,

16; *P*, range = 0.2024 – 0.4972). Percentage of unpopped popcorn from pouches with small and large mesh openings was 1.68 to 1.95% and 1.30 to 2.41%, respectively, among four concentrations (0, 0.7, 1.4, and 2.8 ppm) (Table 2.8). For the popping quality of popcorn treated by spinosad, there were no effects of pouch types, concentrations, and the interaction pouch type and concentration (*F*, range = 0.05 – 5.10; df = 1, 8; *P*, range = 0.0539 – 0.8286). Percentage of unpopped popcorn was 1.76% in control samples and 1.01% in spinosad treated popcorn from pouches with small mesh openings, and were 1.74 and 1.72% in control and treated samples from pouches with large mesh openings, respectively (Table 2.8).

Discussion

There are many factors that determine efficacy of residual contact insecticides including insecticide formulations, insect species, characteristic of the treated surface, exposure durations, temperatures, and application rates (Fang et al., 2002; Toews et al., 2003; Arthur et al., 2009; Jenson et al., 2009; Arthur, 2012; Wijayaratne et al., 2012). Fang et al. (2002) reported that spinosad residues did not significantly degrade over a 12-month period at temperatures from -10 to 32°C and relative humidity from 50 to 70%. Wijayaratne et al. (2012) reported that methoprene was stable within 24 weeks at 20 – 35 °C when applied to concrete or varnished-wood surfaces. Both methoprene and spinosad are susceptible to sunlight (UV light) under outdoor conditions (Quistad et al., 1975; Brunner and Doerr, 1996; Liu et al., 1999). In our study, grain temperatures varied during the six month study period, but relative humidity and moisture content of popcorn were relatively stable. Popcorn samples in mesh pouches were buried beneath the grain surface, and were not directly exposed to sunlight in the current study. Therefore, methoprene and spinosad residues were still effective against insects after six months.

In our study, *P. interpunctella* adults did not emerge in methoprene treated popcorn during the 6-month storage, except in September and October at the concentration of 0.7 ppm. In addition, percentage of damaged kernels caused by adult emergence of *P. interpunctella* were similar among all concentrations and controls. McGregor and Kramer (1975) reported that number of progeny of adults emerging from ten *P. interpunctella* third instars ranged from 185 to 496 in controls, and only extra-large larvae were observed in methoprene treated wheat or corn at the concentration of 2 ppm. Arthur (2016) reported that there was no progeny production of *T. castaneum* in methoprene (2.5 ppm) treated corn, except for the corn stored for 4 months. The test lasted for two years, and treated corn was sampled every 2 months. By contrast, progeny production was 5 – 60 adults in control samples. Arthur (2016) reported similar results that percentage of damaged kernels caused by *T. castaneum* were not significantly different between control and methoprene treatments.

Spinosad can effectively suppress adult emergence from *P. interpunctella* eggs, and there were no adult emergence during the six month study period. Spinosad has shown effectively to suppress *P. interpunctella* larval or adult emergence in wheat and corn (Subramanyam et al., 1999; Fang et al., 2002a; Szabela, 2005; Huang et al., 2007; Huang and Subramanyam 2007). Maier et al. (2006) reported that the residue levels of spinosad showed a 20% decrease after 3 months, and a 35% decrease after 9 months in spinosad treated corn (1 ppm) when moisture content of corn was 14.2 – 15.2% and temperatures ranged from 0 to 30ºC. Huang and Subramanyam (2007) reported that the number of *P. interpunctella* larvae that emerged from eggs in spinosad treated corn (1 ppm) was zero after 21 d, and no adults emerged from eggs after 49 d. Subramanyam et al. (2007) reported that mortality of *R. dominica* adults was 100% after a 14-d exposure to spinosad treated wheat (1 ppm), which were collected monthly from farm bins

for six months. Huang and Subramanyam (2007) reported that the average number of damaged kernels in spinosad-treated corn (0.1 – 2.0 mg/kg) was 0 to 0.2 while in the control it was 73.8 after *P. interpunctella* adults emerged from eggs. The percentage of damaged kernels in spinosad treated popcorn caused by *P. interpunctella* egg-to-adult development was much lower than untreated popcorn for each of the six months.

Average temperatures in July, August and September 2017 were higher than in other months, and warmer weather promotes insect activity (Mellanby, 1939). Therefore, more insects were captured in probe traps, food- and pheromone-baited traps, and sticky traps during the warmer months. Reed et al. (2003) reported that corn and sorghum residue still stored in the bins after grain was discharged in May and June in central Kansas. The mean number of *Cryptolestes* spp., *Sitophilus* spp. and *Tribolium* spp. were 11.9, 5.4, and 3.7 per kilogram of grain residues, respectively. The natural infestation can vary in different sites (Paula et al., 2002). Paula et al. (2002) reported the highest number of insects captured by cage-traps was on conveyor belts and in the receiving and pre-cleaning areas in a paddy rice grain storage facilities in October in southern Brazil. Insect infestation in food plants can arise from many sources (Scott, 1991). Open doors, windows, sewers, and drains, and interplant shipments are potential insect access points (Heaps, 2006). Many insect species can feed on small amounts of food residues in food processing facility. Because the cleaning facility has more food residues, we found different insect species in the cleaning facility compared to storage bins.

Very few insects were found in pouches with large mesh openings recovered from storage bins. The large mesh openings allow insects to freely travel in and out of pouches. Correspondingly, the number of damaged kernels were not significantly among different concentrations at each storage time except in spinosad treatments in September. Damaged

kernels were due to insects feeding and development. These trends of damaged kernels in spinosad treated popcorn had a big difference since low natural infestation was observed in pouches with large mesh-openings.

More unpopped kernels are not desirable when evaluating quality of popcorn. Popping quality of popcorn is affected by popcorn variety, moisture content, kernel size, specific gravity, kernel sphericity, pericarp thickness, translucent and opaque endosperm, and lipid and protein levels (Sweley, 2013). Kernel damage is one of factors affects popping performance of popcorn (Lin and Anatheswaran, 1988; Singh et al 1997). Damaged kernels decrease expansion volume and increase popping time. In our study, damaged kernels caused by insect infestation did not significantly decrease popping quality, because number of damaged kernels was extremely low after six months. Popping quality of treated popcorn was similar compared to control, which indicated that methoprene and spinosad did not affect popcorn inherent characteristics.

In conclusion, storage bins and facility were more prone to insect invasion and infestation during warm seasons. Methoprene and spinosad residues were stable, and were effective in preventing *P. interpunctella* adult emergence consistently over six months. Efficacy of methoprene and spinosad against *P. interpunctella* and their stability in farm-stored popcorn makes them valuable insecticides for stored-product protection.

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Table 2.1. Percentage (mean ± SE) of kernels damaged by *P. interpunctella* after egg-to-adult emergence in untreated and methoprene treated popcorn from small pouches (n = 3).

Sampling time (month)	0 ppm	0.7 ppm	1.4 ppm	2.8 ppm[a]	*F*-value	df	*P*-value
May	5.8 ± 0.2	5.1 ± 0.9	7.9 ± 0.7	6.8 ± 0.2a	0.78	3,8	0.5358
June	6.7 ± 0.9	6.2 ± 0.4	7.2 ± 0.8	7.6 ± 0.2a	1.20	3,8	0.3687
July	6.5 ± 0.3	7.7 ± 0.7	6.3 ± 0.6	5.9 ± 0.7ab	1.14	3,8	0.3901
August	6.5 ± 0.3	7.7 ± 0.7	6.3 ± 0.6	5.9 ± 0.7ab	1.14	3,8	0.3901
September[b]	6.4 ± 0.6A	4.2 ± 0.4B	6.0 ± 0.3A	4.7 ± 0.5bB	4.93	3,8	0.0316
October	6.8 ± 0.1	6.3 ± 0.9	6.5 ± 0.5	7.8 ± 0.5a	1.25	3,8	0.3554
F-value	0.47	2.86	0.42	5.77			
df	5,12	5,12	5,12	5,12			
P-value	0.7930	0.0631	0.8228	0.0061			

[a]Means with lower case letters show significant differences among months ($P < 0.05$; Bonferroni *t* tests).

[b]Means with upper case letters show significant differences among concentrations ($P < 0.05$; Bonferroni *t* tests).

Table 2.2. Percentage (mean ± SE) of kernels damaged by *P. interpunctella* after egg-to-adult emergence in untreated and spinosad treated popcorn from small pouches (n = 3).

Sampling time (month)	0 ppm	1 ppm	*t*-value	df	*P*-value[a]	Reduction of damaged kernels (%)[b]
May	6.8 ± 0.7	0.3 ± 0.2	-6.73	4	0.0025	95.8 ± 2.2
June	5.5 ± 0.6	0.6 ± 0.2	-7.72	4	0.0015	89.5 ± 2.8
July	5.9 ± 0.6	0.5 ± 0.2	-7.15	4	0.0020	92.3 ± 3.5
August	5.9 ± 0.6	0.5 ± 0.2	-7.15	4	0.0020	92.3 ± 3.5
September	5.0 ± 0.6	0.2 ± 0.1	-6.86	4	0.0024	95.5 ± 2.3
October	5.7 ± 0.6	0.2 ± 0.2	-7.38	4	0.0018	96.0 ± 2.7

[a]Difference in number of damaged kernels between 0 and 1 ppm spinosad was for each of the six months ($P < 0.05$; by two-sample *t*-tests).

[b]Differences in reduction of damaged kernels among storage times was not significant ($F = 0.84$; df = 5, 12; $P = 0.5488$).

Table 2.3. Insects captured by 30 perforated probe traps in popcorn storage bins from May to October, 2017.

Species	Number of adults							% of total insects
	May	June	July	August	September	October	Total/species	
T. castaneum	0	1	3	12	185	20	221	32.1
S. paniceum	0	0	0	139	38	31	208	30.2
C. ferrugineus	28	11	21	12	34	50	156	22.6
P. interpunctella	0	2	35	29	20	8	94	13.6
O. surinamensis	3	1	0	0	2	0	6	0.9
C. nodifer	1	1	0	0	0	0	2	0.3
A.verbasci	0	1	0	0	0	0	1	0.2
T. variabile	0	0	0	0	0	1	1	0.2
Total/month	32	17	59	192	279	110	689	100.0

Table 2.4. Number of *P. interpunctella* larvae found in pouches with large mesh openings collected between May and October 2017 (n = 3).

Sampling time (month)	Methoprene				Spinosad	
	0 ppm	0.7 ppm	1.4 ppm	2.8 ppm	0 ppm	1 ppm
May	0	0	0	1	0	0
June	0	0	0	0	0	1
July	5	2	8	3	0	0
August	3	0	3	2	5	7
September	2	3	2	2	2	3
October	1	2	2	2	6	2

Table 2.5. Number (mean ± SE) of damaged kernels per 250 g popcorn treated with methoprene in pouches with large mesh openings.

Sampling time (month)	0 ppm	0.7 ppm[a]	1.4 ppm	2.8 ppm[a]	*F*-value	df	*P*-value[b]
May	5.0 ± 1.2	4.0 ± 0.0ab	5.0 ± 0.6	3.0 ± 1.5ab	0.92	3,8	0.4752
June	4.3 ± 0.9	1.3 ± 0.7b	1.7 ± 0.9	1.7 ± 0.3b	3.70	3,8	0.0616
July	10.3 ± 5.0	5.7 ± 1.5ab	11.7 ± 4.9	6.0 ± 2.1ab	0.66	3,8	0.6004
August	7.7 ± 1.5	8.7 ± 3.2ab	6.7 ± 2.7	7.7 ± 1.5ab	0.12	3,8	0.9443
September	11.0 ± 0.6	10.3 ± 2.0a	10.3 ± 1.7	8.0 ± 1.2a	0.81	3,8	0.5233
October	11.3 ± 2.4	8.0 ± 1.5ab	10.0 ± 1.2	6.3 ± 0.9ab	1.89	3,8	0.2089
F-value	1.60	3.46	2.37	3.58			
df	5,12	5,12	5,12	5,12			
P-value	0.2340	0.0362	0.1026	0.0326			

[a]Means followed by lower-case letters are significantly different among months methoprene concentrations of 0.7 and 2.8 ppm ($P < 0.05$; by Bonferroni *t* tests).

[b]For each of the six months there were no differences among methoprene concentrations ($P > 0.05$; one-way ANOVA).

Table 2.6. Number (mean ± SE) of damaged kernels per 250g popcorn treated with spinosad in pouches with large mesh-openings pouches.

Sampling time (month)	0 ppm	1 ppm	*t*-value	df	*P*-value	Reduction of damaged kernels (%) [a]
May	3.0 ± 1.2	2.3 ± 0.7	-0.50	4	0.6433	22.2 ± 22.2
June	3.7 ± 0.7	2.3 ± 0.7	-1.41	4	0.2302	36.9 ± 18.0
July	3.0 ± 0.6	2.0 ± 1.2	-0.77	4	0.4818	44.4 ± 29.4
August	6.7 ± 0.9	4.7 ± 1.8	-1.01	4	0.3679	36.8 ± 20.3
September[b]	7.3 ± 1.2	2.0 ± 1.0	-3.41	4	0.0270	72.6 ± 13.7
October	8.0 ± 1.5	4.3 ± 1.5	-1.74	4	0.1570	45.8 ± 18.2

[a]The differences in reduction of damaged kernels among storage times were not significant (F = 0.53; df = 5, 12; P = 0.7476; one-way ANOVA).

[b]Number of damaged kernels were different between 0 and 1 ppm spinosad ($P < 0.05$; by two-sample t test).

