Efficacy of selected grain protectants on hulled Kernza®, dehulled Kernza®, and hard red winter wheat against two economically important stored product insect species

by

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Abstract

No grain protectants are currently registered for use on the perennial grass, *Thinopyrum intermedium* (Host) Barkworth & D.R. Dewey (Poales: Poaceae), marketed under the trade name Kernza®. Phosphine is the only fumigant approved for treating perennial grasses. The efficacy of selected commercial formulations of grain protectants namely, deltamethrin, methoprene, methoprene plus deltamethrin, and spinosad on hulled and dehulled Kernza® were evaluated at labeled rates in the laboratory against the lesser grain borer, *Rhyzopertha dominica* (Fabricius) (Coleoptera: Bostrichidae) and rice weevil, *Sitophilus oryzae* (Linnaeus) (Coleoptera: Cucrculionidae). The concentrations of the protectants used were 0.5 and 1 ppm for deltamethrin, 1.25 ppm methoprene, 1.25 ppm methoprene plus 0.5 ppm deltamethrin as a combination product, and spinosad at 1 ppm. At each concentration of an insecticide, 100 g of dehulled Kernza®, hulled Kernza®, and hard red winter wheat were treated and placed in 0.47-L glass mason jars. Into each jar, 50 unsexed adults of mixed ages of *R. dominica* or *S. oryzae* were added, after which the jars were closed with metal lids fitted with wire mesh screens and filter papers. Jars with insects were placed in environmental growth chambers at 28ºC and 65% r.h. Mortality was assessed on independent samples after 7, 14, and, 21 days post-infestation. Mortality of the insects was expressed as percentage of the total insects exposed. In separate replicates, all three grain types were treated with the same protectants and infested as described above. These jars were checked after 42 days to count adult progeny produced. After determination of adult progeny production, the grains were passed through the Boerner divider® to get a working sample of 6 g for wheat and 1.5 g for hulled and dehulled Kernza®. From the working sample, the number of damaged and undamaged kernels were counted, and their weights were measured to determine grain weight loss which was expressed as a percentage. The

results showed that high mortality (>90%) of *R. dominica* adults, low progeny production, and low grain weight loss were observed at 7, 14, and 21 days in dehulled Kernza® and hard red winter wheat at 0.5 and 1 ppm of deltamethrin. The mortality of *S. oryzae* adults was >50% after 14 and 21 days of exposure to the two deltamethrin concentrations on all grain types. No or very low adult progeny numbers were observed in all grain types treated with both concentrations of deltamethrin. Low mortality (<50%) of both insect species were observed in methoprene treated grains at all observation times. However, low adult progeny numbers were obtained in methoprene treated grains despite low mortality of adults. In methoprene plus deltamethrin treatments, the mortality of *R. dominica* adults was ≥ 93% in all grain types at all observation times, whereas the mortality of *S. oryzae* on all grain types and observation times ranged from 60 to 100%. There was no progeny production of *R. dominica* on all grain types in deltamethrin treatments, whereas *S. oryzae* mean progeny production at 7, 14, and 21 days ranged from 0 to 2.4 adults/jar; at 42 days adult progeny production ranged from 0.8 to 21.2 adults/jar. Grain weight loss was <0.2 to 0.9% in treated grains exposed to *R. dominica* and *S. oryzae*. Spinosad caused 100% mortality of *R. dominica* adults and no progeny were produced, and no grain weight loss was observed on all grain types at all observation times. In the case of *S. oryzae*, mean adult mortality at 7, 14, and 21 days in treated grains ranged from 87.2 to 100%, and progeny production and grain weight loss ranged 0 to 21.4 adults/jar and 0%, respectively. Spinosad at 1 ppm, deltamethrin at 0.5 and 1 ppm, methoprene and deltamethrin at 1.25 + 0.5 ppm were most effective grain protectants on Kernza® against *R. dominica.* In conclusion, spinosad at 1 ppm was the most effective grain protectant on Kernza® against *R. dominica* and *S. oryzae.* For dehulled Kernza the efficacy of tested grain protectants could not be satisfactorily gauged because of high mortality of *R. dominica* and *S. oryzae* adults in the control treatments.

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Chapter 1 - Efficacy of selected grain protectants on hulled Kernza®, dehulled Kernza®, and hard red winter wheat against *Rhyzopertha dominica* (Fabricius) (Coleoptera: Bostrichidae)

1.1 Abstract

No grain protectants are currently registered for use on this perennial grass, *Thinopyrum intermedium* (Host) Barkworth & D.R. Dewey) (Poales: Poaceae), marketed under trade name Kernza®. Phosphine is the only fumigant approved for treating perennial grasses. The efficacy of selected commercial formulations of grain protectants, deltamethrin, methoprene, methoprene + deltamethrin, and spinosad on hulled Kernza®, dehulled Kernza® and hard red winter wheat was evaluated in laboratory against lesser grain borer, *Rhyzopertha dominica* (F.). The concentrations of the protectants used were 0.5 and 1 ppm deltamethrin, 1.25 ppm methoprene, 1.25 ppm methoprene + 0.5 ppm deltamethrin as a combination product, and spinosad 1 ppm. Mortality was assessed after 7, 14 and 21 days of the application of protectants and insect introduction. The mortality of the insects was expressed as percentage of the total insects exposed. In separate replicates, grain was infested and observed at 42 d for adult progeny production and weight loss. After determination of adult progeny production, the grains were passed through the Boerner® divider to get a representative sample of 6 g for wheat and 1.5 g for hulled and dehulled Kernza®. The number of damaged kernels was assessed and expressed as a percentage. Deltamethrin at 0.5 and 1 ppm concentrations caused >90% of *R. dominica* adult mortality, result in no progeny production and <2 % grain weight loss of dehulled Kernza®, hulled Kernza®, and hard red winter wheat. Methoprene at 1.25 ppm concentration caused <50% insect mortality, resulting in <15 adult progeny and ≤1.2% grain weight loss of dehulled Kernza® and hard red winter wheat. Methoprene + deltamethrin (1.25 ppm + 0.5 ppm) caused >90% adult mortality. no progeny production in all grain types and ≤0.1 % grain weight loss of dehulled Kernza® and hard red winter wheat. Spinosad at 1 ppm resulted in 100% insect mortality, no adult progeny production and no grain weight loss in all grain types. *R. dominica* adult mortality was

consistently higher in hulled Kernza® in control treatments. Further research under practical field conditions in farmer- managed trials overtime offers a better understanding of efficacy of insecticides against *R. dominica* field populations and physico-chemical study of hulled Kernza® should be done to further examine the cause of high insect mortality.

1.2 Introduction

Kernza® is a trade name for the perennial grain obtained from intensive breeding of intermediate wheatgrass (*Thinopyrum intermedium*) (Host) Barkworth & D. R. Dewey, developed by the scientists at The Land Institute in Salina, Kansas, USA (DeHaan et al., 2014). It is a perennial grain, closely related to annual wheat, that does not require yearly replanting. Its roots can extend over 10 feet into the soil, offering significant environmental advantages such as reducing soil erosion, sequestering carbon, and minimizing nutrient leaching into groundwater. Kernza® serves both as a grain and fodder crop. The first variety, MN-Clearwater, was released by the University of Minnesota in 2019, followed by five additional varieties in 2022, making a total of six approved varieties currently available (Bajgain et al., 2020). Presently, Kernza® is an emerging crop cultivated on a small scale for niche markets. Market demand has surpassed the supply, with small local and organic artisan bakeries and farm-to-table restaurants using Kernza® grain to produce breads, crackers, and pasta (Lanker et al., 2020). Under optimal circumstances, the elongated seed heads of Kernza® have the potential to house more seeds than those of traditional annual wheat. Moreover, scientists strive to enhance the size of the seeds with every breeding cycle. Several efforts have been made to improve the agronomic, compositional, and nutritional qualities of Kernza® compared to annual cereal crops (de Oliveira et al., 2018).

With the improving yield of Kernza®, it is crucial to save it from several stored grain insects (Culman et al., 2013). Globally, more than 1600 insect species are linked to stored products. These pests harm grains and contaminate them, negatively impacting their flavor and odor, making them unfit for consumption (Hagstrum et al., 2013). According to data from the FAO, about 10% of grains are lost globally. Grain insect pests lead to weight loss, fungal growth and increase fatty acid content (Kosewska et al., 2024). Insects like rice weevil, *Sitophilus oryzae* (Linnaeus); lesser grain borer*, Rhyzopertha dominica* (Fabricius) (Coleoptera; Bostrichidae) infest and deteriorate stored cereal grains and other foodstuffs (Yaseen et al., 2019) and Kernza® is also at risk of insect damage resulting in quality and quantity loss.

Lesser grain borer, *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae), is known to be originated in tropical regions of Indian subcontinent. It is an internal pest and both larval and adult stages feed on whole grain and produces insect damaged kernel and is considered one of the most destructive pest of stored – products (Daglish and Nayak, 2018). Female lay eggs loosely on outside of kernel and the first instars tunnel into kernels feeds inside them and complete their development (Jia et al., 2008). They produce large quantity of frass, dust and fine material while feeding. Adults can live long and can disperse by flying to initiate new infestation (Hagstrum and Throne, 1989). The potential food and reproductive source of *R. dominica* includes grains in family Poaceae (e.g., rice, sorghum, wheat, oat, pearl millet, barley) and Leguminosae (e.g., peanuts, beans, chickpeas) (Chittenden, 1911). *R. dominica* reaches its highest reproductive success on dry grains, with a particular preference for wheat. They can move deeper up to 12 m into the grain mass and upward from the infestation in bottom. This ability to move deep within the grain mass and it's cryptic habit makes it's detection difficult (Edde, 2012).