Table 2.7. Popping quality for untreated, methoprene-treated and spinosad treated popcorn in pouches with small and large mesh openings pouches at the end of six months (n = 3)

Unpopped popcorn (%)	Methoprene				Spinosad	
	0 ppm	0.7 ppm	1.4 ppm	2.8 ppm	0 ppm	1 ppm
small pouches	1.95 ± 0.06	1.68 ± 0.20	1.92 ± 0.21	1.73 ± 0.47	1.76 ± 0.26	1.01 ± 0.14
large pouches	1.93 ± 0.17	2.01 ± 0.22	2.41 ± 0.14	1.30 ± 0.11	1.74 ± 0.21	1.72 ± 0.22

Table 2.8. Insects captured by 10 perforated and food- and pheromone-baited traps in popcorn cleaning facility between June and October, 2017.

Species	Number of adults						% of total
	June	July	August	September	October	Total	insects
T. castaneum	152	295	993	1025	320	2785	87.8
S. paniceum	82	58	16	20	3	179	5.6
S. zeamais	0	48	71	35	15	169	5.3
P. interpunctella	2	8	4	1	2	17	0.5
C. ferrugineus	0	0	0	9	0	9	0.3
T. confusum	2	2	4	0	0	8	0.3
O. surinamensis	0	3	0	0	1	4	0.1
Total/month	238	414	1088	1090	341	3171	100.0

Figure 2.1. The average temperature (a) and relative humidity (b) 5 cm below the popcorn grain mass from bins between May and October, 2017 (n = 3).

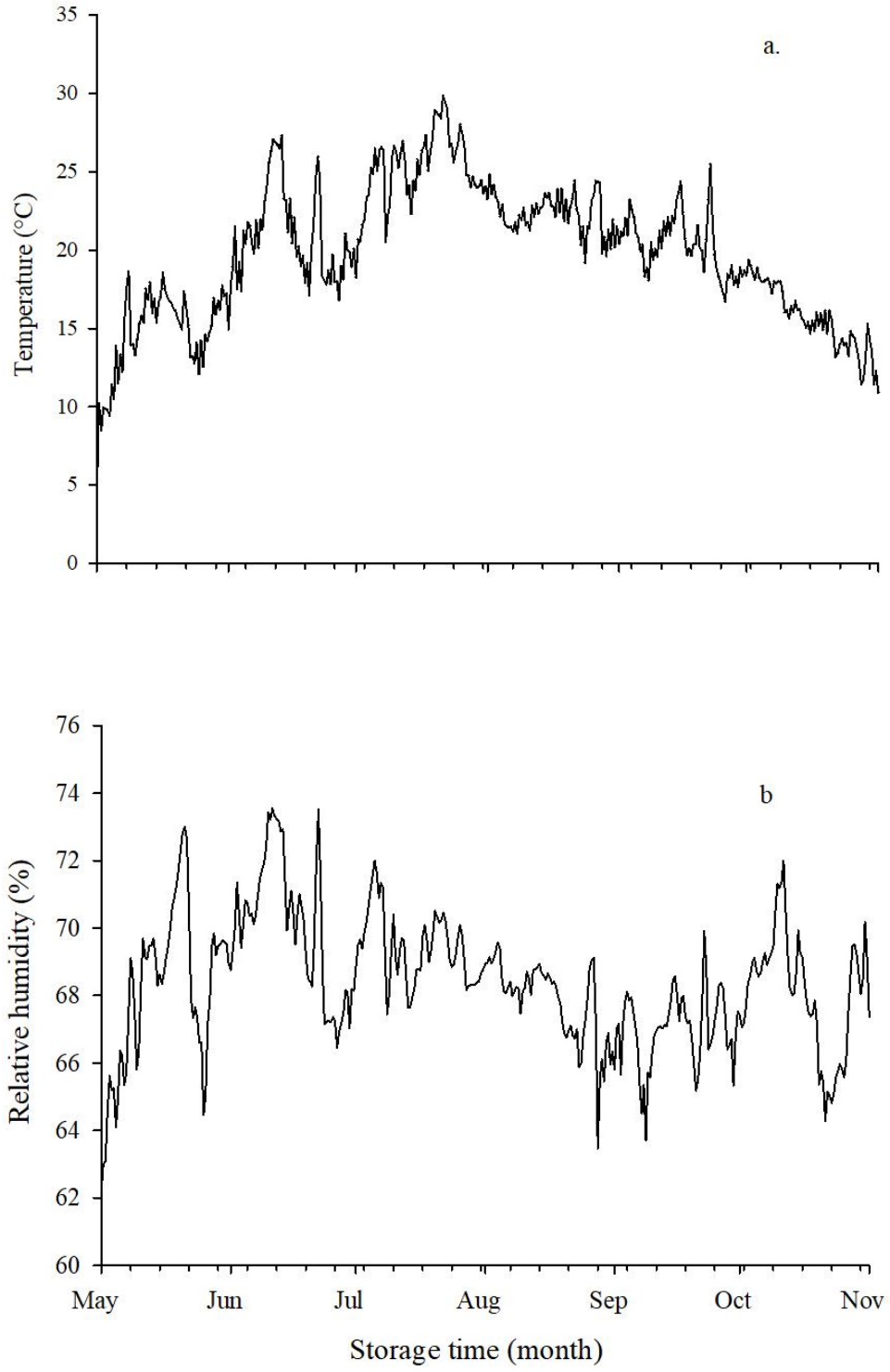


Figure 2.2. Moisture content (mean ± SE) of untreated popcorn in pouches with small mesh openings between May and October, 2017 (n = 3).

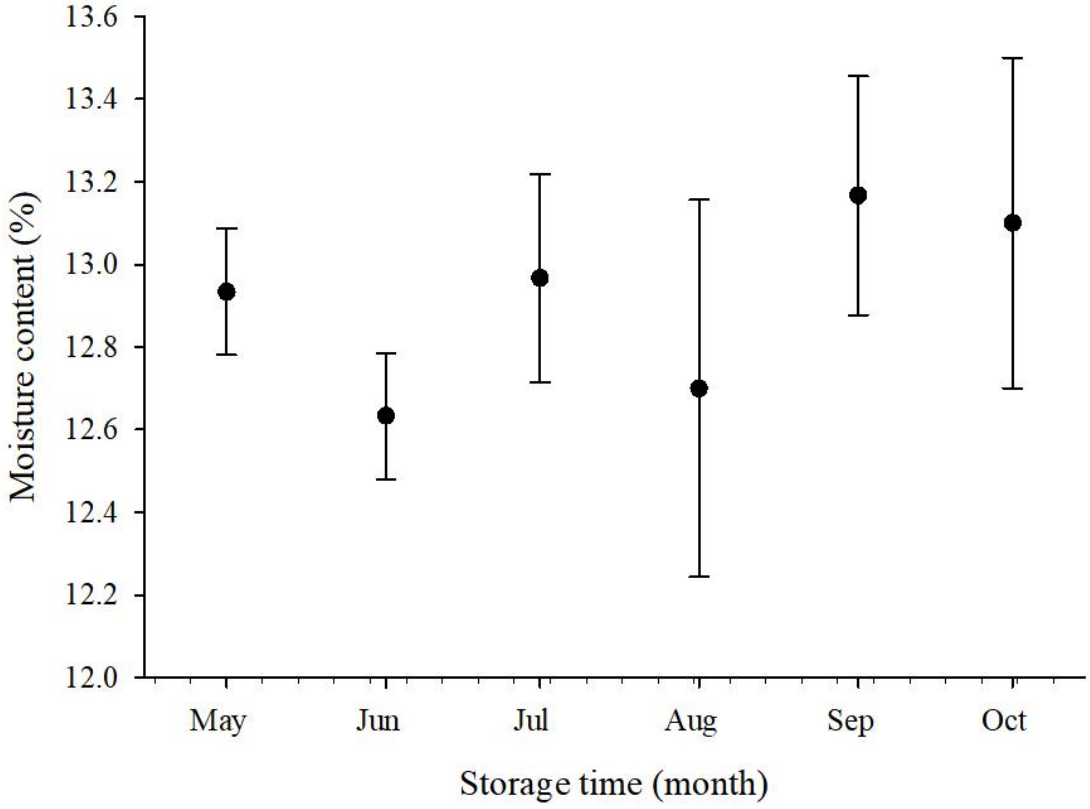
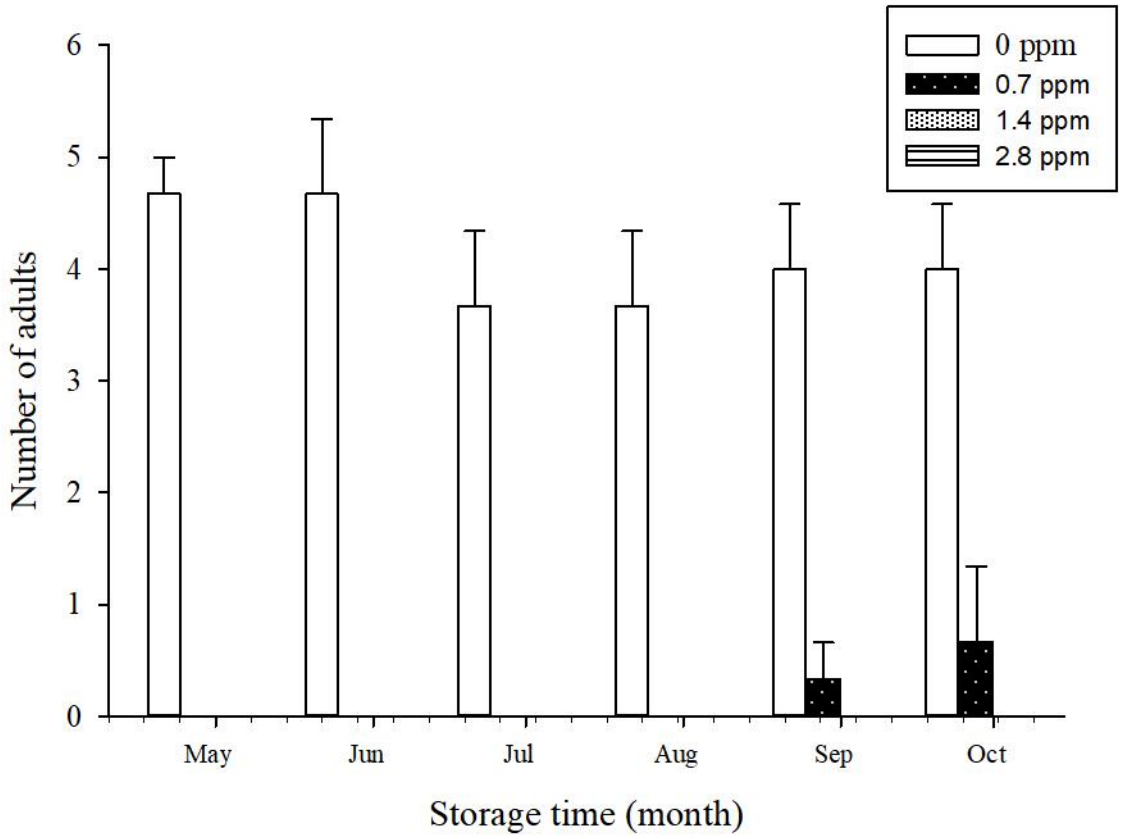
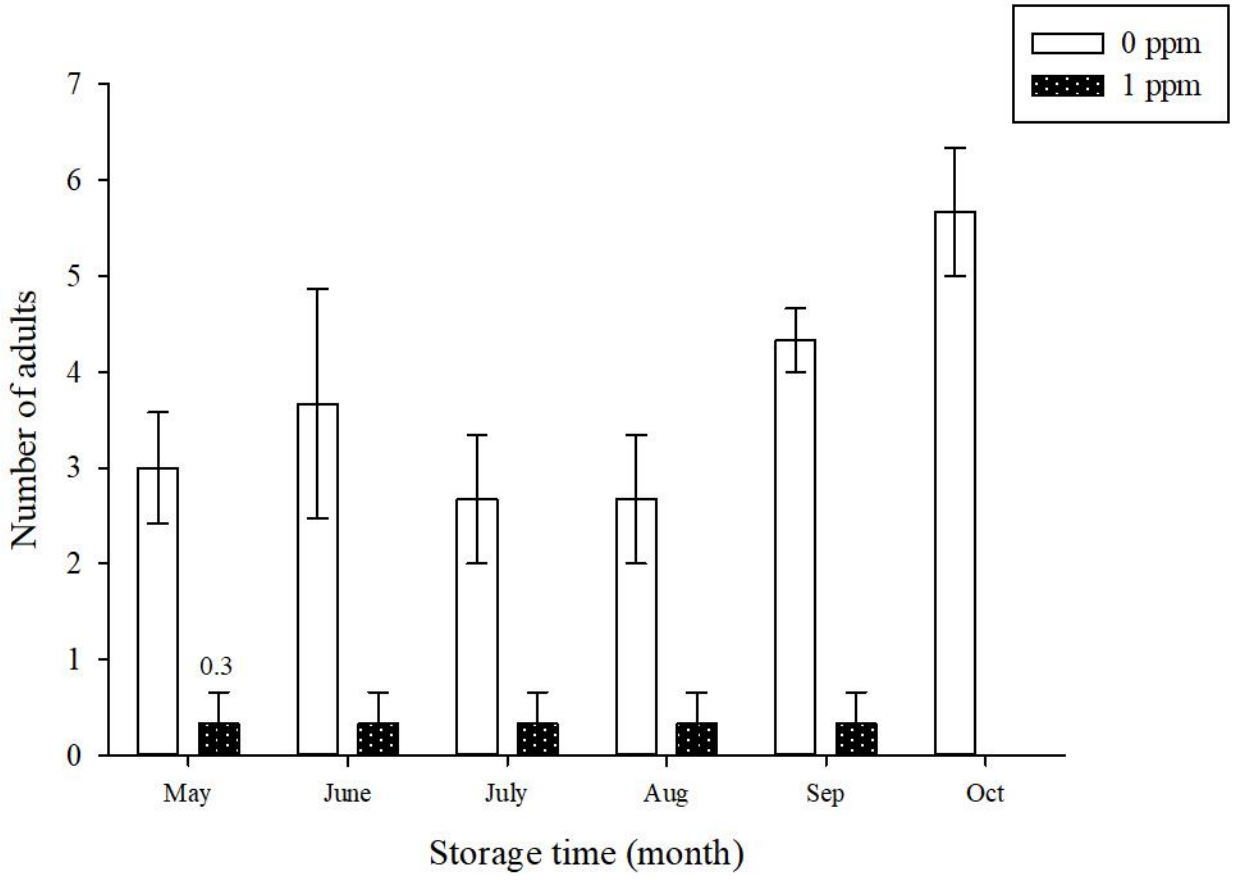


Figure 2.3. Number of (mean ± SE) of *P. interpunctella* adults that emerged from eggs in methoprene treated popcorn stored in bins[a].