Grain protectants are usually used to treat most grains on farms in the US (Subramanyam et al., 2007). Grain protectants are designed to eliminate or significantly reduce insect population growth. The sooner this growth can be delayed, the better. They are liquids or dust applied to newly harvested grains considered for long- or short-term storage. Fumigation can kill the existing population of insects, but it doesn't give residual long- term protection. Insecticide is applied to a moving grain stream at the bottom of the bucket elevator, auger, or belt, ensuring it contacts as many grain kernels as possible while the grain moves to its destination. Non-chemical approaches are not enough to effectively protect against storage pests, making the use of chemical agents essential (Kosewska et al., 2024). To this date no grain protectants are currently registered for Kernza®. Phosphine is the only fumigant approved for treating perennial grasses. Thus far, there have been no experiments performed and literature cited that have evaluated efficacy of existing grain protectants on Kernza® against *R. dominica*. In this study, we evaluated the efficacy of selected grain protectants in hulled and dehulled form of grains against *R. dominica.* Various parameters such as mortality, progeny production and grain weight loss in treated and untreated Kernza® grains in comparison to wheat were investigated.

1.3 Material and methods

1.3.1. Grain commodities

Hulled and dehulled Kernza® (variety: TLI C5) grains from the year 2022 harvest were obtained from the Sustain-A-Grain, McPherson County, Kansas, USA, shipped to the Department of Grain Science and Industry at Kansas State University, Manhattan, KS, USA, and held in freezer at ~-20°C for two weeks to kill live insects. Organic whole grain hard red winter wheat stock (Great River Organic Milling, Fountain City, Wisconsin, USA) (standard control) was compared with Kernza® grains. Kernza® and wheat grain samples were removed from the freezer and acclimatized in plastic bags at room conditions (~24.5 °C and ~76% R.H.) for 1-2 weeks before the experiment. The grain moisture content was measured by air oven method (American Society of Agricultural and Biological Engineers (ASAE, 2000). Both Kernza® and wheat grain samples were equilibrated to 13% moisture (wet basis) content in environmental growth chambers maintained at 28°C and 65% rh. before the experiment.

1.3.2. Insect colonies

Insect cultures of lesser grain borer, *R. dominica* was maintained for more than 20 generations at Department of Grain Science and Industry were used for experiment. Insect cultures were reared in 0.95liter mason glass jars fitted with wire mesh and filter paper lids containing preconditioned organic hard red winter wheat. The glass jars were held inside growth chambers (Model I-36 VL; Percival Scientific, Perry, Iowa, USA) at 28°C, 65% relative humidity (r.h.), and a photoperiod of 14:10 (Light: Dark) h.

1.3.3. Grain protectants

Four selected insecticides were used to assess their efficacy on hulled and dehulled Kernza®. Two insecticide concentrations, 0.5 and 1 ppm of deltamethrin, 1.25 ppm methoprene, 1.25 ppm methoprene + 0.5 ppm deltamethrin as a combination product and 1 ppm spinosad were used. Centynal^{EC} containing 4.75 % active ingredient of deltamethrin, Diacon®-IGR containing 33.6% active ingredient of methoprene and Diacon®-IGR Plus containing 4.75% deltamethrin and 11.4% methoprene were obtained from Central Life Sciences, Illinois, USA. The liquid formulation of Sensat™ containing 8.66% active ingredient of Spinosad was obtained from Bayer Crop Science LP, Research Triangle Park, North Carolina, United States.

1.3.4. Grain treatment, insect exposure, response and grain damage

All grain types were held inside a deep freezer at -13ºC for a week to kill any live insects if present. Then, 100 g grain (13% moisture, wet basis) were transferred to individual 0.45 L glass jars. The glass jars were secured with 250 mm wire mesh screens and filter paper (7 cm diameter) lids to facilitate air diffusion. Grains in the jars were individually treated with prepared insecticide concentrations and untreated were used as control for comparison and treated with same volume of distilled water with Fisherbrand™ adjustable-volume pipette. The jars were manually shaken for 1 min to ensure uniform mixing of insecticide concentrations and distilled water. Fifty unsexed adults of *R. dominica* from culture jars were introduced into each glass jar. After insect introduction jars were covered with lids with wire mesh and filter paper and placed in growth chamber at 28ºC and 65% r.h. Temperature and relative humidity were monitored during the experiment using HOBO data recorders (Onset Computer, Pocasset, MA).

Mortality counts were taken after 7, 14 and 21d after exposure to insecticides. Adults that did not respond when lightly tapped with a camel hairbrush were considered dead. All dead and alive adult beetles were separated from the grain and were discarded. The grains were carefully returned to the individual glass jars and held inside the growth chamber at 28ºC and 65% r.h for additional 28 d. At 42d, adult progeny produced were counted and introduced fifty parental adults were subtracted from overall adult progeny number. Hulled and dehulled Kernza® were passed 6 times and hard red winter wheat was passed 4 times into Boerner® divider (Seedburo Equipment Company, Des Plaines, Illinois, USA) to obtain a working sample of ~1500 mg Kernza® and ~5000 mg wheat to determine grain weight loss. The number of insect damaged kernels out of the total were examined with hand lens for the presence of a hole or burrow and was expressed as a percentage (Boxall, 1986). Kernels with uneven holes about 1 mm wide that get deeper from where the larva entered, spreading around the kernel to the pupation chamber were considered insect damaged kernel (Breese, 1960).

Insect damaged kernels (%) = [Nd ÷ (Nd + Nu)] × 100

Where,

Nu = Number of undamaged kernels

Nd = Number of damaged kernels

The percentage of grain weight loss was calculated based on counting and weighing damaged and un-damaged kernels from each replicate working sub-sample (Boxall, 1986) using a digital weighing balance. The weight loss percentage of grains in each replicate sub-samples was calculated using the following formula:

Weight loss (%) = [(WuNd – WdNu) ÷ (Wu × (Nd + Nu))] × 100

Where,

Wu = Weight of undamaged kernels

Nu = Number of undamaged kernels

Wd = Weight of damaged kernels

Nd = Number of damaged kernels

1.3.5 Statistical analysis

Statistical analysis for mortality (%), total number of progenies produced, and weight loss (%) were performed in quintuplicates using one-way analysis of variance (ANOVA). Three-way ANOVA was performed to check significance of individual and interaction effect of all variables. One -way ANOVA was performed to check significance among grain types and insecticide concentrations. Mortality (%) data was transformed to angular values. The recorded data of adult progeny number was log transformed ($\log_{10}(x + 1)$), for statistical analysis (Zar, 1984). The means were compared with Ryan-Einot-Gabriel-Welsch multiple range (REGWQ) test at $P \leq 0.05$, using the Statistical Analysis System (SAS) Version 9.4, SAS Institute, Cary, North Carolina, USA (SAS Institute, 2012).

1.4 Results

1.4.1. Percentage mortality of *R. dominica* adults exposed to deltamethrin

Three-way ANOVA shows that the main effect of grain type (df = 2, 108), insecticide concentration (df = 2, 108), observation time (df = 2, 108), grain type × insecticide concentration (df = 4, 108), grain type × observation time (df = 4, 108), insecticide concentration × observation time (df = 4, 108) and grain type × insecticide concentration × observation time (df = 8, 108) were all significant (F_{range} = 35.07 – 5218.19; df = 26, 108); $P < 0.0001$) (Table 1.1).

The mortality percentage (Mean ± SE) of *R. dominica* adults in control and following exposure to deltamethrin in hulled, dehulled Kernza® and hard red winter wheat for 7, 14, and 21 days are presented in Table 1.2. One-way ANOVA showed that after 7 d, highest mortality was obtained in 1 ppm treated hulled Kernza® which was statistically similar to 0.5 and 1 ppm treated hulled, dehulled Kernza® and hard red winter wheat (F = 357.2; df = 8, 36; $P < 0.0001$). After 14 d, deltamethrin 0.5 and 1 ppm insecticide concentration killed all exposed *R. dominica.* However, 100% mortality was also found in untreated hulled Kernza® (F = 869.6; df = 8, 36; $P < 0.0001$). Similar trend was observed after 21 d of exposure to insecticide (F = 929.7; df = 8, 36; $P <$ 0.0001).

1.4.2. Adult progeny of *R. dominica* exposed to deltamethrin

Three-way ANOVA shows that the main effect of grain type (df = 2, 144), insecticide concentration (df = 2, 144), observation time (df = 3, 144), grain type × insecticide concentration (df = 4, 144), grain type × observation time (df = 6, 144), insecticide concentration × observation time (df = 6, 144) and grain type × insecticide concentration × observation time (df = 12, 144) were all significant (F_{range} = 10.6 – 5081.7; df = 35, 144); $P < 0.0001$) (Table 1.3).

The adult progeny number of *R. dominica* adults in control hulled, dehulled Kernza® and hard red winter wheat for 7, 14, 21, and 42 days are presented in Table 1.4. After 7d, no progeny was produced in deltamethrin treated grains in all observation period. In control, least progeny was found in hulled Kernza® compared to dehulled Kernza® and hard red winter wheat (F = 324.7; df = 8, 36; P < 0.0001). Similar trend was seen in observation time 14 d (F = 113.8; df = 8, 36; P < 0.0001), 21 d (F = 619.4; df = 8, 36; P < 0.0001) and 42 d (F = 3279.3; df = 8, 36; P < 0.0001).

1.4.3. Weight loss of grain due to *R. dominica* exposed to deltamethrin

Three-way ANOVA shows that the main effect of grain type (df = 2, 140), insecticide concentration (df = 2, 140), observation time (df = 3, 140), grain type × insecticide concentration (df = 4, 140), grain type × observation time (df = 6, 140), insecticide concentration × observation time (df = 6, 140) and grain type × insecticide concentration × observation time (df = 12, 140) were all significant ($F_{, \text{range}}$ = 4.3 – 97.08; df = 35, 140); P < 0.0001) (Table 1.5).

The weight loss of grain due to *R. dominica* adults in control and following exposure to deltamethrin in hulled, dehulled Kernza® and Hard red winter wheat for 7, 14, and 21 days are presented in Table 1.6. Weight loss of both untreated and treated grain after 7 d exposure to *R. dominica* was not statistically significant (F = 1.30; df = 8, 35; P = 0.2740). In untreated grain, weight loss due to insects exposed for 14 d, weight loss was highest in dehulled Kernza® and hard red winter wheat followed by hulled Kernza®. In treated grains, weight loss was statistically similar to untreated hulled Kernza® (F = 6.68; df = 8, 36; P < 0.001). A similar trend was observed at 21 d (F = 5.57; df = 8, 34; P = 0.0002). After 42 d continuous exposure, highest weight loss was obtained in untreated hard red winter wheat (5.4 ± 0.4) followed by untreated dehulled Kernza® (2.9 ± 0.4) and 0.5 ppm treated dehulled Kernza® (1.5 ± 0.5) (F = 48.81; df = 8, 36; $P < 0.001$).

1.4.4. Percentage mortality of *R. dominica* adults exposed to methoprene

Three-way ANOVA shows that the main effect of grain type (df = 2, 72), insecticide insecticide concentration (df = 1, 72), observation time (df = 2, 72), grain type × insecticide concentration (df = 2, 72), grain type × observation time (df = 4, 72) and insecticide concentration × observation time (df = 2, 72) and grain type × insecticide concentration × observation time (df = 4, 72) were significant ($F_{,\ range}$ = 7.5 – 833.3; df = 17, 72); $P < 0.05$) (Table 1.7).

The mortality percentage of *R. dominica* adults in control and following exposure to methoprene in hulled, dehulled Kernza® and hard red winter wheat for 7, 14, and 21 days are presented in Table 1.8. In control, higher mortality of insects was observed in hulled Kernza® than dehulled Kernza® and hard red winter wheat and trend was similar in all observation times ($F_{,\ range}$ = 96.14 – 220.81; df = 5, 24; $P < 0.0001$). In treated grains after 7 d the highest mortality was obtained in hulled Kernza® followed by dehulled Kernza®. After 14 d exposure, again highest mortality was obtained in hulled Kernza® followed by hard red winter wheat followed by dehulled Kernza® (F = 96.14; df = 5, 24; $P < 0.001$). Similarly, after 21 d, highest mortality was observed in hulled Kernza®, followed by dehulled Kernza® and hard red winter wheat (F = 220.81; df = 5, 24; $P < 0.001$).

1.4.5. Adult progeny of *R. dominica* exposed to methoprene

Three-way ANOVA shows that the main effect of grain type (df = 2, 96), insecticide concentration (df = 1, 96), observation time (df = 3, 96), grain type × insecticide concentration (df = 2, 96), grain type × observation time (df = 6, 96) and insecticide concentration ×

observation time (df = 6, 96) and grain type × insecticide concentration × observation time (df = 6, 96) were significant ($F_{,\ range}$ = 8.3 – 432.5; df = 23, 96); P < 0.0001 (Table 1.9).

The adult progeny number of *R. dominica* adults in control and methoprene treated hulled, dehulled Kernza® and Hard red winter wheat for 7, 14, 21, and 42 days are presented in Table 1.10. After 7 d, in control, highest progeny number was obtained at dehulled Kernza® followed by hard red winter wheat followed by hulled Kernza® (F = 12.26; df = 5, 24; P < 0.001). After 14 d and 42 d, the highest progeny number was obtained in hard red winter wheat followed by dehulled Kernza® and hulled Kernza® ($F_{,\ range}$ = 58.13 – 89.59; df = 5, 24; P < 0.0001). However, after 21 d, the highest progeny number was seen on hard red winter wheat which was statistically like progeny number in dehulled Kernza® followed by hulled Kernza® (F = 64.46; df = 5, 24; P < 0.001). In treated grains, after 7, 21, and 42 d, highest progeny number was seen on hard red winter wheat in compared to dehulled and hulled Kernza® ($F_{,\ range}$ = 12.26 – 89.59; df = 5, 24; P = < 0.0001). After 14 d of exposure, the progeny production was highest in dehulled Kernza® followed by hulled Kernza® and hard red winter wheat (F = 58.13; df = 5, 24; P < 0.001).

1.4.6. Weight loss of grain due to *R. dominica* exposed to methoprene

Three-way ANOVA shows that the main effect of grain type (df = 2, 96), insecticide concentration (df = 1, 96), observation time (df = 3, 96), grain type × insecticide concentration (df = 2, 96), grain type × observation time (df = 6, 96) and insecticide concentration × observation time (df = 6, 96) were significant ($F_{,\ range}$ = 7.7 – 79.2; df = 23, 96); P < 0.0001 (Table 1.11).

The weight loss of grain due to *R. dominica* adults in control and following exposure to methoprene in hulled, dehulled Kernza® and Hard red winter wheat for 7, 14, 21, and 42 d are presented in Table 1.12. After 7, 21, and 42 d the highest weight loss in control treatment was obtained in hard red winter wheat followed by dehulled Kernza® which was not statistically significant with hulled Kernza® (F_{range} = 8.47 – 41.72; df = 5, 24; P < 0.0001). Weight loss of methoprene treated grain was low and was significantly similar among all grain types (F_{range} = 8.47 – 41.72; df = 5, 24; P < 0.0001).

1.4.7. Percentage mortality of *R. dominica* adults exposed to methoprene + deltamethrin

Three-way ANOVA shows that the main effect of grain type (df = 2, 72), insecticide concentration (df = 1, 72), observation time (df = 2, 72), grain type × insecticide concentration (df = 2, 72), insecticide concentration × observation time (df = 2, 72), and grain type × insecticide concentration × observation time (df = 4, 72) were all significant (F_{range} = 6.9 – 4288.4; df = 17, 72); P < 0.05 (Table 1.13).

The mortality percentage of *R. dominica* adults in control and following exposure to methoprene in hulled, dehulled Kernza® and Hard red winter wheat for 7, 14, and 21 days are presented in Table 1.14. In control, higher mortality (80.8 to 99.2 %) of insects was observed in hulled Kernza® than dehulled Kernza® and hard red winter wheat after 7 d of exposure to insects (F = 373.89; df = 5, 24; P < 0.001). Similar trend was obtained after 14 d (F = 462.5; df = 5, 24; P < 0.001) and 21 d (F = 504.9; df = 5, 24; P < 0.001). In treated grains after 7 d exposure, the highest mortality of insects was obtained in hard red winter wheat which was statistically similar to mortality in hulled Kernza® (F = 373.8; df = 5, 24; P < 0.001). However, after 14 and 21 d of exposure, there was 100 % mortality of insects and insecticide performance was equal in all three grains (F_{range} = 462.54 – 504.95; df = 5, 24; P = < 0.0001).

1.4.8. Adult progeny of *R. dominica* exposed to methoprene + deltamethrin

Three-way ANOVA shows that the main effect of grain type (df = 2, 96), insecticide concentration (df = 1, 96), observation time (2, 96), grain type × insecticide concentration (df = 2, 96), grain type × observation time (df = 6, 96), insecticide concentration × observation time (df = 2, 96), and grain type × insecticide concentration × observation time (df = 6, 96) were all significant (F_{range} = 27.7 – 4605.7; df = 23, 96); $P < 0.0001$ (Table 1.15).

The adult progeny number of *R. dominica* adults in control and methoprene + deltamethrin treated hulled, dehulled Kernza® and hard red winter wheat for 7, 14, 21, and 42 days are presented in Table 1.16. In control after 7 and 14 d, mortality was highest in hard red winter wheat followed by dehulled Kernza® and hulled Kernza® (F_{range} = 115.93 – 501.39; df = 5, 24; $P < 0.0001$). After 21 d, highest progeny was obtained in hard red winter wheat which was statistically like dehulled Kernza® (F = 94.81; df = 5, 24; $P < 0.001$). Similarly, after 42 d, highest progeny was obtained in hard red winter wheat followed by dehulled Kernza® and hulled Kernza® (F = 4461.14; df = 5, 24; $P < 0.001$). There was no progeny production in methoprene + deltamethrin treated grains.

1.4.9. Weight loss of grain due to *R. dominica* exposed to methoprene + deltamethrin

Three-way ANOVA shows that the main effect of grain type (df = 2, 95), insecticide concentration (df = 1, 95), observation time (2, 95), grain type × insecticide concentration (df = 2, 95), grain type × observation time (df = 6, 95) insecticide concentration × observation time (df = 3, 95), and grain type × insecticide concentration × observation time (df = 6, 95) were all significant (F_{range} = 2.21 – 184.3; df = 23, 95); $P < 0.0001$ (Table 1.17).

The weight loss of grain due to *R. dominica* adults in control and following exposure to methoprene+ deltamethrin in hulled, dehulled Kernza® and hard red winter wheat for 7, 14, 21, and 42 d are presented in Table 1.18. In control highest grain weight loss was obtained in hard red winter wheat in all observation time and it was statistically similar to grain weight loss in dehulled Kernza® at 7 and 14 d exposure to insects ($F_{,\ range}$ = 8.98 – 10.95; df = 5, 24; P < 0.0001). However, in control at 21 and 42 d, there was significant difference in grain weight loss between hard red winter wheat, dehulled Kernza® and hulled Kernza® ($F_{,\ range}$ = 10.84 – 61.48; df = 5, 24; P < 0.0001). There was no grain weight loss observed at all observation periods post treatment with methoprene + deltamethrin ($F_{,\ range}$ = 8.9 – 61.5; df = 5, 24; P < 0.0001).