[a]No adults emerged in methoprene-treated popcorn between May and August.

Figure 2.4. Number of (mean ± SE) of *P. interpunctella* adults that emerged from eggs in spinosad treated popcorn stored in bins[a].



[a]No adults emerged in spinosad-treated popcorn in October.

Figure 2.5. Number of *P. interpunctella* adults captured by 30 sticky traps in popcorn storage bins between June and October, 2017.

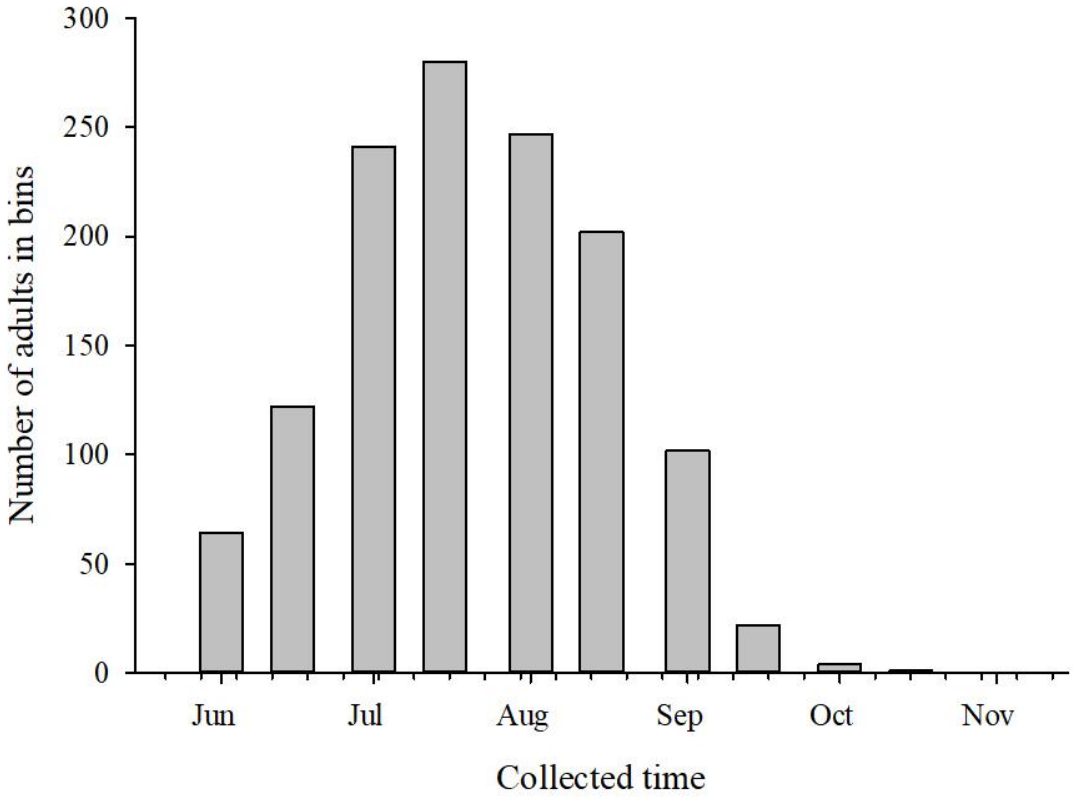
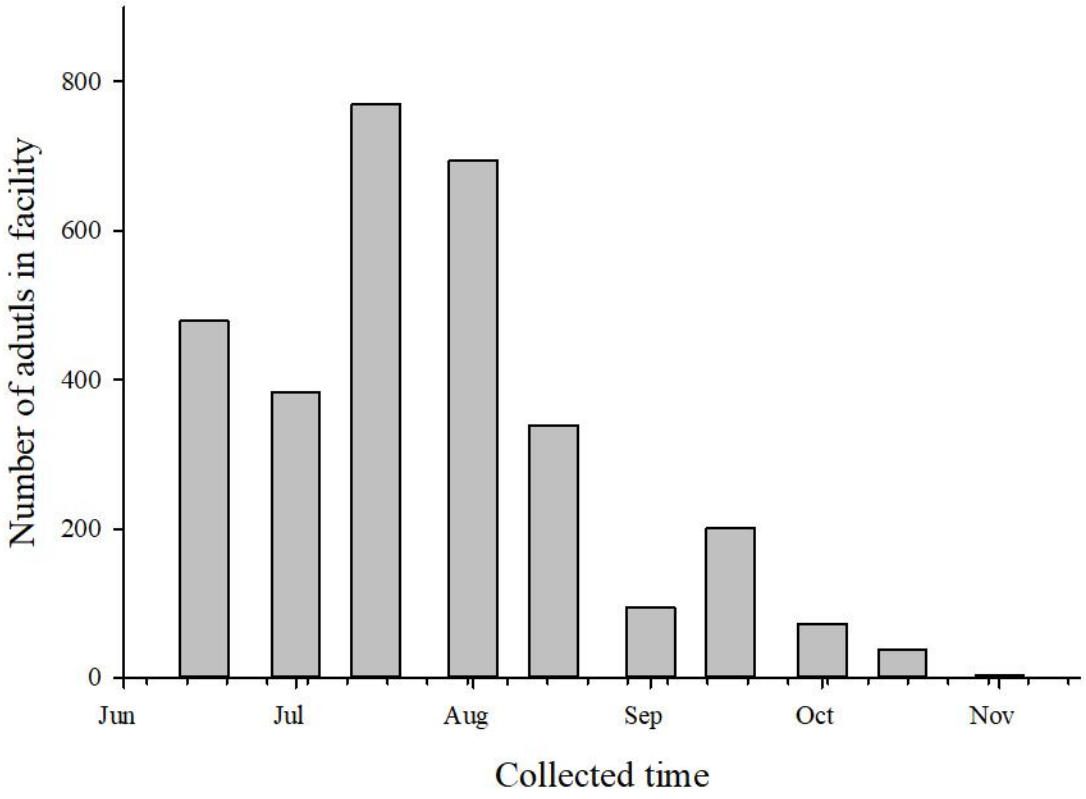


Figure 2.6. Number of *P. interpunctella* adults captured in 10 sticky traps in popcorn cleaning facility between June and October, 2017[a].



[a]One trap was missing during 8/1-8/15 collection time.

Chapter 3 - Efficacy of spinosad against *Sitophilus zeamais* in popcorn

Abstract

Spinosad is a risk-reduced insecticide, and is effective in management of a variety of stored-product insect pests. This study was aimed at determining the efficacy of spinosad applied to popcorn against the maize weevil, *Sitophilus zeamais* Motschulsky, by evaluating the post-exposure mortality and progeny production. Adults of a field strain (TX) were exposed to spinosad treated popcorn at the labeled rate of 1 ppm. Fifty unsexed adults (1-2 wk old) were placed in 100 g of untreated and spinosad-treated popcorn, respectively. Exposure times were 1, 4, 8, 12, 24, 72, 120, 168 and 336 h. Adults were transferred to 100 g clean untreated popcorn soon after the intended exposure. Mortality was assessed at 0, 7, 14, and 21 d after transfer. Progeny were counted after 42 d of transferring to clean popcorn. Adults that emerged from treated popcorn were counted after 42 d. Samples were held in an environmental growth chamber at 28°C and 65% r.h. Damaged kernels were counted after the progeny or adult emergence assessment. Zero day mortality of *S. zeamais* was less than 10% after exposure to spinosad for 1 to 12 h. Mortality increased as exposure time increased, and ranged from 94 to 100% when the exposure time was 120 to 336 h. Complete progeny reduction was achieved after a 168-h exposure and the reduction of damaged kernel of clean popcorn was 100% after a 336-h exposure. The reduction of adult emergence and damaged kernels in original treated popcorn was over 90% after a 120-h exposure. There were no significant differences among the 0, 7, 14, and 21 d mortality at each spinosad exposure time. Spinosad was effective in controlling *S. zeamais* adults, and can significantly suppress the progeny production in popcorn.

Introduction

Popcorn is a variety of corn, which expands and puffs up when heated. The popcorn sales had a consistent growth from 1950s to early 1990s (Sweley et al., 2013). In 2015, popcorn sales in North America accounted for 61% of the world-wide sale, and the consumption of popped popcorn was 13 billion quarts or 42 quarts per capita in the U.S (www.popcorn.org.). Popcorn is vulnerable to infestation by internal-feeding stored-product insects, especially weevils of the genus *Sitophilus* (Coleoptera: Curculionidae) (Suleiman et al., 2015). The maize weevil, *Sitophilus zeamais* Motschulsky, is a common insect pest found in various grains, particularly in stored corn in the warm regions (Rees, 1996). Suleiman et al (2015) reported that one kilogram of yellow and white popcorn with 15.5% of moisture content was infested by 87 weevils, and after a 90-d incubation at 27°C, the average number of live weevils found in yellow and white popcorn was 135 and 215, respectively.

Spinosad is a mixture of natural substances isolated from the fermentation products of the bacterium *Saccharopolyspora spinose* (Mertz and Yao, 1990). Spinosyns A and D are two insecticidal ingredients with a ratio of 85:15 in the final spinosad product (Kirst et al., 1992). Spinosad has been studied as a grain protectant to control numerous key stored-product insect pests in a wide range of grains (Subramanyam et al., 1999; 2002). It has low mammalian toxicity and is environmentally benign (Thompson et al., 2000; Cleveland et al., 2001). In 2005, spinosad was registered by the United States Environmental Protection Agency (EPA) at the concentration of 1 ppm of grain as grain protectant for wheat, corn, barley, millets, oats, rice, and sorghum (EPA Reg. No. 264-995). However, the commercial formulation, Sensat^{TM}, was not available as a grain protectant until 2018.

Several laboratory and field studies showed spinosad to be effective in controlling insects associated with various stored grains. Spinosad was effective against adults of the lesser grain borer, *Rhyzopertha dominica* (Fabricius); rice weevil, *Sitophilus oryzae* (Linnaeus); *S. zeamais*; rusty grain beetle, *Cryptolestes ferrugineus* (Stephens); sawtooth grain beetle, *Oryzaephilus surinamensis* (Linnaeus); red flour beetle, *Tribolium castaneum* (Herbst); confused flour beetle, *Tribolium confusum* (Jacquelin du Val); and the immature stage of Indian meal moth, *Plodia interpunctella* (Hübner) (Fang et. al., 2002a; Fang et. al., 2003; Huang and Subramanyam, 2007; Subramanyam et. al., 2007; Subramanyam et. al., 2012; Subramanyam et. al., 2014). Spinosad killed all adults of *R. dominica* after a 7-d exposure and suppressed the egg-to-adult emergence by 93-100% in four classes of wheat, namely hard red winter, hard red spring, soft red winter, and durum wheats at the labeled rate of 1 ppm. The mortality of *S. oryzae, O. surinamensis,* and *T. castaneum* was 100% in spinosad treated durum wheat at 1 ppm concentration (Fang et. al., 2002a). Another study from Huang et al. (2007) reported that after a 14-d of exposure to 1 ppm spinosad treated hard white winter wheat, all adults of *R. dominica, C. ferrugineus, S. oryzae,* and *S. zeamais,* and 92 – 96% of *T. castaneum* and *T. confusum* were killed. No live larvae of *P. interpunctella* emerged from eggs (Huang et al., 2007). Similar trends were observed in spinosad treated corn at 1 ppm (Huang and Subramanyam, 2007). Huang and Subramanyam (2007) reported that adult mortality of *C. ferrugineus, R. dominica*, *O. surinamensis*, *S. oryzae*, and *S. zeamais* were 98 – 100%, while the adult mortality of *T. castaneum* was 84% after a 12-d exposure to 1 ppm spinosad. The number of larvae and adults that emerged from eggs of *P. interpunctella* were zero after a 21-d and 49-d exposure to spinosad.

The immediate and delayed toxicity effects of spinosad to field strains of *S. zeamais* are unknown. Therefore, laboratory experiments were designed to evaluate the effectiveness of

spinosad against one field strain of *S. zeamais* in popcorn at labeled rate of 1 ppm. In addition, the effect of spinosad on adult progeny production and reduction of damaged kernels were also determined.

Materials and Methods

Insect rearing, spinosad formulations, and popcorn. Cultures of *S. zeamais* were reared in 0.95-L glass jars with 250 g of organic yellow corn (Heartland Mills, Marienthal, KS, USA) with a moisture content of 12.5% (wet basis). Culture jars were kept in a growth chamber at 28°C and 65% r.h. The field strain of *S. zeamais* was collected from farm-stored corn in Texas, USA in 2011. The liquid formulation of spinosad (Sensat^{TM}) containing 8.66 % active ingredient (a.i.) was obtained from Bayer Crop Science (Durham County, NC, USA). The insecticide was diluted with distilled water to make the final solution. Spinosad was applied at the labeled rate of 1 ppm. Yellow Tenderflake popcorn was purchased from Popcorn County, USA (North Loup, NE, USA). Popcorn was frozen for two weeks at -13ºC to kill any live insects prior to bioassays. Sensat^{TM} liquid of 5.77 ml was diluted with distilled water in a 500 ml volumetric flask to obtain the spinosad concentration of 1 mg (a.i.)/ml. Spinosad solution in 100µl was applied to 100g of popcorn. Untreated popcorn (control) received 100µl of distilled water. Popcorn in containers was shaken manually after addition of spinosad solution or distilled water.

Bioassays. One hundred grams of untreated and spinosad treated popcorn held in a 150-ml plastic container. Each container was capped with a lid that had a 10-mm round hole drilled in the middle and covered with 250 (µm) mesh screen to ensure air circulation and prevent insect escape. After adding 50 unsexed, 1-2 week old *S. zeamais* adults, containers were placed in an

environmental growth chamber set at 28°C and 65% r.h. Exposure times were 1, 4, 8, 12, 24, 72, 120, 168, and 336 h. Each spinosad concentration (0 and 1 ppm) and exposure time combination was replicated three times. After the intended exposure, adults were transferred to 100 g clean popcorn in plastic containers. Zero-day adult mortality of *S. zeamais* was assessed at the time of transfer. Post-exposure mortality was assessed 7, 14, and 21 d after transfer to clean popcorn. Adults that failed to respond when prodded with a Camel's brush were considered dead. Adult progeny production in clean popcorn from transferred adults was determined at 42 d. Adults that emerged from eggs laid by the 50 introduced adults in the original 100g of popcorn were assessed after a 42-d incubation. Kernels with feeding and adult emergence holes (insect damaged kernels) were considered as damaged. Damaged kernels from clean popcorn were evaluated when adult progeny was determined. Damaged kernels from original popcorn were evaluated when after emergence of adults.

Data analysis. Mortality of *S. zeamais* adults was expressed as a percentage: [(number dead ÷ 50) × 100], and corrected for mortality in the control treatment (Abbott, 1925). One-way analysis of variance (ANOVA) was used to analyze significant differences in corrected mortality data among different exposure times. Means were separated using Bonferroni *t* tests at $\alpha = 0.05$ (SAS Institute, 2013). The significant difference in cumulative post-exposure mortality was analyzed by Cochran-Armitage test (Cochran, 1954; Armitage, 1955). If the 95% confidence interval (CI) of the Pearson and Spearman correlation coefficient does not contain zero, there is a significant difference in post-exposure mortality counted at each insecticide and concentration (SAS Institute, 2013).

Adult progeny were calculated as: (total number of adults in clean popcorn after the 42-d of incubation – 50 originally added adults). Percentage reduction in adult progeny production in

spinosad treatments relative to the production in controls was calculated as: (1 – treatment progeny ÷ control progeny) ×100. Adult emergence included the total number of adults that emerged in original untreated and treated popcorn after a 42-d incubation. Percentage reduction of adult emergence in treatments relative to the emergence in controls was calculated as: (1 – number of adults that emerged in treatments ÷ number of adults that emerged in the control treatment) ×100. The reduction in number of kernels damaged relative to control treatment was calculated as: (1 – number of damaged kernels in treatment ÷ number of damaged kernels in the control treatment) × 100. Progeny production, adult emergence, and number of damaged kernels were transformed to $\log_{10}(x+1)$ scale prior to subjecting data to two-way and one-way ANOVA. Two-way ANOVA was used to determine differences in progeny production, adult emergence, and number of damaged kernels between concentrations and exposure times and the interaction of concentration and exposure time. If one-way ANOVA significant, Bonferroni *t* tests to determine significant difference at each exposure time. Percentage in reduction in adult progeny production, adult emergence, and damaged kernels were transformed to angular values (Zar, 1984) to normalize treatment variances prior to one-way ANOVA and Bonferroni *t* tests to separate means among exposure times.

Results

Mortality of *S. zeamais* in spinosad treated popcorn. Control mortality of *S. zeamais* adults in untreated popcorn over different exposure times ranged from 0 to 13.3% (Table 3.1). Corrected 0 d mortality of *S. zeamais* was less than 10% after exposure to spinosad for 1 to 12 h. However, 0 d mortality increased as exposure time increased, and mortality was 94.6 to 100% when exposure times were 120 to 336 h. (Table 3.2). The 95% confidence interval (CI) for the

Pearson and Spearman correlation coefficients contained zero for all exposure times according to Cochran-Armitage test results. There was no cumulative post-exposure mortality for each exposure time. One-way ANOVA indicated that the effect of exposure times on corrected mortality was significant (F range = 159.95 - 182.52; df = 8, 18; P <.0001).

Insecticidal effect of spinosad on progeny production and damaged kernels in clean popcorn. Two-way ANOVA indicated that there were significant effects between spinosad concentrations (F = 277.12; df = 1, 36; P < 0.0001), among exposure times (F = 50.99; df = 8, 36; P <.0001), and the interaction of concentration and exposure time (F = 43.98; df = 8, 36; P <.0001) in adult progeny production of *S. zeamais*. Progeny production significantly decreased when *S. zeamais* were exposed to spinosad treated popcorn for 24 h. Progeny reduction increased as exposure time increased and reached 100% after a 168-h exposure (Table 3.3). There were significant differences in number of damaged kernels between spinosad concentrations (F = 231.48; df = 1, 36; P <.0001), and among exposure times (F = 47.27; df = 8, 36; P <.0001). The concentration and exposure time interaction also was significant (F = 40.29; df = 8, 36; P <.0001). Reduction of damaged kernels was 44.5 – 100% after 24 to 336 h exposure (Table 3.4). Reduction of damaged kernels was not very significant at shorter exposure times.

Effect of spinosad on adult emergence and damaged kernels in original popcorn. Egg-to-adult emergence in original popcorn after 42 d showed trend similar to that observed in *S. zeamais* adult progeny production in clean popcorn (Table 3.5). Two-way ANOVA indicated there were significant differences between spinosad concentrations (F = 44.42; df = 1, 36; P < 0.0001) and exposure times (F = 42.39; df = 8, 36; P < 0.0001); the interaction of concentration and exposure time also was significant (F = 16.59; df = 8, 36; P < 0.0001). Reduction in adult emergence was 91.7% after a 120-h exposure, and reached to 96.2% after a 168-h exposure,

which was significantly higher than other exposure times. Two-way ANOVA of number of damaged kernels in original popcorn was significant between spinosad concentrations (F = 45.29; df = 1, 36; $P < 0.0001$) and among exposure times (F = 46.61; df = 8, 36; $P < 0.0001$). The concentration and exposure time interaction also was significant (F = 18.52; df = 8, 36; $P <$ 0.0001). Reduction of damaged kernels in original containers was 33.7, 90.4, and 96.8%, after a 72, 120, and 336-h exposure, respectively, to spinosad treated popcorn. Reduction of damaged kernels at longer exposure times was significantly higher than at shorter exposure times (Table 3.6).

Discussion

In the present investigation, mortality of a field strain of *S. zeamais* after exposure to popcorn treated with 1 ppm spinosad ranged from 8.7 to 100%. Mortality increased as exposure time increased between 12 and 336 h. The efficacy of spinosad in killing stored-product insect pests can be affected by several factors including insect species and strains, and types of commodity (Huang et al 2007; Huang and Subramanyam, 2007; Boina et al. 2012). In spinosad treated wheat at 1 ppm, mortality of *R. dominica* adults was 100% after a 3-d exposure (Boina et al. 2012); mortality of *C. ferrugineus* and *S. oryzae* adults reached 100% after a 7-d exposure (Huang et al 2007); mortality of *S. zeamais* adults reached to 100% after a 14-d exposure (Huang et al 2007); and mortality of *T. castaneum* and *T. confusum* adults were 94.8% and 96%, respectively, after a 14-d exposure (Huang et al 2007). In spinosad treated corn at 0.5 ppm, Huang and Subramanyan (2007) reported that mortality of a laboratory strain of *S. oryzae* adults was 100% after a 12-d exposure. They reported complete mortality of *S. zeamais* adults was achieved in spinosad treated corn at the concentration of 1 ppm after a 14-d exposure.

The characteristics of kernel surface may affect the efficacy of spinosad against insects due to spinosad residue coverage, distribution, and retention (Amos et al., 1986). Huang and Subramanyam (2003) first reported the effect of grain types on the insecticidal efficacy of spinosad. *Corcyra cephalonica* (Stainton), the rice moth, was more susceptible to spinosad treated corn than sunflower seeds at rates of 0.5 and 1 ppm, respectively. The number of larvae that emerged from 50 eggs of *C. cephalonica* was zero at concentrations of 0.5 and 1 ppm, respectively, after a 21-d exposure to spinosad treated corn. Similarly, there were no adults that emerged from eggs after a 49-d exposure to spinosad treated corn at these concentrations. In spinosad treated sunflower seeds, the number of larvae and adults that emerged from eggs were 1.0 and 0.4, respectively, at a concentration of 0.5 ppm. By contrast, the number of larvae were 0.2 after 21 d and adults were zero after 49 d. The efficacy of spinosad against adults of a laboratory strain of *S. oryzae* varied among wheat classes (Fang et. al, 2002). Fang et al (2002) reported that mortality of *S. oryzae* adults after exposure to spinosad treated durum, hard red spring, hard red winter, and soft red winter wheats at the concentration of 1 ppm were 100, 76.1, 70.3, and 69.0%, respectively. Toews and Subramanyam (2003) reported the mortality of *R. dominica* varied depending on the condition of the wheat treated with spinosad at the concentration of 0.1 ppm. More than 97% of *R. dominica* adults were dead in spinosad treated whole kernels of hard red winter wheat with 13% moisture content. Mortality was 42% in cracked wheat which were wheat fractions retained on the sieve screen with 841-µm openings. Only 19% of insects died in spinosad treated whole-wheat flour sifted through the screen with 250-µm openings. Huang and Subramanyam (2007) reported that with the same moisture content (13%) and spinosad concentration (1 ppm), treated wheat rendered a lower *S. oryzae* mortality compared to treated corn (9.5% vs. 29.5%) after a 40-h exposure.

Delayed toxicity effects of spinosad vary with species, types of grain, and environmental conditions. *R. dominica* adults showed delayed toxicity responses to spinosad in several studies (Getchell and Subramanyam, 2008; Athanassiou et. al 2010; Boina et al. 2012). Boina et al. (2012) reported that mortality of *R. dominica* adults was to 100% after 5 d, when the initial mortality was zero after exposure to spinosad treated wheat (1 ppm) for 1 h at 28°C and 65% r.h. Athanassiou et. al, (2010) reported that the immediate mortality of *S. oryzae* adults was 9.5% after exposure to spinosad treat wheat (1 ppm) for 40 h, and delayed mortality reached to 50.5% after 7 d at 27.5°C and 75% r.h. *S. oryzae* showed similar trends when exposed to spinosad treated corn (1 ppm); the immediate mortality was 29.5% and the delayed mortality was 46.0%. However, Getchell and Subramanyam (2008) reported that there was no delayed toxicity effect in the case of *S. oryzae* adults exposed to wheat, corn, and sorghum treated with 1 ppm spinosad at 28°C and 65% r.h. Our study reported here showed no significant delayed toxicity effects of spinosad with the field strain of *S. zeamais*.

Our study showed that higher adult mortality led to lower progeny production and less damaged kernels in spinosad treatments. Similar trends were reported in Huang et al. (2007). The mortality of *S. zeamais* adults was 0.01 and 100 % in control and spinosad treated hard white wheat at concentrations 1.0 ppm after a 14-d exposure; average progeny production was 676.7 and 3.0 adults after 49 d in control and spinosad treatments, respectively. The average number of damaged kernels in control and spinosad treatments was 57.3 and 0, respectively, at 49 d. Similarly, mortality of *S. oryzae* adults was 10.8% in the control treatment and 100% in wheat treated with 1 ppm spinosad. The average adult progeny production was 647.3 and 11.3 in control and spinosad treatments, respectively. The average number of damaged kernels in control and spinosad treatments was 49.3 and 0.7, respectively (Huang et al., 2007).

In our study, the reduction of *S. zeamais* adult emergence and damaged kernels increased as immediate mortality increased. *S. zeamais* is an internal feeder, and its larval development occurs inside the kernels. The progeny of *S. zeamais* can only come in contact with the spinosad after adults emerged in the original plastic containers. Both reduction of egg-to-adult emergence of *S. zeamais* adults and reduction of damaged kernels were around 30% after a 3-d exposure, and over 90% after a 5-d exposure. These results suggested that spinosad was effective in killing the adults before females had a chance to mate and lay eggs inside the kernels. Similar results for *P. interpunctella* were reported by Fang et al. (2002a). Larval mortality of *P. interpunctella* was 83.2% in 0.1 ppm spinosad treated hard red winter wheat, and the average number of adults that emerged was 8.4, along with 28.7% of damaged kernels. When exposed to spinosad treated wheat (1 ppm), larval mortality of *P. interpunctella* was 98.8%, and average number of adults that emerged was 1.2, with only 0.8% of damaged kernels.

In conclusion, spinosad at the labeled rate of 1 ppm produced complete control of *S. zeamais* adults in popcorn. Spinosad can deliver high mortality of *S. zeamais* adults and completely suppress adult progeny production, and significantly reduce the number of damaged kernels. Spinosad is an ideal insecticide to manage *S. zeamais* in stored popcorn.

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Table 3.1. Mean ± SE mortality of *S. zeamais* adults in control samples after 0, 7, 14, and 21d of incubation in clean popcorn.

Exposure time (h)	Cumulative post-exposure mortality (% mean ± SE) [a]			
	0 d	7 d	14 d	21d
1	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
4	0.0 ± 0.0	2.0 ± 2.0	4.0 ± 4.0	4.0 ± 4.0
8	0.0 ± 0.0	1.3 ± 1.2	2.0 ± 2.0	4.0 ± 2.0
12	0.0 ± 0.0	1.3 ± 1.2	2.0 ± 2.0	3.3 ± 3.1
24	0.0 ± 0.0	2.7 ± 1.2	4.0 ± 2.0	4.7 ± 2.3
72	1.3 ± 2.3	2.0 ± 2.0	2.7 ± 1.2	4.7 ± 1.2
120	2.0 ± 2.0	5.5 ± 1.2	5.5 ± 1.2	7.3 ± 1.2
168	4.0 ± 5.3	7.3 ± 5.0	10.0 ± 6.9	13.3 ± 5.0
336	0.7 ± 1.2	1.3 ± 1.2	2.0 ± 2.0	2.0 ± 2.0

[a]Each mean is based on $n = 3$.