1.4.10. Percentage mortality of *R. dominica* adults exposed to spinosad

Three-way ANOVA shows that the main effect of grain type (df = 2, 72), insecticide concentration (df = 1, 72), observation time (df = 2, 72), grain type × insecticide concentration (df = 2, 72), insecticide concentration × observation time (2, 72), grain type × observation time (df = 4, 72) and grain type × insecticide concentration × observation time (df = 4, 72) were all significant ($F_{,\ range}$ = 18 – 2558.4; df = 23, 96; P < 0.0001) (Table 1.19).

The mortality percentage of *R. dominica* adults in control and following exposure to spinosad in hulled, dehulled Kernza® and hard red winter wheat for 7, 14, and 21 days are presented in Table 1.20. One-way ANOVA showed that in control after 7 d, 100% of insect adult was found in hulled Kernza® compared to dehulled Kernza® and hard red winter wheat mortality (F = 1542.28; df = 5, 24; P <0.0001). Whereas after 14 and 21 d, highest mortality was again seen in hulled Kernza® among all grain type, but the mortality was 22.4 % and 37.6% respectively ($F_{,\ range}$ = 49.91 – 274.66; df = 5, 24; P <0.0001. In spinosad treated grains 100% mortality was obtained in all grain type in all observation time (F_{range} = 49.91 – 1542.28; df = 5, 24; P < 0.0001).

1.4.11. Adult progeny of *R. dominica* exposed to spinosad

Three-way ANOVA shows that the main effect of grain type (df = 2, 96), insecticide concentration (df = 1, 96), observation time (df = 3, 96), grain type × insecticide concentration (df = 2, 96), grain type × observation time (df = 6, 96), insecticide concentration × observation time (df = 3, 96) and grain type × insecticide concentration × observation time (df = 6, 96) were all significant (F_{range} = 15.3 – 3449.2; df = 23, 96; P < 0.0001) (Table 1.21).

The progeny number of *R. dominica* adults in control and following exposure to spinosad in hulled, dehulled Kernza® and hard red winter wheat for 7, 14,21, and 42 days are presented in Table 1.22. One-way ANOVA showed that in control highest progeny was obtained in hard red winter wheat, followed by dehulled Kernza® and hulled Kernza® respectively after 7, 14 and 21 d (F_{range} = 114.3 – 398.15; df = 5, 24; P < 0.0001). After 42 d of continuous infestation highest progeny was seen in hard red winter wheat followed by hulled Kernza® and dehulled Kernza® (F = 1522.55; df = 5, 24; P < 0.0001). In spinosad treated grains 100% mortality was obtained in all grain type in all observation time (F_{range} = 114.3 – 1522.55; df = 5, 24; P < 0.0001).

1.4.12. Weight loss of grain due to *R. dominica* exposed to spinosad

Three-way ANOVA shows that the main effect of grain type (df = 2, 96), insecticide concentration (df = 1, 96), observation time (df = 3, 96), grain type × insecticide concentration (df = 2, 96), grain type × observation time (df = 6, 96), insecticide concentration × observation time (df = 3, 96) and grain type × insecticide concentration × observation time (df = 6, 96) were all significant (F_{range} = 1 – 26.1; df = 23, 96; P < 0.0001) (Table 1.23).

The weight loss of grain due to *R. dominica* adults in control and following exposure to spinosad in hulled, dehulled Kernza® and hard red winter wheat after 7, 14, 21, and 42 d are presented in Table 1.24. One-way ANOVA showed that highest weight loss was obtained in hard red winter wheat compared to both type of Kernza® in all observation time and weight loss increased as there was increase in observation time (F_{range} = 10.96 – 127.16; df = 5, 24; $P <$ 0.0001). In spinosad treated grains no weight loss was seen in hard red winter wheat and dehulled Kernza®, but much less weight loss (0.1-0.8%) was found in hulled Kernza®.

1.5 Discussion

Deltamethrin's mode of action is thought to be mainly central in action, or at least originate in higher nerve centers of the brain. Death of insects seems to be due to irreversible damage to the nervous system occurring when poisoning lasts more than a few hours (Rehman et al., 2014). Deltamethrin can poison insects through cuticular penetration or oral ingestion. Known for its broad-spectrum control, deltamethrin is regarded as the most potent among synthetic pyrethroids. Technical grade deltamethrin has a purity of over 98% and is chemically stable, minimizing concerns about exposure to its breakdown products.

Deltamethrin, under the trade name Centynal (Wellmark International, Schaumburg, Illinois), was approved in 2012 by the United States Environmental Protection Agency (U.S.-EPA) for treatment of barley, corn, oats, popcorn, rice, rye, sorghum, and wheat intended for storage and for treatment of empty storage facilities receiving these grains. Centynal is the only formulation of deltamethrin currently available in the United States for treatment of empty storage structures and stored grain (Sehgal and Subramanyam, 2014). The current research examined the activity of 0.5 and 1 ppm deltamethrin insecticide concentration in Kernza® grains with compared to wheat. The study by (Trostanetsky et al., 2023), also concluded that significant mortality of *R. dominica* was observed at insecticide concentration of 0.5 and 1 ppm deltamethrin in wheat after 7 and 14 d exposure. However, in hulled Kernza® the mortality was also higher in control and was not statistically different from treated grains. Numerous past studies have reported that hulled grains prevent insect pest penetration and damage (Chanbang et al., 2008; Kumar et al., 2020). Likewise, 99-100% mortality of *R. dominica* and no progeny emergence when exposed to 0.25 ppm deltamethrin in wheat was found by (Pražić Golić et al., 2018).

Insect growth and development is regulated by juvenile hormone secreted by corpora allata and ecdysone hormone by prothoracic glands (Wijayaratne et al., 2018). Juvenile hormone synthesized in corpora allata is transported through hemolymph by binding proteins to target tissues to regulate function (Hidayat and Goodman, 1994). Several analogues of juvenile hormone have been developed based on chemical modifications. Juvenile hormone analogues have low concentration toxicity (Wright, 1976). When JH or JHAs are externally applied to insects, the usual regulation of JH levels within their bodies is disrupted, resulting in various morphological and physiological changes compared (Athanassiou et al., 2011).

Our result showed that there was not high mortality (<50%) of *R. dominica* in S-Methoprene treated dehulled Kernza® and hard red winter wheat in all observation period, thus high progeny production was expected. However, there was very little progeny production in S-Methoprene treated grains in all observation period compared to control. IGR does not kill the insects directly or is not necessarily toxic to its target, but it cause abnormality and affects survival of the insects (Siddall, 1976).

In our study with mixture of deltamethrin and S-Methoprene, we found high insect mortality (93 to 100 %) was obtained in all grain type in all observation period and there was no progeny production and no grain weight loss in treated grains. Similar results was also obtained by (Arthur, 2019) where there was no weight loss and no progeny production of *R. dominica* when 0.5ppm deltamethrin + 1.25 ppm Methoprene was applied on wheat and brown rice.

Spinosyns are a distinctive group of insecticides derived through fermentation of naturally occurring soil actinomycete, *Saccharopolyspora spinosa* known for their strong effectiveness and reduced environmental impact(Kirst, 2010; Mertz and Yao, 1990). Spinosyns target the nicotinic acetylcholine and gamma-aminobutyric acid (GABA) receptor sites within the insect nervous system. To date, they have not shown cross-resistance with any other known insecticide (Kirst, 2010). Currently, Spinosad is registered in many countries as a grain protectant at a maximum labeled use concentration of 1 ppm (1 mg ai/kg of grain) and with the Maximum Residue Level (MRL) or tolerance on grains set at 1 or 1.5 ppm (Hertlein et al., 2011). Spinosad residues remain very stable on grains stored in bins, providing protection for periods ranging from 6 months to 2 years. Various factors influencing the overall effectiveness of Spinosad include the insect pest species, pest life stage, grain type, grain variety, and formulation type (Hertlein et al., 2011).

In our study, we found that 1 ppm Spinosad caused 100 % mortality of *R. dominica* in all grain type in all observation times. (Vayias et al., 2010) found that 1 ppm Spinosad is highly effective against *R. dominica* and provided protection for a period of storage for 4 months and Spinosad performance was not influenced by grain type. Similar results were obtained by (Huang et al., 2007), where Spinosad was more effective against stored insect pests at 1 ppm insecticide concentration than 0.1 and 0.5 ppm.

In conclusion, Deltamethrin at 0.5 and 1 ppm concentrations caused >90% of *R. dominica* adult mortality, result in no progeny production and <2 % grain weight loss of dehulled

Kernza®, hulled Kernza®, and hard red winter wheat. Methoprene at 1.25 ppm concentration caused <50% insect mortality, resulting in <15 adult progeny and ≤1.2% grain weight loss of dehulled Kernza® and hard red winter wheat. Methoprene + deltamethrin (1.25 ppm + 0.5 ppm) caused >90% adult mortality resulting in no progeny production in all grain types, and there was ≤0.1 % grain weight loss of dehulled Kernza® and hard red winter wheat. Spinosad at 1 ppm resulted in 100% insect mortality, no adult progeny production and no grain weight loss in all grain types. *R. dominica* adult mortality was consistently higher in hulled Kernza® in control treatments. Additional research under practical field conditions in farmer-managed trials overtime offers a better understanding of efficacy of insecticides against *R. dominica* field populations. Physical and chemical study on hulled Kernza® to explore causes of high insect mortality can be done.