Table 3.2. Mean ± SE corrected mortality of *S. zeamais* adults assessed 0, 7, 14, and 21 d after exposure to popcorn treated with 1 ppm spinosad.

Exposure time (h)	Cumulative post-exposure mortality (% mean ± SE, n = 3)[a]			
	0 d	7 d	14 d	21d
1	0.0 ± 0.0 c	2.0 ± 1.5 d	4.7 ± 2.4 d	4.7 ± 2.4 cd
4	0.7 ± 0.7 c	1.4 ± 1.4 d	1.4 ± 1.4 d	1.4 ± 1.4 d
8	0.0 ± 0.0 c	10.8 ± 3.1 cd	12.2 ± 2.4 cd	10.4 ± 2.4 cd
12	8.7 ± 2.7 c	24.4 ± 3.6 c	23.8 ± 3.6 c	22.8 ± 3.7 c
24	44.7 ± 9.6 b	56.8 ± 8.3 b	57.6 ±8.5 b	58.0 ± 9.2 b
72	86.0 ± 0.0 a	93.9 ± 2.0 a	94.5 ± 1.4 a	95.1 ± 0.7 a
120	94.6 ± 2.5 a	97.2 ± 0.7 a	97.9 ± 0.0 a	98.6 ± 0.7 a
168	97.9 ± 0.0 a	98.6 ± 0.7 a	98.5 ± 0.7 a	99.2 ± 0.8 a
336	100.0 ± 0.0 a	100.0 ± 0.0 a	100.0 ± 0.0 a	100.0 ± 0.0 a

[a]Mortality followed by different lower-case letters are significantly different among exposure times ($P < 0.05$; by Bonferroni t tests).

Table 3.3. Progeny production (mean ± SE, n = 3) by *S. zeamais* adults exposed to untreated and spinosad treated popcorn after 42 d in clean popcorn.

Exposure time (h)	0 ppm	1 ppm	*t*-value	df	*P*-value	Progeny reduction (%)[a]
1	129.0 ± 15.1	113.7 ± 25.4	0.66	4	0.5443	11.9 ± 19.7 bc
4	83.7 ± 13.6	123.7 ± 31.3	-1.02	4	0.3671	-47.2 ± 37.3 c
8	135.0 ± 21.0	117.3 ± 8.7	0.66	4	0.5467	13.1 ± 6.5 bc
12	132.3 ± 21.8	153.0 ± 12.6	-0.85	4	0.4434	-2.0 ± 16.5 c
24	74.7 ± 24.3	39.0 ± 5.3	1.17	4	0.3062	48.0 ± 7.1 b
72[b]	73.0 ± 19.9	2.3 ± 0.9	7.40	4	0.0018	96.8 ± 1.2 a
120[b]	79.3 ± 10.2	2.7 ± 1.5	5.94	4	0.0040	96.6 ± 1.8 a
168[b,c]	139.7 ± 16.3	0.0 ± 0.0	43.55	2	0.0005	100.0 ± 0.0 a
336[b,c]	84.7 ± 6.4	0.0 ± 0.0	57.47	2	0.003	100.0 ± 0.0 a

[a]Means followed by different lower-case letters are significantly different among exposure times ($P < 0.05$; by Bonferroni *t* tests).

[b]Progeny production between 0 and 1 ppm spinosad was significant ($P < 0.05$; by two-sample *t* tests).

[c]Variances were unequal; *t*-values were determined by Satterthwaite method. All others had equal variance.

Table 3.4. Number (mean ± SE, n = 3) of kernels damaged by *S. zeamais* after exposure to untreated and treated popcorn after 42 d in clean popcorn[a].

Exposure time (h)	0 ppm	1 ppm	*t*-value	df	*P*-value	Reduction of damaged kernels in clean containers (%)[b]
1	391.7 ± 36.9	317.7 ± 79.2	1.02	4	0.3636	18.9 ± 20.2 cd
4	219.3 ± 35.3	336.0 ± 68.7	-1.50	4	0.2075	-53.2 ± 31.3 d
8	376.7 ± 22.2	327.0 ± 33.6	1.24	4	0.2826	13.2 ± 8.9 cd
12	368.3 ± 42.6	398.7 ± 33.4	-0.58	4	0.5942	-8.2 ± 9.1 d
24	205.3 ± 40.4	114.0 ± 16.7	2.41	4	0.0738	44.5 ± 8.1 bc
72	209.0 ± 47.6	28.7 ± 4.1	6.62	4	0.0027	86.3 ± 1.9 ab
72[c]	240.3 ± 25.8	7.3 ± 3.7	4.57	2.07	0.0418	96.9 ± 1.5 a
120[c,d]	363.3 ± 37.8	1.0 ± 1.0	11.45	4	0.0003	99.7 ± 0.3 a
168[c]	196.3 ± 11.1	0.0 ± 0.0	92.18	2	0.0001	100.0 ± 0.0 a

[a]Insect damaged kernels are expressed as number per 100 g of popcorn.

[b]Means followed by different lower-case letters are significantly different among exposure times ($P < 0.05$; by Bonferroni *t* tests).

[c]The number of kernels damaged were significantly different between 0 and 1 ppm spinosad ($P < 0.05$; by two-sample *t* tests).

[d]Variances were unequal; *t*-values were determined by Satterthwaite method. All others had equal variance.

Table 3.5. Adult emergence (mean ± SE, n = 3) of *S. zeamais* from the original plastic containers containing untreated and spinosad treated popcorn after 42 d.

Exposure time (h)	0 ppm	1 ppm	*t*-value	df	*P*-value	Adult emergence reduction (%)[a]
1[b]	0.0 ± 0.0	0.0 ± 0.0	n/a	n/a	n/a	n/a
4	0.7 ± 0.3	0.7 ± 0.7	0.22	4	0.8356	4.8 ± 95.2 ab
8	2.7 ± 0.7	2.7 ± 1.2	0.20	4	0.8477	1.2 ± 44.5 ab
12	1.7 ± 0.7	1.0 ± 0.6	0.83	4	0.4547	41.2 ± 34.0 ab
24	2.0 ± 0.6	13.7 ± 4.2	-4.69	4	0.0094	-583.3 ± 208.8 b
72	22.3 ± 7.5	15.0 ± 2.5	0.86	4	0.4375	32.7 ± 11.3 ab
120	32.0 ± 4.04	2.7 ± 1.8	4.29	4	0.0128	91.7 ± 5.5 a
168[c]	123.7 ± 7.3	4.7 ± 3.2	6.22	2.05	0.0234	96.2 ± 2.6 a
336	250.0 ± 47.7	11.7 ± 5.2	7.28	4	0.0019	95.3 ± 2.1 a

[a]Means followed by different lower-case letters are significantly different among exposure times ($P < 0.05$; by Bonferroni *t* tests).

[b]No statistic was generated by SAS as all values were 0.

[c]Variances were unequal; *t* value was determined by Satterthwaite method. All others had equal variance.

Table 3.6. Number (mean ± SE, n = 3) of kernels damaged by *S. zeamais* in untreated and spinosad treated popcorn in original containers after 42 d[a].

Exposure time (h)	0 ppm	1 ppm	*t*-value	df	*P*-value	Reduction of damaged kernels in original containers (%)[b]
1	0.7 ± 0.3	0.7 ± 0.3	0.00	4	1.0000	0.50 ± 49.8 ab
4	1.3 ± 0.3	2.0 ± 1.5	0.00	4	1.0000	-50.4 ± 114.9 b
8	3.3 ± 0.3	4.3 ± 0.3	-2.18	4	0.0946	-30.1 ± 10.0 b
12	2.7 ± 1.7	2.3 ± 1.3	0.09	4	0.9307	12.6 ± 49.9 ab
24	4.0 ± 0.6	18.7 ± 6.7	-3.66	4	0.0216	-336.7 ± 169.1 b
72	30.7 ± 9.8	20.3 ± 2.6	1.00	4	0.3726	33.7 ± 8.5 ab
120[c]	52.3 ± 7.5	5.0 ± 1.7	6.49	4	0.0029	90.4 ± 3.3 a
168[c,d]	176.7 ± 8.3	7.0 ± 4.0	6.73	2.04	0.0204	96.0 ± 2.3 a
336[c]	454.3 ± 42.4	14.7 ± 4.7	11.63	4	0.0003	96.8 ± 1.0 a

[a]Insect damaged kernels are expressed as number per 100g of popcorn.

[b]Means followed by different lower-case letters are significantly different among exposure times ($P < 0.05$; by Bonferroni *t* tests).

[c]There were significant differences in damaged kernels between 0 and 1 ppm spinosad ($P < 0.05$; by two sample *t* tests).

[d]Variances were unequal; *t* value was determined by Satterthwaite method. All others had equal variance.

Chapter 4 - Efficacy of methoprene, deltamethrin, and deltamethrin plus methoprene against *Sitophilus zeamais* in popcorn

Abstract

The efficacy of deltamethrin and methoprene at labeled rates, applied alone and in combination, was evaluated against a field strain of the maize weevil, *Sitophilus zeamais* Motschulsky in popcorn 28°C and 65% r.h. Fifty unsexed adults of *S. zeamais* (1-2 wk old) were introduced into 100g of untreated and insecticide treated popcorn. The exposure times were 1, 4, 8, 12, 24, 72, 120, 168 and 336 h. Adults were transferred to 100g clean untreated popcorn after the intended exposure. The mortality was assessed at 0, 7, 14, and 21 d after transferring, except those exposed to methoprene where only immediate mortality was determined. Adult progeny production was counted after 42 d of transferring surviving adults to clean popcorn. Adult emergence was evaluated after 42 d in the original popcorn sample. Damaged kernels were counted after the progeny or adult emergence assessment. In methoprene treated popcorn, *S. zeamais* showed low mortality, little progeny reduction, and high adult emergence. Meanwhile, the delayed toxicity of deltamethrin and deltamethrin plus methoprene was not significant for *S. zeamais* in popcorn. Seven day mortality of *S. zeamais* in deltamethrin-treated popcorn increased as exposure time increased. The range of *S. zeamais* mortality was 4.0 to 28.6% and 3.3 to 67.1% when exposed to 0.5 and 1.0 ppm deltamethrin, respectively, for 72 to 336 h. The highest progeny reduction was 70.8% and reduction in adult emergence was 96% popcorn treated with 1 ppm deltamethrin after a 336-h exposure. The 7 d mortality in deltamethrin plus methoprene treatments reached 43.6 and 70.5% after exposure to 0.5 deltamethrin plus 1.25 ppm methoprene and 1.0 deltamethrin plus 2.5 ppm methoprene, respectively, after a 336-h exposure. The highest

progeny reduction (96.2%) was achieved after exposure to 0.5 deltamethrin plus 1.25 ppm methoprene at 336-h in clean popcorn. The highest adult emergence reduction (99%) was observed in 1.0 deltamethrin plus 2.5 ppm methoprene treated popcorn in original containers at 336-h. In conclusion, methoprene alone cannot control *S. zeamais* adults, but when used along with deltamethrin, the insecticidal effect is significantly improved.

Introduction

Popcorn has been one of the first cereal snack food prepared by humans (Rooney and Serna-Saldivar, 1987), and became a commercial commodity in the United States by the 1890s (Brunson and Richardson, 1958). The maize weevil, *Sitophilus zeamais* Motschulsky, is an important and cosmopolitan insect pest of stored commodities (Hagstrum and Subramanyam, 2006; Santos et al. 1990). Popcorn is a specialty type of corn and it is vulnerable to insect infestation, especially with weevils of the genus *Sitophilus* (Coleoptera: Curculionidae) (Suleiman et al., 2015). Suleiman et al. (2015) reported that one kilogram of the yellow and white popcorn infested by 87 weevils after a 90-d incubation at 27°C produced yellow and white popcorn was 135 and 215 adults in yellow and white popcorn, respectively.

Chemical control is a traditional method for stored-product protection against insect pests, and residual insecticides can provide long-term protection (Arthur, 1996; White and Leesch, 1996; Arthur and Subramanyam, 2012). The combination of deltamethrin and methoprene, under the trade name Diacon® IGR Plus (Central Life Sciences, Schaumburg, IL), was approved to treat barley, corn, oats, popcorn, rice, rye, sorghum and wheat at the rate of 0.5 and 1.0 ppm of deltamethrin plus 1.25 and 2.5 ppm of methoprene in 2016 by the United States Environmental Protection Agency (EPA Reg. Number: 89459-86).

Several studies showed the effectiveness of deltamethrin or deltamethrin combined with other insecticides against insects associated with stored-grains (Arthur 1997a; Daglish, 1998; Kljajić and Perić, 2009). A dustable powder of deltamethrin at the concentration of 0.5 ppm was 100% effective against a laboratory strain of the granary weevil, *Sitophilus granarius* (Linnaeus), after 7 and 14 d of exposure in soft wheat of 12% moisture content at 25 ± 1ºC and 60 ± 5% r.h. (Kljajić and Perić, 2009). The dry formulation of deltamethrin (0.05% dust) at the application

rate of 3.54 g/m^2 caused 100% mortality of the lesser grain borer, *Rhyzopertha dominica* (Fabricius), and red flour beetle, *Tribolium castaneum* (Herbst) adults on concrete surfaces after a 24-h exposure (Arthur 1997a). Daglish (1998) reported using deltamethrin combined with other insecticides to kill adults of *R. dominica.* Wheat and corn were treated separately by emulsifiable concentrations of deltamethrin + piperonyl butoxide (0.25 + 8 ppm), chlorpyrifos-methyl + deltametrhin + piperonly butoxide (10 + 0.25 + 8 ppm), and pirimiphos-methyl + deltamethrin + piperonly butoxide (6 + 0.25 + 8 ppm). The mortality of *R. dominica* adults was over 99% after a 2-week exposure when treated wheat and corn was stored for 30 and 36 weeks, respectively.