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Table 1.1 Three-way ANOVA statistics for mortality of *R. dominica* exposed to deltamethrin.

Source	df	Mean Square	*F*-value	*P*-value*
Grain type	2	1.02	314.06	< 0.0001
Insecticide concentration	2	17.05	5218.19	< 0.0001
Observation time	2	0.25	77.41	< 0.0001
Grain type × Insecticide concentration	4	1.01	310.88	< 0.0001
Grain type × Observation time	4	0.24	75.92	< 0.0001
Insecticide concentration × Observation time	4	0.11	35.07	< 0.0001
Grain type × Insecticide concentration × Observation time	8	0.23	71.87	< 0.0001
Error	108	0.0033		

*Significant ($P < 0.05$)

Table 1.2 Mortality of *R. dominica* adults exposed for 7, 14, and 21 d to untreated and deltamethrin treated dehulled Kernza®, hulled Kernza®, and hard red winter wheat.

Grain type	Insecticide concentration (ppm)	% Mean ± SE mortality at[a]:		
		7 d	14 d	21 d
Dehulled Kernza®	0	5.2 ± 1.3 c	1.2 ± 0.8 b	17.2 ± 1.7 b
Dehulled Kernza®	0.5	99.6 ± 0.4 a	100.0 ± 0.0 a	99.6 ± 0.4 a
Dehulled Kernza®	1.0	99.2 ± 0.4 a	99.6 ± 0.4 a	100.0 ± 0.0 a
Hulled Kernza®	0	16.0 ± 3.3 b	100.0 ± 0.0 a	76.8 ± 2.7 a
Hulled Kernza®	0.5	98.0 ± 0.9 a	100.0 ± 0.0 a	100.0 ± 0.0 a
Hulled Kernza®	1.0	100.0 ± 0.0 a	100.0 ± 0.0 a	100.0 ± 0.0 a
Hard red winter wheat	0	5.2 ± 0.4 c	1.2 ± 0.8 b	10.8 ± 0.8 c
Hard red winter wheat	0.5	98.8 ± 0.8 a	100.0 ± 0.0 a	100.0 ± 0.0 a
Hard red winter wheat	1.0	99.6 ± 0.4 a	100.0 ± 0.0 a	100.0 ± 1.0 a

[a]Means among grain type and insecticide concentration at each observation time followed by different letters are significantly different ($P < 0.05$; by REGWQ) test.

Table 1.3 Three-way ANOVA statistics for adult progeny production of *R. dominica* exposed to deltamethrin.

Source	df	Mean Square	*F*- value	*P*- value*
Grain	2	2.2	215.9	< 0.0001
Insecticide concentration	2	52.7	5081.7	< 0.0001
Observation time	3	2.2	215.3	< 0.0001
Grain type × Insecticide concentration	4	2.2	215.9	< 0.0001
Grain type × Observation time	6	0.1	10.6	< 0.0001
Insecticide concentration × Observation time	6	2.2	215.3	< 0.0001
Grain type × Insecticide concentration × Observation time	12	0.1	10.7	< 0.0001
Error	144	0.01		

* Significant ($P < 0.05$)

Table 1.4 Adult progeny production of *R. dominica* exposed for 7, 14, 21, and 42 d to untreated and deltamethrin treated dehulled Kernza®, hulled Kernza®, and hard red winter wheat.

Grain type	Insecticide concentration (ppm)	Mean ± SE mortality at[a]:			
		7 d	14 d	21 d	42 d
Dehulled Kernza®	0	7.0 ± 1.8 b	53.4 ± 8.6 a	110.8 ± 8.6 b	328.4 ± 23.7 b
Dehulled Kernza®	0.5	0.0 ± 0.0 d	0.0 ± 0.0 d	0.0 ± 0.0 d	0.0 ± 0.0 d
Dehulled Kernza®	1.0	0.0 ± 0.0 d	0.0 ± 0.0 d	0.0 ± 0.0 d	0.0 ± 0.0 d
Hulled Kernza®	0	0.0 ± 0.0 c	3.8 ± 1.7 b	24.0 ± 4.5 c	104.8 ± 4.6 c
Hulled Kernza®	0.5	0.0 ± 0.0 d	0.0 ± 0.0 d	0.0 ± 0.0 d	0.0 ± 0.0 d
Hulled Kernza®	1.0	0.0 ± 0.0 d	0.0 ± 0.0 d	0.0 ± 0.0 d	0.0 ± 0.0 d
Hard red winter wheat	0	45.6 ± 21.6 a	72.4 ± 8.5 a	200.8 ± 30.2 a	435.8 ± 49.1 a
Hard red winter wheat	0.5	0.0 ± 0.0 d	0.0 ± 0.0 d	0.0 ± 0.0 d	0.0 ± 0.0 d
Hard red winter wheat	1.0	0.0 ± 0.0 d	0.0 ± 0.0 d	0.0 ± 0.0 d	0.0 ± 0.0 d

[a]Means among grain type and insecticide concentration at each observation time followed by different letters are significantly different ($P < 0.05$; by REGWQ) test.

Table 1.5 Three-way ANOVA statistics for weight loss of dehulled Kernza®, hulled Kernza®, and hard red winter wheat due to *R. dominica* after exposure to deltamethrin.

Source	df	Mean Square	*F*- value	*P*-value*
Grain type	2	11.241	25.24	< 0.0001
Insecticide concentration	2	43.236	97.08	< 0.0001
Observation time	3	6.8622	15.41	< 0.0001
Grain type × Insecticide concentration	4	8.5523	19.20	< 0.0001
Grain type × Observation time	6	2.7856	6.25	< 0.0001
Insecticide concentration × Observation time	6	3.9141	8.79	< 0.0001
Grain type× Insecticide concentration × Observation time	12	1.8984	4.26	< 0.0001
Error	140	0.4453		

.* Significant ($P < 0.05$)

Table 1.6 Grain weight loss of untreated and deltamethrin treated dehulled Kernza®, hulled Kernza®, and hard red winter wheat after exposure to *R. dominica* adults for 7, 14, 21, and 42 d.

Grain type	Insecticide concentration (ppm)	% Mean ± SE weight loss at[a]:			
		7 d	14 d	21 d	42 d
Dehulled Kernza®	0	0.9 ± 0.2	1.3 ± 0.2 a	2.8 ± 1.1 a	2.9 ± 0.4 b
Dehulled Kernza®	0.5	0.1 ± 0.0	0.4 ± 0.5 b	1.7 ± 0.4 ab	1.5 ± 0.5 c
Dehulled Kernza®	1.0	0.1 ± 0.1	0.0 ± 0.0 b	0.2 ± 0.2 b	0.0 ± 0.0 d
Hulled Kernza®	0	0.2 ± 0.1	0.2 ± 0.1 b	0.5 ± 0.2 b	0.8 ± 0.3 cd
Hulled Kernza®	0.5	0.0 ± 0.0	0.0 ± 0.0 b	0.0 ± 0.0 b	0.0 ± 0.0 d
Hulled Kernza®	1.0	0.1 ± 0.1	0.0 ± 0.0 b	0.0 ± 0.0 b	0.1 ± 0.1 d
Hard red winter wheat	0	1.6 ± 0.4	2.1 ± 0.8 a	1.1 ± 0.2 ab	5.4 ± 0.4 a
Hard red winter wheat	0.5	0.0 ± 0.0	0.0 ± 0.0 b	0.0 ± 0.0 b	0.0 ± 0.0 d
Hard red winter wheat	1.0	0.0 ± 0.0	0.0 ± 0.0 b	0.0 ± 0.0 b	0.0 ± 0.0 d

[a]Means among grain type and insecticide concentration at each observation time followed by different letters are significantly different ($P < 0.05$; by REGWQ) test.

Table 1.7 Three-way ANOVA statistics for mortality of *R. dominica* exposed to methoprene.

Source	df	Mean Square	*F* -value	*P*-value*
Grain type	2	10.9	833.3	< 0.0001
Insecticide concentration	1	1.0	83.3	< 0.0001
Observation time	2	0.75	62.5	< 0.0001
Grain type × Insecticide concentration	2	0.09	7.5	0.0009
Grain type × Observation time	4	0.08	6.6	< 0.0001
Insecticide concentration × Observation time	2	0.04	3.33	0.0214
Grain type × Insecticide concentration × Observation time	4	0.10	8.33	< 0.0001
Error	72	0.012		

* Significant ($P < 0.05$)

Table 1.8 Mortality of *R. dominica* adults exposed for 7, 14, and 21 d to untreated and methoprene treated dehulled Kernza®, hulled Kernza®, and hard red winter wheat.

Grain type	Insecticide concentration (ppm)	% Mean ± SE mortality at[a]:		
		7 d	14 d	21 d
Dehulled Kernza®	0	1.2 ± 0.8 b	3.2 ± 1.2 c	4.0 ± 1.4 c
Dehulled Kernza®	1.25	3.6 ± 1.6 a	7.6 ± 1.1 c	36.0 ± 2.6 a
Hulled Kernza®	0	80.8 ± 3.7 a	86.0 ± 0.8 a	99.2 ± 0.5 a
Hulled Kernza®	1.25	91.6 ± 2.4 a	90.4 ± 4.5 a	99.2 ± 0.4 a
Hard red winter wheat	0	0.4 ± 0.4 b	3.2 ± 1.0 c	8.0 ± 2.6 c
Hard red winter wheat	1.25	2.8 ± 1.2 b	42.8 ± 6.6 b	30.8 ± 3.9 b

[a]Means among grain type and insecticide concentration at each observation time followed by different letters are significantly different ($P < 0.05$; by REGWQ) test.