Methoprene, a juvenile hormone analog, is another chemical used for stored-grain protection. Methoprene disrupts the development of immature stages and reduces the adult survival rate (Arthur, 2001). The adult emergence from fifth instars of the Indian meal moth, *Plodia interpunctella* (Hübner), was 30 to 40% while the adult emergence in controls was over 90% when exposed to methoprene treated Kraft paper at the application rate of 0.3 µg (AI) /cm^2 for 0.5, 1, and 2 h at 28ºC (Jenson et al., 2009). In another study, Jenson et al (2010a) reported that adult emergence was 92.5%, when *P. interpunctella* larvae were exposed to esfenvalerate alone at the application rate of 1.04 ml/m^3. By contrast, adult emergence from larvae after exposure to the combination of methoprene (1.07 mg/ m^3) and esfenvalerate (1.04 ml/m^3) treated shed at their respective application rates was only 0.91%.

Unlike laboratory strains, field strains are less susceptible to insecticides (Guedes, 1995; Lorini and Galley, 1999; Perez-Mendoza, 1999; Daglish and Nayak, 2018). Sehgal and Subramanyam (2014) reported that the mortality of six field strains of *T. castaneum* and three field strains of the sawtoothed grain beetle, *Oryzaephilus surinamensis* (Linnaeus) after a 7-d

exposure to deltamethrin treated wheat at 0.5 ppm was significantly lower than the laboratory strains. Collins (1990) reported that the LC_{50} of pyrethroid-resistant strain of *T. castaneum* was 59 ppm, compared to the susceptible strain which had an LC_{50} of 0.062 ppm in wheat at 25 ± 1ºC and 55 ± 2 % r.h. Guedes et al. (1995) demonstrated that six out of 12 field strains of *S. zeamais* had resistance to deltamethrin with the mortality ranging from 0 to 45% after exposure to insecticide-impregnated filter paper with a deltamethrin concentration of 2.50 mg/L.

Therefore, the evaluation of deltamethrin plus methoprene against field strains of insects is important to verify its performance in the field. The objectives of this study were to 1) determine the mortality of a field strain of *S. zeamais* adults in methoprene, deltamethrin, and deltamethrin plus methoprene treated popcorn at different exposure times; 2) evaluate adult progeny production at each exposure time; and 3) assess adult emergence in methoprene, deltamethrin, and deltamethrin plus methoprene treated popcorn.

Materials and Methods

Insect rearing, insecticides, and popcorn. Cultures of *S. zeamais* were reared in 0.95-L glass jars with approximately 250g of organic yellow corn (Heartland Mills, Marienthal, KS, USA) with a moisture content of 12.5% (wet basis). All cultures were reared in a growth chamber at 28°C and 65% r.h. A field strain of *S. zeamais* was collected from the farm-stored corn in Texas, USA in 2011. A liquid formulation of deltamethrin (Centynal™) containing 4.75% active ingredient (AI) was obtained from Central Life Sciences (Schaumburg, IL, USA). To obtain final deltamethrin solutions of 0.5 and 1.0 mg/ml, distilled water was mixed with 5.26 and 10.53 ml Centynal™ liquid, respectively, in a 500 ml volumetric flask. A liquid formulation of *S*-methoprene (Diacon®-IGR) containing 33.6 % (AI) was obtained from Central Life

Sciences. To obtain final methoprene solutions of 0.7, 1.4, and 2.8 mg/ml, distilled water was mixed with 1.04, 2.08, and 4.17 ml Diacon®-IGR liquid, respectively, in a 500 ml volumetric flask. A liquid formulation of deltamethrin plus *S*-methoprene (Diacon®-IGR Plus) containing 4.75% (AI) deltamethrin and 11.40% (AI) *S*-methoprene was obtained from Central Life Sciences. To obtain final deltamethrin solutions of 0.5 mg/ml deltamethrin plus 1.25 mg/ml methoprene and 1.0 mg/ml deltamethrin plus 2.5 mg/ml methoprene, distilled water was mixed with 5.26 and 10.53 ml Diacon®-IGR Plus liquid, respectively, in the 500 ml volumetric flask. Yellow Tenderflake popcorn was purchased from Popcorn County, USA (North Loup, NE, USA). Popcorn was frozen for two weeks at -13 ºC to kill any live insects present prior to use in tests.

Bioassays. One hundred microliters of deltamethrin, methoprene and deltamethrin plus methoprene final solution or distilled water (control treatment) were added to 100 g of popcorn held in 150-ml plastic containers. Each container was capped by a lid having one 10-mm round hole drilled in the middle and covered with 250 (µm) mesh screen to ensure air circulation and prevent insect escape. The plastic containers were shaken manually for 1 minute to ensure uniform distribution of the insecticide on popcorn kernels. After adding 50 unsexed, 1-2 week old *S. zeamais* adults from cultures, containers were kept in an environmental growth chamber set at 28°C and 65% r.h. The exposure times were 1, 4, 8, 12, 24, 72, 120, 168, and 336 h. Each insecticide-exposure time combination was replicated three times. After exposure, adults were transferred to 100 g of clean popcorn and kept in plastic containers.

In the methoprene bioassays, zero day adult mortality was assessed at the time of transfer to clean popcorn. Methoprene does not cause significant adult mortality based on previous studies (Oberlander and Silhacek, 2000; Daglish, 2008). Therefore mortality was not re-checked

after the initial assessment. Adult progeny production in the clean popcorn was determined at 42 d after transfer. Adult emergence in the original untreated and treated popcorn was assessed after 42 d of incubation. In the deltamethrin and deltamethrin plus methoprene treatments, zero day adult mortality was also assessed at the time of transferring. Post-exposure mortality was assessed 7, 14, and 21 d after transferring to clean popcorn. Adults that failed to respond when prodded with a Camel's hair brush were considered dead. Adult progeny production in the clean popcorn was determined at 42 d after transfer. Adult emergence in the original untreated and treated popcorn was assessed after 42 d of incubation. Kernels with feeding and adult emergence holes were considered as damaged. Damaged kernels from the clean popcorn were evaluated at the same time when the progeny was counted. Damaged kernels in the original popcorn were evaluated when adult emergence was assessed.

Data analysis. Mortality of *S. zeamais* adults in treatments was calculated as a percentage: [(number of dead adults ÷ /50) × 100], and corrected for control mortality (Abbott, 1925). One-way analysis of variance (ANOVA) was used to analyze significant differences in corrected mortality among different exposure times for each insecticide and concentration. Means were separated by using Bonferroni *t* tests at $\alpha = 0.05$ (SAS Institute, 2013). The significance of cumulative post-exposure mortality was analyzed by Cochran-Armitage test (Cochran, 1954; Armitage, 1955). If the 95% confidence interval (CI) of the Pearson and Spearman correlation coefficient does not contain zero, there is a significant difference in post-exposure mortality for each insecticide and concentration (SAS Institute, 2013).

The adult progeny production in clean popcorn was calculated as: (total number of adults in clean popcorn after 42 d – 50 originally added adults). The reduction in adult progeny production relative to production in the control treatment was calculated as: (1 – treatment

progeny ÷ control progeny) ×100. Adult emergence was reported as the total number of adults that emerged in the original untreated and treated popcorn after 42 d. Damaged kernels were expressed as a percentage: (number of damaged kernels ÷ total number of kernels) × 100. The percentage of progeny reduction and percentage of damaged kernels were transformed to angular values (Zar, 1984), and adult emergence data were transformed to $\log_{10}(x+1)$ scale to normalize treatment variances prior to subjecting data to ANOVA. Means were separated by Bonferroni *t* tests at $\alpha = 0.05$ (SAS Institute, 2013).

Results

Efficacy of methoprene, deltamethrin, and deltamethrin plus methoprene against *S. zeamais*. According to Cochran-Armitage test results of deltamethrin treatments, the 95% confidence interval (CI) for the Pearson and Spearman correlation coefficients contained zero for all exposure times at the concentration of 0.5 and 1.0 ppm, respectively. The same results were found in deltamethrin plus methoprene treatments. Therefore, the cumulative post-exposure mortality showed no delayed toxicity effects among 0, 7, 14, and 21 d assessment times either in deltamethrin and deltamethrin plus methoprene treated popcorn. For deltamethrin and deltamethrin plus methoprene treatments, 7 d mortality results were reported in Tables 4.1 and 4.2.

The control mortality of *S. zeamais* ranged from 0.0 to 4.7 %, 0 to 8.7%, and 0.0 to 2.0% in deltamethrin, methoprene, and deltamethrin plus methoprene treatments across all exposure times, respectively (Table 4.1). Corrected mortality of *S. zeamais* after exposure to deltamethrin, methoprene, and deltamethrin plus methoprene treated popcorn is shown in Table 4.2. In deltamethrin and deltamethrin plus methoprene treatments, mortality increased as exposure time

increased, except in methoprene treatments. Deltamethrin and deltamethrin plus methoprene caused significantly higher mortality of *S. zeamais* than methoprene alone after 72 – 336 h exposure. The 0 d mortality of *S. zeamais* was less than 4.4% among three concentrations of methoprene after 1 – 336 h of exposure. The 7 d mortality of *S. zeamais* was above 10% after a 120-h exposure in both deltamethrin and deltamethrin plus methoprene treatments. The highest 7 d mortality (67.1% and 70.5%) was achieved when adults were exposed to concentrations of 1 ppm deltamethrin and 1 ppm deltamethrin plus 2.5 ppm methoprene, respectively, after a 336-h exposure.

Efficacy of insecticides in suppressing adult progeny production. In methoprene treated popcorn, the average progeny reduction of *S. zeamais* ranged from -67.4 to 26.2 %, -27.4 to 15.2 % and -113.0 to 18.8 % at concentrations of 0.7, 1.4 and 2.8 ppm, respectively (Table 4.3). The negative values indicated that more progeny were produced in the treatments compared to control. Progeny reduction was significantly different among exposure times except for 1.4 ppm treatment. The highest progeny reduction was achieved after 120-h and 168-h exposure, at concentrations of 0.7 and 2.8 ppm, respectively.

In deltamethrin treatments the highest progeny reductions were 53.3 and 70.8% when adults were exposed to 0.5 and 1.0 ppm treated popcorn, respectively, at 336 h. Only at a concentration of 1.0 ppm, progeny reduction was significantly different among all exposure times. There were no significant differences in progeny reduction between 0.5 and 1.0 ppm concentrations at each exposure time (Table 4.3).

In deltamethrin plus methoprene treatments, the highest progeny reduction (96.2%) was achieved when exposed to 0.5 deltamethrin plus 1.25 ppm methoprene after 336 h of exposure.

The progeny reduction in deltamethrin and deltamethrin plus methoprene was significantly higher than in methoprene treated popcorn at 336-h (Table 4.3).

Effects of insecticides on egg-to-adult emergence. In methoprene treated popcorn, two-way ANOVA showed that adult emergence was significant among exposure times (F = 98.81; df =8, 72; $P < 0.0001$), but not among concentrations and the interaction of concentration and exposure time (F range = 1.29 – 1.45; df, concentration = 3, 72; df, interaction = 24, 72; P, range = 0.2011 – 0.2351) (Table 4.4). The number of adults that emerged increased as exposure time increased for each concentration, and the 336-h exposure resulted the highest adult emergence, since more eggs were laid by *S. zeamais* during this long exposure time. Number of adults that emerged was similar between control and treatments for each exposure time except at 336 h, where treatments had significantly more adults emerged than the control.

In deltamethrin treatments, two-way ANOVA of adult emergence data indicated significant differences between concentrations, among exposure times, and interaction of concentration and exposure time (F range = 4.77 – 108.75; df, concentration = 2, 54; df, exposure time = 8, 54; df, interaction = 16, 54; $P < 0.0001$) (Table 4.5). More adults emerged as exposure time increased. The number of adults that emerged in control samples was significantly higher than in treatments at exposure times of 24-336 h. Higher concentrations resulted in lower adult emergence, however, the differences were not statistically significant, except at 336-h.

In deltamethrin plus methoprene treatments, two-way ANOVA of adult emergence was significant among concentrations, exposure times, and the interaction of concentration and exposure time (F range = 6.72 – 58.29; concentration = 2, 54; df, exposure time = 8, 54; df, interaction= 16, 54; $P < 0.0001$) (Table 4.5). Similar to results with deltamethrin, the number of adults that emerged in control samples was significantly higher than in treatments after 24 – 336

h of exposure, and there were no significant differences between the two concentrations tested at each exposure time.

Damaged kernels in original and clean popcorn. In the original methoprene treated popcorn, there were significant differences in percentage of damaged kernels among concentrations, exposure times, and the interaction of concentration and exposure time (F range = 3.52 – 220.96; df, concentration = 3, 72; df, exposure time = 8, 72; df, interaction = 24, 72; $P <$ 0.0086; two-way ANOVA) (Table 4.6). Damaged kernels (%) increased as the exposure time increased in the original popcorn. There were no significant differences among concentrations for each exposure time except at 336 h. Damaged kernels were 47.0 – 83.3% in treatments, which was significantly higher than in the control (37.7%) after a 336-h exposure. Damaged kernels after 42-d incubation in clean popcorn is shown in Table 4.6. Two-way ANOVA showed significant effects of among concentrations and exposure time (F range = 2.91 – 7.28; df, concentration = 3, 72; df, exposure time = 8, 72; $P < 0.0403$; two-way ANOVA), but the interaction of concentration and exposure time was not significant ($F = 1.07$; df = 24, 72; $P =$ 0.3962). Damaged kernels were significantly different among exposure times at the methoprene concentration of 0.7 and 2.8 ppm. The highest percentage of damaged kernels was 83.3% and 78.0% at concentrations of 0.7 and 2.8 ppm, respectively. Damaged kernels were not different among methoprene concentrations at each exposure time.

In original deltamethrin treated popcorn, there were significant effects in damaged kernels among concentrations, exposure times, and the interaction of concentration and exposure time (F range = 18.22 – 185.41; df, exposure time = 8, 54; df, concentration = 2, 54; df, interaction = 16, 54; $P < 0.0001$; two-way ANOVA) (Table 4.7). The percentage of damaged kernels in control samples was significantly higher than in treatments after 24 – 336 h of

exposure. At the low deltamethrin concentration samples generally had higher percentage of damaged kernels, but differences were not significant, except at 336 h where the percentage of damaged kernels was significantly higher in 0.5 ppm samples than in 1.0 ppm samples. Damaged kernels increased as exposure times increased at all concentrations. The highest percentage of damaged kernels was 77.5, 27.2 and 9.5%, when exposed to deltamethrin concentrations of 0, 0.5, and 1.0 ppm, respectively, at 336 h. In clean popcorn two-way ANOVA indicated the significant effects of among concentrations and exposure times on the percentage of damaged kernels (F range = 7.92 – 9.09; df, exposure time = 8, 54; df, concentration = 2, 54; $P <=$ 0.0010), but interaction of concentration and exposure time was not significant (F = 1.30; df = 16, 54; P = 0.2320) (Table 4.7). Damaged kernels were significantly different among exposure times at the concentration of 0 and 1.0 ppm, respectively. More kernels were damaged at lower than high concentrations, and this trend was significant only at 12 h of exposure, where the percentage of damaged kernels was 63.1, 48.7 and 38.0% at concentrations of 0, 0.5, and 1.0 ppm, respectively.

In the original deltamethrin plus methoprene treated popcorn, differences in damaged kernels were significant among concentrations, exposure times, and the interaction of concentration and exposure time (F range = 8.28 – 63.65; df, concentration = 2, 54; df, exposure time = 8, 54; df, interaction= 16, 54; $P < 0.0001$; two-way ANOVA) (Table 4.8). There were significantly more damaged kernels in treatments than control samples; however, the percentage of damaged kernels were similar between the concentrations tested at 24 – 336 h exposures. More damaged kernels were observed with an increase in exposure time at each concentration. The highest percentage of damaged kernels was 69.1, 14.0, and 5.4% in control, low, and high concentration treatments, respectively, at 336 h. In clean popcorn, percentage of damaged

kernels were significant among concentrations, exposure times, and the interaction of concentration and exposure time (F range = 1.97 – 19.66; df, concentration = 2, 54; df, exposure time = 8, 54; df, interaction = 16, 54; $P < 0.0331$; two-way ANOVA) (Table 4.8). The percentage of damaged kernels in control samples was significantly higher than in treatments after 72, 120, and 336 h of exposure. However, no significant differences were found between the concentrations tested.

Discussion

The present study indicated that methoprene did not cause mortality of *S. zeamais* adults. Similar results were reported by Daglish (2008), who indicated the mortality of *S. oryzae* to be 0.7% in methoprene treated wheat (0.6 ppm) after a 2-wk exposure. Athanassiou et al. (2011) found that the granary weevil, *Sitophilus granarius* (Linnaeus) adults showed 1.1 and 4.4% mortality in methoprene treated wheat at concentrations of 1 and 5 ppm, respectively; while the mortality in the control was 1.1%. *S. zeamais* is an internal insect species, and females oviposit directly inside the kernels. Immature stages complete inside the kernels, therefore are not subjected to pesticide residues from the grain surface. In our study, adult progeny production and emergence were not significantly suppressed in methoprene treated popcorn. Athanassiou et al. (2011) reported adult progeny production of *S. granariu*s was 119 and 103 in methoprene treated wheat at concentrations of 1 and 5 ppm, respectively; while the adult progeny in control were 134.

Since methoprene cannot control *S. zeamais*, deltamethrin and deltamethrin plus methoprene were evaluated in our study. The TX strain of *S. zeamais* showed highest mortality of 67.1 and 70.5% in deltamethrin and deltamethrin plus methoprene treatments, respectively, at

the highest label rates after a 336 h of exposure. Previous studies have shown that the efficacy of deltamethrin can vary between insecticide formulations, insect strains, insect species, and exposure times (Jain and Yadav, 1989; Arthur 1994; Ceruti and Lazzari, 2005; Kavallieratos et al., 2015). Deltamethrin wettable powder formulation was more effective than emulsifiable concentrate based on the results of Jain and Yadav (1989). The cowpea weevil, *Callosobruchus maculatus* Fabricius and the adzuki bean weevil, *Callosobruchus chinensis* Linnaeus were killed completely when exposed to 10 mg/m^2 wettable powder of deltamethrin on glass, cement, aluminum, tile, painted and unpainted plywood, mud, black polythene, filter paper, and jute surfaces; while emulsifable concentrate of deltamethrin only caused 100% mortality for *C. maculatus* and *C. chinensis* on glass, aluminum, tile, and unpainted plywood at the same concentration. The study by Ceruti and Lazzari (2005) reported that a dust formulation of deltamethrin achieved 100% mortality of *S. zeamais* within 6 d of exposure on treated corn at a concentration of 0.5 ppm and in 3 d at a concentration of 1.0 ppm. Arthur (1994) used emulsifiable concentrate of deltamethrin to treat wheat or corn at the concentration of 0.5 ppm and stored for up to 10 months, and bioassays with treated grain was conducted every two months. The mortality of *S. oryzae* adults was 15 to 45% when exposed to treated wheat after a 5-d exposure. The survival of *S. zeamais* ranged from 3.5 to 23.5% after a 5-d exposure in treated corn, while the survival rate ranged from 92 to 96.5% on untreated corn.

Our study showed no delayed toxicity effects in *S. zeamais* either in deltamethrin or deltamethrin plus methoprene treatments. Similar results at short exposure times were reported by Kavallieratos et al (2015), where they reported immediate mortality and 7-d mortality of *R. dominica*, *S. granarius*, and *T. castaneum* after exposure to 0.5 ppm deltamethrin treated brown

rice for 24 h. They reported that the mortality of adults observed immediately and after 7 d for all three species did not exceed 7%.

The highest progeny reduction at each concentration of deltamethrin and deltamethrin plus methoprene treatments is shown after a 336 h exposure. The low progeny production in deltamethrin and deltamethrin plus methoprene treated popcorn was probably due to high mortality at longer exposure treatments, since less eggs were laid by the fewer surviving adults. Arthur (1994) reported that no progeny production of *S. zeamais* were observed in deltamethrin treated corn at concentration of 0.5, 0.75 and 1.0 ppm. Athanassiou et al. (2004) reported that progeny of *S. oryzae* were 67.7 and 5.3 after 33 d exposure to deltamethrin treated wheat at concentrations of 0.125 and 0.25 ppm, respectively. However, Kavallieratos et al. (2015), reported progeny production of *S. oryzae* and *S. zeamais* to beover 300 adults in 0.5 ppm deltamethrin treated rice, and it did not differ from progeny produced in the control treatment. Daglish (1998) reported no progeny production of *S. oryzae* after exposure to chlorpyrifos-methyl (10 ppm) + methoprene (1 ppm) and chlorpyrifos-methyl (10 ppm) + deltamethrin (0.25 ppm) + piperonyl butoxide (8 ppm) treated wheat.

Adult emergence in the original treated popcorn indicated that *S. zeamais* adults can mate and lay eggs inside popcorn during insecticide exposure. Very few adults emerged in a 1 to 12 h treatments, which may be due to limited time for oviposition before the removal of parental adults. The current study showed that *S. zeamais* adult emergence reduction was significant in deltamethrin, and deltamethrin plus methoprene treatments at 24 – 336 h exposures. Evans (1985) reported that no larvae, pupae or adults emerged from *T. castaneum* eggs in deltamethrin treated corn at the concentration of 1 ppm after a 70-d exposure, while there were 73 larvae and pupae and 53 adults in control samples. In Jenson's et al. (2010b) study, the percentage of adult

emergence from eggs of *P. interpunctella* was around 40%, when 3% pyrethrin was applied alone to a protein bar, and the adult emergence reduced to 10% when 3% pyrethrin plus methoprene was applied.

Damaged kernels in clean popcorn were caused by surviving *S. zeamais* adults and their progeny. Insect population density is one of the factors contributing to grain damage (Tefera et al., 2011). *S. zeamais* populations increased to 35, 122, and 174 in 200 g corn after 60 d of storage when the original adults were 5, 25, and 50, respectively. Meanwhile the percentage of damaged kernels was 3.5, 7.4 and 11.4%, respectively (Tefera et al, 2011). In the current study, damaged kernels in original containers were mostly caused by egg-to-adult development. Ileke and Oni (2011) reported that the percentage of damaged kernels was 0.9 and 5.3% in plant powder (*Garcina kola* and *Moringa oleifera*) treated wheat after the 42 d exposure, while it was 22.7% in controls.

In conclusion, our results showed that deltamethrin and deltamethrin plus methoprene were more effective than methoprene alone against TX strain of *S. zeamais*. Progeny production, adult emergence, and damaged kernels were reduced significantly in deltamethrin and deltamethrin plus methoprene treated popcorn. Since *S. zeamais* adults did not achieve complete mortality, it is necessary to combine deltamethrin and deltamethrin plus methoprene with other insecticides to successfully control the field strain of *S. zeamais* in popcorn.

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Table 4.1. Mortality (%mean ± SE, *n* = 3) of *S. zeamais* adults in control samples corresponding to methoprene, deltamethrin, and deltamethrin plus methoprene treatments after 0, 7, and 7 d, respectively.

Exposure time (h)	Methoprene	Deltamethrin	Deltamethrin plus methoprene
1	0.0 ± 0.0	1.3 ± 1.3	0.0 ± 0.0
4	0.0 ± 0.0	1.3 ± 0.7	2.0 ± 0.0
8	0.0 ± 0.0	0.0 ± 0.0	1.3 ± 0.7
12	1.3 ± 1.3	1.3 ± 1.3	0.0 ± 0.0
24	0.0 ± 0.0	0.7 ± 0.7	0.0 ± 0.0
72	1.3 ± 0.7	0.0 ± 0.0	0.0 ± 0.0
120	0.0 ± 0.0	0.7 ± 0.7	0.0 ± 0.0
168	8.7 ± 4.7	1.3 ± 0.7	1.3 ± 1.3
336	2.7 ± 1.3	4.7 ± 2.4	0.7 ± 0.7

Table 4.2. Corrected mortality (% mean ± SE, *n* = 3) of *S. zeamais* adults in methoprene, deltamethrin, and deltamethrin plus methoprene treated popcorn after 0, 7, and 7 d, respectively.

Exposure time (h)	Methoprene (ppm)			Deltamethrin (ppm)		Deltamethrin plus methoprene (ppm)	
	0.7	1.4	2.8	0.5 [a]	1.0[a]	0.5 D + 1.25 M[a]	1.0 D + 2.5 M[a]
1	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.7 ± 1.2b	0.0 ± 0.0b	0.7 ± 0.7b	4.0 ± 3.1c
4	0.0 ± 0.0	0.0 ± 0.0	2.0 ± 1.2	0.0 ± 0.0b	0.0 ± 1.4b	0.7 ± 0.7b	0.0 ± 0.0c
8	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	2.0 ± 0.0b	2.7 ± 1.8b	0.7 ± 0.0b	2.0 ± 0.7c
12	0.0 ± 0.0	0.0 ± 1.4	0.0 ± 0.0	0.0 ± 0.0b	0.7 ± 1.2b	0.7 ± 0.7b	0.7 ± 0.7c
24	0.7 ± 0.7	1.3 ± 0.7	0.0 ± 0.0	3.3 ± 1.2b	2.7 ± 1.3b	3.3 ± 1.3b	4.7 ± 1.8c
72[b]	0.0 ± 0.0B	0.0 ± 0.0B	0.0 ± 1.4B	4.0 ± 1.2bAB	3.3 ± 1.3bAB	3.3 ± 1.3bAB	6.7 ± 1.3cA
120[b]	1.3 ± 0.7B	4.0 ± 1.2B	0.7 ± 0.7B	9.4 ± 1.2bAB	14.1 ± 7.0bAB	13.3 ± 2.4bAB	22.7 ± 0.7bA
168[b]	0.0 ± 0.0D	4.4 ± 4.8D	0.0 ± 0.0D	27.7 ± 4.1aBC	50.7 ± 10.6aAB	33.1 ± 4.2aABC	57.4 ± 6.2aA
336[b]	0.0 ± 0.0C	0.7 ± 1.8C	2.1 ± 3.0C	28.6 ± 4.4aB	67.1 ± 2.5aA	43.6 ± 5.3aB	70.5 ± 4.1aA

[a]Means followed by different lower-case letters are significantly different among exposure times at a concentration ($P < 0.05$; by Bonferroni *t* tests); [b]Means followed by different upper-case letters are significantly different among concentrations among insecticide concentrations at a given exposure time ($P < 0.05$; by Bonferroni *t* tests).

Table 4.3. Progeny reduction % (mean + SE, *n* = 3) at 42 d of *S. zeamais* after exposure to methoprene, deltamethrin, and deltamethrin plus methoprene and incubated in clean popcorn for 42 d.