Table 1.9 Three-way ANOVA statistics for adult progeny production of *R. dominica* exposed to methoprene.

Source	df	Mean Square	*F* - value	*P* - value*
Grain	2	7.6	176.7	< 0.0001
Insecticide concentration	1	18.6	432.5	< 0.0001
Observation time	3	2.8	65.12	< 0.0001
Grain type × Insecticide concentration	2	0.7	16.27	< 0.0001
Grain type × Observation time	6	0.3	6.97	< 0.0001
Insecticide concentration × Observation time	3	0.4	9.30	< 0.0001
Grain type × Insecticide concentration × Observation time	6	0.36	8.37	< 0.0001
Error	96	0.043		

* Significant ($P < 0.05$)

Table 1.10 Adult progeny production of *R. dominica* exposed for 7, 14, 21, and 42 d to untreated and methoprene treated dehulled Kernza®, hulled Kernza®, and hard red winter wheat.

Grain type	Insecticide concentration (ppm)	Mean ± SE adult progeny production at[a]:			
		7 d	14 d	21 d	42 d
Dehulled Kernza®	0	14.6 ± 1.3 a	12.2 ± 1.5 b	52.2 ± 4.2 a	125.6 ± 19.8 b
Dehulled Kernza®	1.25	1.6 ± 0.5 c	2.0 ± 0.5 cd	2.4 ± 0.6 d	13.6 ± 1.7 c
Hulled Kernza®	0	4.8 ± 1.8 bc	3.2 ± 0.8 c	6.2 ± 1.7 c	9.4 ± 1.7 c
Hulled Kernza®	1.25	1.2 ± 0.5 c	0.6 ± 0.4 de	1.6 ± 0.6 d	1.4 ± 0.9 d
Hard red winter wheat	0	12.0 ± 0.7 a	57.8 ± 2.3 a	105 ± 5.8 a	294.4 ± 5.7 a
Hard red winter wheat	1.25	9.2 ± 1.8 ab	1.0 ± 0.6 e	12.4 ± 0.7 b	15.6 ± 1.6 c

[a]Means among grain type and insecticide concentration at each observation time followed by different letters are significantly different ($P < 0.05$; by REGWQ) test.

Table 1.11 Three-way ANOVA statistics for weight loss of dehulled Kernza®, hulled Kernza®, and hard red winter wheat due to *R. dominica* after exposure to methoprene.

Source	df	Mean Square	*F* - value	*P* – value*
Grain type	2	26.297	67.68	< 0.0001
Insecticide concentration	1	30.795	79.27	< 0.0001
Observation time	3	8.4492	21.75	< 0.0001
Grain type × Insecticide concentration	2	12.814	32.98	< 0.0001
Grain type × Observation time	6	3.0245	7.78	< 0.0001
Insecticide concentration × Observation time	3	6.0053	15.46	< 0.0001
Grain type × Insecticide concentration × Observation time	6	0.8364	2.15	0.0543
Error	96	0.3885		

* Significant ($P < 0.05$)

Table 1.12 Grain weight loss of untreated and methoprene treated dehulled Kernza®, hulled Kernza®, and hard red winter wheat after exposure to *R. dominica* adults for 7, 14, 21, and 42 d.

Grain type	Insecticide concentration (ppm)	% Mean ± SE weight loss at[a]:			
		7 d	14 d [b]	21 d	42 d
Dehulled Kernza®	0	0.9 ± 0.3 b	0.4 ± 0.1	0.7 ± 0.3 b	2.9 ± 0.4 b
Dehulled Kernza®	1.25	0.1 ± 0.1 b	0.2 ± 0.1	0.4 ± 0.2 b	0.4 ± 0.1 c
Hulled Kernza®	0	0.2 ± 0.1 b	0.2 ± 0.1	0.6 ± 0.2 b	0.6 ± 0.2 c
Hulled Kernza®	1.25	0.7 ± 0.1 b	0.7 ± 0.4	0.2 ± 0.1 b	0.2 ± 0.2 c
Hard red winter wheat	0	2.9 ± 0.7 a	0.9 ± 0.4	3.3 ± 0.3 a	5.1 ± 0.5 a
Hard red winter wheat	1.25	0.8 ± 0.2 b	0.3 ± 0.2	1.1 ± 0.8 b	1.2 ± 0.2 c

[a]Means among grain type and insecticide concentration at each observation time followed by different letters are significantly different ($P < 0.05$; by REGWQ) test.

[b] There was no significant difference in weight loss among grain type and insecticide concentration after 14 d of exposure to insects.

Table 1.13 Three-way ANOVA statistics for mortality of *R. dominica* exposed to methoprene + deltamethrin (1.25 + 0.5 ppm).

Source	df	Mean Square	*F* -value	*P*-value
Grain type	2	3.4	653.8	< 0.0001
Insecticide concentration	1	22.3	4288.4	< 0.0001
Observation time	2	0.25	48.1	< 0.0001
Grain type × Insecticide concentration	2	3.08	592.3	< 0.0001
Grain type × Observation time	4	0.0125	2.40	0.059
Insecticide concentration × Observation time	2	0.03627	6.97	0.001
Grain type × Insecticide concentration × Observation time	4	0.1845	35.4	< 0.0001
Error	72	0.0052		

* Significant ($P < 0.05$)

Table 1.14 Mortality of *R. dominica* adults exposed for 7, 14, and 21 d to untreated and methoprene + deltamethrin (1.25 + 0.5 ppm) treated dehulled Kernza®, hulled Kernza®, and hard red winter wheat.

Grain type	Insecticide concentration (ppm)	% Mean ± SE mortality at[a]:		
		7 d	14 d	21 d
Dehulled Kernza®	0	1.2 ± 0.8 d	3.2 ± 1.2 c	4.0 ± 1.4 b
Dehulled Kernza®	1.25 + 0.5	93.6 ± 2.4 c	97.6 ± 0.7 a	100.0 ± 0.0 a
Hulled Kernza®	0	80.8 ± 3.7 c	86.0 ± 0.8 b	99.2 ± 0.5 a
Hulled Kernza®	1.25 + 0.5	98.4 ± 0.4 ab	100.0 ± 0.0 a	100.0 ± 0.0 a
Hard red winter wheat	0	0.4 ± 0.4 d	3.2 ± 1.0 c	8.0 ± 2.6 b
Hard red winter wheat	1.25 + 0.5	100.0 ± 0.0 a	100.0 ± 0.0 a	100.0 ± 0.0 a

[a]Means among grain type and insecticide concentration at each observation time followed by different letters are significantly different ($P < 0.05$; by REGWQ) test.

Table 1.15 Three-way ANOVA statistics for adult progeny production of *R. dominica* exposed to methoprene + deltamethrin (1.25 + 0.5 ppm).

Source	df	Mean Square	*F*- value	*P*- value
Grain	2	3.7	288.17	< 0.0001
Insecticide concentration	1	59.5	4605.7	< 0.0001
Observation time	3	3.15	243.59	< 0.0001
Grain type × Insecticide concentration	2	3.72	288.17	< 0.0001
Grain type × Observation time	6	0.358	27.72	< 0.0001
Insecticide concentration × Observation time	3	3.14	243.59	< 0.0001
Grain type × Insecticide concentration × Observation time	6	0.358	27.72	< 0.0001
Error	96	0.012		

[*] Significant ($P < 0.05$)

Table 1.16 Adult progeny production of *R. dominica* adults exposed for 7, 14, 21, and 42 d to untreated and methoprene + deltamethrin (1.25 + 0.5 ppm) treated dehulled Kernza®, hulled Kernza®, and hard red winter wheat.

Grain type	Insecticide concentration (ppm)	Mean ± SE adult progeny production at[a]:			
		7 d	14 d	21 d	42 d
Dehulled Kernza®	0	8.4 ± 1.5 b	24.2 ± 2.8 b	53.4 ± 8.6 a	202.2 ± 20.6 b
Dehulled Kernza®	1.25 + 0.5	0.0 ± 0.0 c	0.0 ± 0.0 c	0.0 ± 0.0 c	0.0 ± 0.0 c
Hulled Kernza®	0	0.0 ± 0.0 c	0.4 ± 0.2 c	3.8 ± 1.7 b	181.4 ± 8.1 b
Hulled Kernza®	1.25 + 0.5	0.0 ± 0.0 c	0.0 ± 0.0 c	0.0 ± 0.0 c	0.0 ± 0.0 c
Hard red winter wheat	0	45.6 ± 3.4 a	52.0 ± 1.1 a	64.8 ± 8.4 a	279.2 ± 7.9 a
Hard red winter wheat	1.25 + 0.5	0.0 ± 0.0 c	0.0 ± 0.0 c	0.0 ± 0.0 c	0.0 ± 0.0 c

[a]Means among grain type and insecticide concentration at each observation time followed by different letters are significantly different ($P < 0.05$; by REGWQ) test.

Table 1.17 Three-way ANOVA statistics for weight loss of dehulled Kernza®, hulled Kernza®, and hard red winter wheat due to *R. dominica* after exposure to methoprene + deltamethrin (1.25 + 0.5 ppm).

Source	df	Mean Square	*F* - value	*P* – value*
Grain type	2	18.554	37.91	< 0.0001
Insecticide concentration	1	90.199	184.3	< 0.0001
Observation time	3	3.8457	7.86	< 0.0001
Grain type × Insecticide concentration	2	18.837	38.49	< 0.0001
Grain type × Observation time	6	1.0799	2.21	0.0489
Insecticide concentration × Observation time	3	4.1141	8.41	< 0.0001
Grain type × Insecticide concentration × Observation time	6	1.061	2.17	0.0528
Error	95	0.4894		

* Significant ($P < 0.05$)

Table 1.18 Grain weight loss of untreated and methoprene + deltamethrin (1.25 + 0.5 ppm) treated dehulled Kernza®, hulled Kernza® and hard red winter wheat after exposure to *R. dominica* adults for 7, 14, 21, and 42 d.

Grain type	Insecticide concentration (ppm)	% Mean ± SE weight loss at[a]:			
		7 d	14 d	21 d	42 d
Dehulled Kernza®	0	1.3 ± 0.3 ab	1.8 ± 0.5 a	0.8 ± 0.2 b	2.9 ± 0.4 b
Dehulled Kernza®	1.25 + 0.5	0.1 ± 0.0 b	0.2 ± 0.2 b	0.1 ± 0.1 b	0.1 ± 0.1 c
Hulled Kernza®	0	0.2 ± 0.1 b	0.2 ± 0.3 b	0.7 ± 0.3 b	0.6 ± 0.2 c
Hulled Kernza®	1.25 + 0.5	0.0 ± 0.0 b	0.0 ± 0.0 b	0.0 ± 0.0 b	0.0 ± 0.0 c
Hard red winter wheat	0	2.4 ± 0.7 a	2.5 ± 0.6 a	2.7 ± 0.6 a	5.1 ± 0.5 a
Hard red winter wheat	1.25 + 0.5	0.0 ± 0.0 b	0.0 ± 0.0 b	0.0 ± 0.0 b	0.0 ± 0.0 c

[a]Means among grain type and insecticide concentration at each observation time followed by different letters are significantly different ($P < 0.05$; by REGWQ) test.

Table 1.19 Three-way ANOVA statistics for mortality of *R. dominica* exposed to spinosad.

Source	df	Mean Square	*F* -value	*P*-value*
Grain type	2	1.61	128.8	< 0.0001
Insecticide concentration	1	31.98	2558.4	< 0.0001
Observation time	2	0.225	18	< 0.0001
Grain type × Insecticide concentration	2	1.6129	129	< 0.0001
Grain type × Observation time	4	0.3469	27.75	< 0.0001
Insecticide concentration × Observation time	2	0.2254	18.03	< 0.0001
Grain type × Insecticide concentration × Observation time	4	0.3469	27.75	< 0.0001
Error	72	0.0125		

* Significant ($P < 0.05$)

Table 1.20 Mortality of *R. dominica* adults exposed for 7, 14, and 21 d to untreated and spinosad treated dehulled Kernza®, hulled Kernza®, and hard red winter wheat.

Grain type	Insecticide concentration (ppm)	Mean ± SE mortality at: [a]		
		7 d	14 d	21 d
Dehulled Kernza®	0	1.6 ± 0.9 b	0.8 ± 0.8 d	12.8 ± 2.6 bc
Dehulled Kernza®	1	100.0 ± 0.0 a	100.0 ± 0. 0 a	100.0 ± 0.0 a
Hulled Kernza®	0	100.0 ± 0.0 a	22.4 ± 2.7 b	37.6 ± 14.7 b
Hulled Kernza®	1	100.0 ± 0.0 a	100.0 ± 0.0 a	100.0 ± 0.0 a
Hard red winter wheat	0	0.0 ± 0.0 c	1.6 ± 0.9 c	3.2 ± 1.9 c
Hard red winter wheat	1	100.0 ± 0.0 a	100.0 ± 0.0 a	100.0 ± 0.0 a

[a]Means among grain type and insecticide concentration at each observation time followed by different letters are significantly different ($P < 0.05$; by REGWQ) test.

Table 1.21 Three-way ANOVA statistics for adult progeny production of *R. dominica* exposed to spinosad.

Source	df	Mean Square	*F*- value	*P* – value*
Grain	2	4.08	136	< 0.0001
Insecticide concentration	1	110.17	3672.3	< 0.0001
Observation time	3	1.12	37.3	< 0.0001
Grain type × Insecticide concentration	2	4.04	134.6	< 0.0001
Grain type × Observation time	6	0.48	16	< 0.0001
Insecticide concentration × Observation time	3	1.12	37.3	< 0.0001
Grain type × Insecticide concentration × Observation time	6	0.48	16	< 0.0001
Error	96	0.03		

* Significant ($P < 0.05$)

Table 1.22 Adult progeny production of *R. dominica* exposed for 7, 14, 21, and 42 d to untreated and spinosad treated dehulled Kernza®, hulled Kernza®, and hard red winter wheat.

Grain type	Insecticide concentration (ppm)	Mean ± SE adult progeny production at[a]:			
		7 d	14 d	21 d	42 d
Dehulled Kernza®	0	66.4 ± 25.4 b	76.4 ± 11.4 b	53.4 ± 8.6 b	134.8 ± 17.1 b
Dehulled Kernza®	1	0.0 ± 0.0 c	0.0 ± 0.0 d	0.0 ± 0.0 c	0.0 ± 0.0 c
Hulled Kernza®	0	1.6 ± 1.1 c	6.4 ± 2.0 c	95.6 ± 24.0 b	156.4 ± 6.7 b
Hulled Kernza®	1	0.0 ± 0.0 c	0.0 ± 0.0 d	0.0 ± 0.0 c	0.0 ± 0.0 c
Hard red winter wheat	0	296.4 ± 25.6 a	318.4 ± 22.3 a	351.6 ± 26.7 a	483.2 ± 59.1 a
Hard red winter wheat	1	0.0 ± 0.0 c	0.0 ± 0.0 d	0.0 ± 0.0 c	0.0 ± 0.0 c

[a]Means among grain type and insecticide concentration at each observation time followed by different letters are significantly different ($P < 0.05$; by REGWQ) test.

Table 1.23 Three-way ANOVA statistics for weight loss of dehulled Kernza®, hulled Kernza®, and hard red winter wheat due to *R. dominica* after exposure to spinosad.

Source	df	Mean Square	*F* - value	*P* – value*
Grain type	2	253.64	16.7	< 0.0001
Insecticide concentration	1	396.52	26.1	< 0.0001
Observation time	3	26.901	1.7	< 0.0001
Grain type × Insecticide concentration	2	253.64	16.7	< 0.0001
Grain type × Observation time	6	15.152	1	< 0.0001
Insecticide concentration × Observation time	3	26.90	0.9	< 0.0001
Grain type × Insecticide concentration × Observation time	6	26.901	1.7	< 0.0001
Error	96	15.152		

* Significant ($P < 0.05$)

Table 1.24 Grain weight loss of untreated and spinosad treated dehulled Kernza®, hulled Kernza®, and hard red winter wheat after exposure to *R. dominica* adults for 7, 14, 21, and 42 d.

Grain type	Insecticide concentration (ppm)	% Mean ± SE weight loss at[a]:			
		7 d	14 d	21 d	42 d
Dehulled Kernza®	0	0.3 ± 0.1 b	0.9 ± 0.2 b	0.8 ± 0.3 b	2.6 ± 0.5 b
Dehulled Kernza®	1	0.0 ± 0.0 b	0.0 ± 0.0 b	0.0 ± 0.0 b	0.0 ± 0.0 b
Hulled Kernza®	0	0.2 ± 0.1 b	0.1 ± 0.1 b	0.8 ± 0.2 b	0.3 ± 0.1 b
Hulled Kernza®	1	0.0 ± 0.0 b	0.0 ± 0.0 b	0.0 ± 0.0 b	0.0 ± 0.0 b
Hard red winter wheat	0	4.6 ± 0.8 a	8.4 ± 0.7 a	8.8 ± 2.6 a	15.8 ± 2.3 a
Hard red winter wheat	1	0.0 ± 0.0 b	0.0 ± 0.0 b	0.0 ± 0.0 b	0.0 ± 0.0 b

[a]Means among grain type and insecticide concentration at each observation time followed by different letters are significantly different ($P < 0.05$; by REGWQ) test.

Chapter 2 - Efficacy of selected grain protectants on hulled Kernza®, dehulled Kernza® and hard red winter wheat against *Sitophilus oryzae* (Linneaus) (Coleoptera: Curculionidae)

2.1 Abstract

The efficacy of selected commercial formulations of grain protectants, deltamethrin, methoprene and methoprene + deltamethrin, and spinosad on hulled Kernza®, dehulled Kernza® and hard red winter wheat was evaluated in laboratory against Rice weevil, *Sitophilus oryzae* (Linneaus). To date, no grain protectants are currently registered for use on this perennial grass (*Thinopyrum intermedium*) [Order: Poales; Family: Poaceae] (Host) Barkworth & D.R. Dewey), marketed under the trade name Kernza®. Phosphine is the only fumigant approved for treating perennial grasses. The concentrations of the protectants used were 0.5 and 1 ppm deltamethrin, 1.25 ppm methoprene, 1.25 ppm methoprene + 0.5 ppm deltamethrin as a combination product and spinosad 1 ppm. Mortality was assessed after 7, 14 and 21 days of the application of protectants and insect introduction. The mortality of the insects was expressed as percentage of the total insects exposed. In separate replicates, grain was infested and observed after 42 d for adult progeny production and weight loss. After determination of adult progeny production, the grains were passed through the Boerner® divider to get a representative sample of 6 g for wheat and 1.5 g for hulled and dehulled Kernza®. The number of damaged kernels was assessed and expressed as a percentage. Among grain protectants used, deltamethrin at 0.5 and 1 ppm gave >50% *S. oryzae* adult mortality after 14 and 21 d resulting in <10 adult progeny production and <0.5% grain weight loss in all grain types. Methoprene at 1.25 ppm did not cause significant mortality of *S. oryzae* but caused low progeny numbers in dehulled Kernza® and hard red winter wheat. Adult mortality was 60-100 %, progeny production was < 21 and grain weight loss was 0.2 to 0.7 % in methoprene + deltamethrin treatment in all grain types. Spinosad at 1 ppm caused >87 % adult mortality of *S. oryzae* resulting in progeny production less than 6 and there was no

grain weight loss. *S. oryzae* adult mortality was consistently higher in hulled Kernza® in control treatments for all insecticides.