Exposure time (h)	Methoprene (ppm)			Deltamethrin (ppm)		Deltamethirn plus methoprene (ppm)	
	0.7[a]	1.4	2.8[a]	0.5	1.0[a]	0.5 D + 1.25 M[a]	1.0 D + 2.5 M[a]
1[b]	–46.8 ± 5.4abB	–27.4 ± 25.3AB	–6.8 ± 23.1aAB	36.1 ± 10.6A	46.9 ± 1.5abA	43.0 ± 19.2abA	47.2 ± 6.4aA
4	–8.7 ± 15.2ab	3.5 ± 25.4	–12.7 ± 6.5a	–2.7 ± 20.2	–2.7 ± 0.9b	–4.3 ± 19.9b	34.0 ± 20.4ab
8[b]	–32.0 ± 27.3abB	–18.7 ± 14.4AB	–26.3 ± 3.8abAB	46.8 ± 5.8A	42.5 ± 13.0abAB	11.1 ± 23.1bAB	44.7 ± 13.7aAB
12[b]	12.1 ± 18.3abA	13.0 ± 1.6AB	–2.6 ± 8.9aAB	28.8 ± 5.2AB	41.4 ± 6.5abAB	-9.7 ± 8.0bB	21.0 ± 10.0abAB
24[b]	–4.6 ± 14.8abA	12.3 ± 1.4AB	–27.4 ± 11.1abAB	22.9 ± 9.0AB	34.8 ± 17.9abAB	–19.6 ± 5.2bAB	-53.2 ± 28.2bB
72	11.1 ± 13.0ab	–24.0 ± 31.5	–24.9 ± 16.2ab	23.3 ± 33.7	40.6 ± 17.9ab	43.3 ± 17.5ab	48.3 ± 20.8a
120	26.2 ± 2.8a	11.5 ± 19.7	–33.0 ± 22.9ab	14.9 ± 20.4	12.8 ± 2.6ab	34.4 ± 3.6ab	37.0 ± 6.7a
168	–14.6 ± 22.6ab	–16.5 ± 52.5	18.8 ± 20.4a	10.1 ± 20.0	54.4 ± 21.4ab	18.9 ± 26.4ab	38.4 ± 21.2a
336[b]	–67.4 ± 9.0bBC	15.2 ± 15.1BC	–113.0 ± 33.4bC	53.3 ± 32.6A	70.8 ± 8.6aA	96.2 ± 3.2aA	75.6 ± 3.6aA

[a]Means followed by different lower-case letters are significantly different among exposure times at a concentration($P < 0.05$; by Bonferroni *t* tests); [b]Means followed by different upper-case letters are significantly different among concentrations at a given exposure time ($P < 0.05$; by Bonferroni *t* tests).

Table 4.4. *S. zeamais* adult emergence (mean ± SE, *n* = 3) after exposure to methoprene treated popcorn and incubated for 42 d in original popcorn.

Exposure time (h)	Popcorn originally treated with methoprene (ppm)			
	0[a]	0.7[a]	1.4[a]	2.8[a]
1	1.3 ± 1.3d	0.0 ± 0.0e	0.0 ± 0.0e	0.0 ± 0.0d
4	0.3 ± 0.3d	0.7 ± 0.7e	0.0 ± 0.0e	0.0 ± 0.0d
8	5.7 ± 5.2bcd	2.3 ± 0.9de	1.0 ± 0.0de	1.0 ± 0.6cd
12	1.3 ± 0.9cd	0.7 ± 0.7e	0.7 ± 0.7de	0.3 ± 0.3d
24	9.0 ± 1.5abcd	12.0 ± 6.7cd	4.7 ± 2.0cd	9.0 ± 5.0bc
72	10.3 ± 5.8bcd	33.0 ± 6.1bc	31.7 ± 7.4b	33.3 ± 4.3b
120	30.0 ± 5.5abc	19.0 ± 7.5bc	19.3 ± 10.0bc	26.0 ± 7.6b
168	50.0 ± 18.5ab	58.0 ± 2.6ab	24.0 ± 5.3b	23.3 ± 12.8b
336[b]	121.0 ± 18.6aB	250.3 ± 22.0aA	156.7 ± 26.9aAB	236.7 ± 19.3aA

[a]Means followed by different lower-case letters are significantly different among exposure times at a concentration($P < 0.05$; by Bonferroni *t* tests); [b]Means followed by different upper-case letters are significantly different among concentrations at a given exposure time ($P < 0.05$; by Bonferroni *t* tests).

Table 4.5. *S. zeamais* adult emergence (mean ± SE, $n = 3$) after exposure to deltamethrin, and deltamethrin plus methoprene treated popcorn and incubated for 42 d in original popcorn.

Exposure time (h)	Deltamethrin (ppm)			Deltamethrin plus methoprene (ppm)		
	0[a]	0.5[a]	1.0[a]	0[a]	0.5 D + 1.25 M[a]	1.0 D + 2.5 M[a]
1	0.0 ± 0.0e	0.0 ± 0.0c	0.0 ± 0.0c	0.0 ± 0.0e	0.0 ± 0.0d	0.0 ± 0.0b
4	0.3 ± 0.3e	0.0 ± 0.0c	0.0 ± 0.0c	0.0 ± 0.0e	0.0 ± 0.0d	0.0 ± 0.0b
8	0.7 ± 0.7e	0.0 ± 0.0c	0.0 ± 0.0c	1.0 ± 0.0eA	0.0 ± 0.0dB	0.0 ± 0.0bB
12	0.3 ± 0.3e	0.0 ± 0.0c	0.0 ± 0.0c	0.0 ± 0.0e	0.0 ± 0.0d	0.0 ± 0.0b
24[b]	9.3 ± 1.8dA	1.0 ± 1.0cB	2.0 ± 1.0bAB	10.0 ± 2.1dA	5.3 ± 2.0bcdAB	0.7 ± 0.7bB
72[b]	35.7 ± 7.0cAB	6.0 ± 5.0bcAB	2.3 ± 0.7bB	48.0 ± 10.5cA	1.7 ± 1.7cdB	6.7 ± 2.0abAB
120[b]	93.7 ± 17.3bcA	12.7 ± 7.6bcB	4.3 ± 1.3bB	107.7 ± 17.1bcA	17.3 ± 13.3abcB	1.0 ± 0.6abB
168[b]	155.7 ± 18.6abA	26.3 ± 12.5abB	20.0 ± 3.5aB	120.0 ± 27.8bA	32.3 ± 19.3abAB	5.0 ± 2.6abB
336[b]	320.0 ± 15.3aA	129.7 ± 12.4aB	32.7 ± 6.8aC	270.7 ± 38.3aA	58.7 ± 14.4aAB	25.0 ± 20.0aB

[a]Means followed by different lower-case letters are significantly different among exposure times at the same concentration ($P < 0.05$; by Bonferroni *t* tests); [b]Means followed by different upper-case letters are significantly different among concentrations at the same exposure time in deltamethrin or deltamethrin plus methoprene treatments ($P < 0.05$; by Bonferroni *t* tests).

Table 4.6. Damaged kernels (% mean ± SE, *n* = 3) in original and clean popcorn at 42 d after exposure to methoprene.

Exposure time (h)	Original popcorn (ppm)				Clean popcorn (ppm)			
	0 [a]	0.7 [a]	1.4 [a]	2.8 [a]	0 [a]	0.7 [a]	1.4 [a]	2.8 [a]
1	0.3 ± 0.3d	0.0 ± 0.0e	0.0 ± 0.0d	0.0 ± 0.0d	50.0 ± 3.5	77.7 ± 3.9ab	58.7 ± 9.1	55.3 ± 13.0ab
4	0.0 ± 0.0d	0.3 ± 0.3de	0.0 ± 0.0d	0.0 ± 0.0d	56.7 ± 11.0	63.7 ± 7.8ab	60.0 ± 13.5	65.0 ± 2.1ab
8	2.7 ± 1.3cd	0.7 ± 0.3de	0.3 ± 0.3d	0.3 ± 0.3d	60.0 ± 7.5	83.3 ± 8.7a	69.3 ± 6.4	66.3 ± 1.2ab
12	1.0 ± 0.0d	0.0 ± 0.0e	0.0 ± 0.0d	0.0 ± 0.0d	77.3 ± 5.8	77.3 ± 7.7ab	74.0 ± 2.6	78.0 ± 6.0a
24	3.0 ± 1.0bcd	4.3 ± 2.0cd	1.7 ± 0.7cd	2.3 ± 1.3cd	59.0 ± 6.7	60.7 ± 10.4ab	45.3 ± 0.3	71.7 ± 2.7a
72	4.0 ± 1.0bcd	10.3 ± 1.7bc	9.0 ± 1.7bc	11.3 ± 2.4b	60.7 ± 12.9	61.3 ± 13.7ab	70.0 ± 14.2	75.7 ± 3.8a
120	9.3 ± 1.3bc	7.0 ± 2.0bc	5.7 ± 2.7bc	8.3 ± 2.4bc	51.3 ± 17.1	39.7 ± 2.4b	50.0 ± 4.7	67.0 ± 8.9ab
168	14.3 ± 4.1b	16.3 ± 0.9b	9.7 ± 0.9b	9.7 ± 2.7b	40.3 ± 4.1	44.0 ± 2.9ab	44.0 ± 14.4	35.3 ± 7.4b
336[b]	37.7 ± 5.7aC	76.7 ± 6.9aAB	47.0 ± 8.3aBC	83.3 ± 3.3aA	37.0 ± 7.2	57.0 ± 6.1ab	29.3 ± 4.7	61.3 ± 7.7ab

[a]Means followed by different lower-case letters are significantly different among exposure times at the same concentration ($P < 0.05$; by Bonferroni *t* tests); [b]Means followed by different upper-case letters are significantly different among concentrations at a given exposure time in original treated popcorn ($P < 0.05$; by Bonferroni *t* tests). There were no significant differences on clean popcorn.

Table 4.7. Damaged kernels (% mean ± SE, *n* = 3) at 42 d in original and clean popcorn after exposure to deltamethrin.

Exposure time (h)	Original popcorn (ppm)			Clean popcorn (ppm)		
	0 [a]	0.5 [a]	1.0 [a]	0 [a]	0.5	1.0 [a]
1	0.0 ± 0.0e	0.0 ± 0.0d	0.0 ± 0.0b	33.8 ± 6.3bc	32.3 ± 2.2	28.7 ± 0.9ab
4	0.2 ± 0.2de	0.0 ± 0.0d	0.0 ± 0.0b	38.3 ± 9.0abc	42.9 ± 5.7	40.6 ± 1.1ab
8	0.4 ± 0.4de	0.0 ± 0.0d	0.2 ± 0.2b	55.6 ± 5.8abc	34.4 ± 4.2	41.4 ± 5.8ab
12	0.7 ± 0.4de	0.0 ± 0.0d	0.0 ± 0.0b	63.1 ± 3.9abcA	48.7 ± 0.2B	38.0 ± 2.2abC
24 [b]	2.6 ± 0.3cdA	0.4 ± 0.2cdB	0.4 ± 0.2bB	61.5 ± 1.8abc	60.8 ± 8.2	41.8 ± 10.1ab
72 [b]	7.1 ± 1.4cA	1.7 ± 1.1bcB	1.1 ± 0.4bB	72.4 ± 13.3ab	51.2 ± 15.3	62.9 ± 13.9a
120 [b]	22.1 ± 4.1bA	2.6 ± 1.0bcB	1.3 ± 0.4bB	61.5 ± 14.5abc	67.8 ± 15.6	60.1 ± 10.0ab
168 [b]	33.6 ± 3.9bA	5.1 ± 0.7bB	6.4 ± 0.6aB	78.7 ± 1.4a	59.1 ± 17.9	26.2 ± 10.8ab
336 [b]	77.5 ± 1.9aA	27.2 ± 2.8aB	9.5 ± 3.3aC	27.9 ± 1.2c	18.0 ± 7.9	10.5 ± 2.3b

[a]Means followed by different lower-case letters are significantly different among exposure times at the same concentration ($P < 0.05$; by Bonferroni *t* tests); [b]Means followed by different upper-case letters are significantly different among concentrations at a given exposure time in original treated or clean popcorn ($P < 0.05$; by Bonferroni *t* tests).

Table 4.8. Damaged kernels (% mean ± SE, *n* = 3) in original and clean popcorn at 42 d after exposure to deltamethrin plus methoprene.

Exposure time (h)	Original popcorn (ppm)			Clean popcorn (ppm)		
	0 [a]	0.5 D + 1.25 M [a]	1.0 D + 2.5 M [a]	0 [a]	0.5 D + 1.25 M [a]	1.0 D + 2.5 M [a]
1	0.0 ± 0.0d	0.0 ± 0.0c	0.0 ± 0.0b	33.8 ± 6.3ab	32.3 ± 2.2a	28.7 ± 0.9ab
4	0.2 ± 0.2d	0.0 ± 0.0c	0.0 ± 0.0b	38.3 ± 9.0ab	42.9 ± 5.7a	40.6 ± 1.1ab
8	0.8 ± 0.2cd	0.2 ± 0.2c	0.2 ± 0.2ab	55.6 ± 5.8ab	34.4 ± 4.2a	41.4 ± 5.8ab
12	0.4 ± 0.2d	0.0 ± 0.0c	0.0 ± 0.0b	63.1 ± 3.9ab	48.7 ± 0.2a	38.0 ± 2.2a
24 [b]	1.5 ± 0.2cdA	0.9 ± 0.2bcAB	0.2 ± 0.2abB	61.5 ± 1.8a	60.8 ± 8.2a	41.8 ± 10.1a
72 [b]	10.6 ± 1.7bcA	0.7 ± 0.4bcB	2.8 ± 1.1abB	72.4 ± 13.3aA	51.2 ± 15.3aB	62.9 ± 13.9aB
120 [b]	23.7 ± 5.7bA	5.1 ± 3.3abB	0.9 ± 0.3abB	61.5 ± 14.5aA	67.8 ± 15.6aB	60.1 ± 10.0aB
168 [b]	23.4 ± 6.0bA	5.5 ± 2.1abB	1.3 ± 0.7abB	78.7 ± 1.4a	59.1 ± 17.9a	26.2 ± 10.8ab
336 [b]	69.1 ± 15.0aA	14.0 ± 2.0aB	5.4 ± 3.9aB	27.9 ± 1.2bA	18.0 ± 7.9bB	10.5 ± 2.3bB

[a]Means followed by different lower-case letters are significantly different among exposure times at the same concentration ($P < 0.05$; by Bonferroni *t* tests); [b]Means followed by different upper-case letters are significantly different among concentrations at a given exposure exposure time in original treated or clean popcorn ($P < 0.05$; by Bonferroni *t* tests).