Further research under practical field conditions in farmer- managed trials overtime offers a better understanding of efficacy of insecticides against *S. oryzae* field populations. Additionally, physico-chemical study of hulled Kernza® should be done to further examine the cause of high insect mortality.

2.2 Introduction

Kernza®, a newly developed grain by the Land Institute is an intermediate wheatgrass (*Thinopyrum intermedium*) (Host) Barkworth & D. R. Dewey, widely adapting, high yielding that is used as forage grass and is excellent feed for livestock (Hendrickson et al., 2005). It is 20 - 50% smaller than wheat grains and has high hull to kernel weight ratio (30% hull to 70% kernel) but the plant produces more seeds in compared to annual wheat (Penoza, 2024). Kernza® is highly nutritious grain as it has 50% more protein, 65% more ash content, and 129% more dietary fiber than whole wheat flour. It is also rich with high vitamins and micronutrients than whole wheat (AURI, 2022; Craine and DeHaan, 2024). Presently, Kernza® is an emerging crop cultivated on a small scale for niche markets. Market demand has surpassed the supply, with small local and organic artisan bakeries and farm-to-table restaurants using Kernza® grain to produce breads, crackers, and pasta (Lanker et al., 2020). Scientists strive to enhance the size of the seeds with every breeding cycle. With the improving yield of Kernza®, it is crucial to save it from several stored grain insects (Culman et al., 2013).

Rice weevil, *Sitophilus oryzae* (Linnaeus) (Coleoptera; Curculionidae) is among the most notorious insects infesting stored grains worldwide (Gowda et al., 2019). Rice weevil reproduces rapidly and has the potential to spread globally. Adult insects bore into grains and lay their eggs inside. As the eggs hatch into larvae, they consume the interior of the grains, leaving them hollow. This can cause significant loss in quality and quantity of grain (Zhang et al., 2021). It is known to cause both vertical (from top to bottom) and horizontal infestation (from harvest operation) to stored grain . It has been reported to cause damage ranging from 10-65% in short-term storage and up to 80% in long-term storage conditions (Park et al., 2004).

No grain protectants are currently registered for use on Kernza®. Phosphine is the only fumigant approved for treating perennial grasses. Grain protectants are insecticides that are incorporated into grains directly to protect grains from insects and mites. Application Insecticide concentrations are typically selected to ensure that residual protection lasts until the point of consumption. This extended protection is often a key benefit in managing grain storage systems (Bengston, 1985). Grain protectants are liquids or dust applied to newly harvested grains considered for long- or short-term storage. Fumigation can kill the existing population of insects, but it doesn't give residual long- term protection. The varying effectiveness of insecticides across different grains should be investigated in greater detail, as it could result in the need for different application Insecticide concentrations for each grain type (Ksoura et al., 2022).

Insecticide is applied to a moving grain stream at the bottom of the bucket elevator, auger, or belt, ensuring it contacts as many grain kernels as possible while the grain moves to its destination (Studebaker, 2024). Thus far, there have been no experiments performed and literature cited that have evaluated efficacy of existing grain protectants on Kernza® against *S. oryzae.* In this study, we evaluated the efficacy of selected grain protectants in hulled and dehulled form of grains against *S. oryzae.* Parameters such as mortality, progeny production and grain weight loss in treated and untreated Kernza® grains in comparison to wheat were evaluated.

2.3 Material and methods

2.3.1. Grain commodities

Hulled and dehulled Kernza® (variety: TLI C5) grains from the year 2022 harvest were obtained from the Sustain-A-Grain, McPherson County, Kansas, USA, shipped to the Department of

Grain Science and Industry at Kansas State University, Manhattan, KS, USA, and held in freezer at ~-20°C for two weeks to kill live insects. Organic whole grain hard red winter wheat stock (Great River Organic Milling, Fountain City, Wisconsin, USA) (standard control) was compared with Kernza® grains. Kernza® and wheat grain samples were removed from the freezer and acclimatized in plastic bags at room conditions (~24.5 °C and ~76% R.H.) for 1-2 weeks before the experiment. The grain moisture content was measured by air oven method (American Society of Agricultural and Biological Engineers (ASAE, 2000). Both Kernza® and wheat grain samples were equilibrated to 13% moisture (wet basis) content in environmental growth chambers maintained at 28°C and 65% rh. before the experiment.

2.3.2. Insect colonies

Insect cultures of lesser grain borer, *S. oryzae* was maintained for more than 20 generations at Department of Grain Science and Industry were used for experiment. Insect cultures were reared in 0.95liter mason glass jars fitted with wire mesh and filter paper lids containing preconditioned organic hard red winter wheat. The glass jars were held inside growth chambers (Model I-36 VL; Percival Scientific, Perry, Iowa, USA) at 28°C, 65% relative humidity (r.h.), and a photoperiod of 14:10 (Light: Dark) h.

2.3.3. Grain protectants

Four selected insecticides were used to assess their efficacy on hulled and dehulled Kernza® against *S. oryzae*. Two insecticide concentrations, 0.5 and 1 ppm of deltamethrin, 1.25 ppm methoprene, 1.25 ppm methoprene + 0.5 ppm deltamethrin as a combination product and 1 ppm spinosad concentration were used. Centynal^{EC} containing 4.75 % active ingredient of deltamethrin, Diacon®-IGR containing 33.6% active ingredient of methoprene and Diacon®-IGR Plus containing 4.75% deltamethrin and 11.4% methoprene were obtained from Central Life

Sciences, Illinois, USA. The liquid formulation of Sensat™ containing 8.66% active ingredient of Spinosad was obtained from Bayer Crop Science LP, Research Triangle Park, North Carolina, United States.

2.3.4. Grain treatment, insect exposure, response and grain damage

All grain types were held inside a deep freezer at -13ºC for a week to kill any live insects if present. Then, 100 g grain (13% moisture, wet basis) were transferred to individual 0.45 L glass jars. The glass jars were secured with 250 mm wire mesh screens and filter paper (7 cm diameter) lids to facilitate air diffusion. Grains in the jars were individually treated with prepared insecticide concentrations and untreated grains were used as control for comparison and treated with same volume of distilled water with Fisherbrand™ adjustable-volume pipette. The jars were manually shaken for 1 min to ensure uniform mixing of insecticide concentrations and distilled water. Fifty unsexed adults of *S. oryzae* from culture jars were introduced into each glass jar. After insect introduction jars were covered with lids with wire mesh and filter paper and placed in growth chamber at 28ºC and 65% r.h. Temperature and relative humidity were monitored during the experiment using HOBO data recorders (Onset Computer, Pocasset, MA).

Mortality counts were taken after 7, 14 and 21d after exposure to insecticides. Adults that did not respond when lightly tapped with a camel hairbrush were considered dead. All dead and alive adult beetles were separated from the grain and were discarded. The grains were carefully returned to the individual glass jars and held inside the growth chamber at 28ºC and 65% r.h for additional 28 d. At 42d, adult progeny produced were counted and introduced fifty parental adults were subtracted from overall adult progeny number. Hulled and dehulled Kernza® were passed 6 times and hard red winter wheat was passed 4 times into Boerner® divider (Seedburo Equipment Company, Des Plaines, Illinois, USA) to obtain a working sample of ~1500 mg

Kernza® and ~5000 mg wheat to determine grain weight loss. The number of insect damaged kernels out of the total were examined with hand lens for the presence of a hole or burrow and was expressed as a percentage (Boxall, 1986). Kernels with uneven holes about 1 mm wide that get deeper from where the larva entered, spreading around the kernel to the pupation chamber were considered insect damaged kernel (Breese, 1960).

Insect damaged kernels (%) = $[Nd \div (Nd + Nu)] \times 100$

Where,

Nu = Number of undamaged kernels

Nd = Number of damaged kernels

The percentage of grain weight loss was calculated based on counting and weighing damaged and un-damaged kernels from each replicate working sub-sample (Boxall, 1986) using a digital weighing balance. The weight loss percentage of grains in each replicate sub-samples was calculated using the following formula:

Weight loss (%) = $[(WuNd - WdNu) \div (Wu \times (Nd + Nu))] \times 100$

Where,

Wu = Weight of undamaged kernels

Nu = Number of undamaged kernels

Wd = Weight of damaged kernels

Nd = Number of damaged kernels

2.3.5. Statistical analysis

Statistical analysis for mortality (%), total number of progenies produced, and weight loss (%) were performed in quintuplicates using one-way analysis of variance (ANOVA). Three-way ANOVA was performed to check significance of individual and interaction effect of all variables. One -way ANOVA was performed to check significance among grain types and insecticide concentrations. Mortality (%) data was transformed to angular values. The recorded data of adult progeny number was log transformed ($\log_{10}(x + 1)$), for statistical analysis (Zar, 1984). The means were compared with Ryan-Einot-Gabriel-Welsch multiple range (REGWQ) test at $P \leq 0.05$, using the Statistical Analysis System (SAS) Version 9.4, SAS Institute, Cary, North Carolina, USA (SAS Institute, 2012).

2.4 Results

2.4.1. Percentage mortality of *S. oryzae* adults exposed to deltamethrin

The main effects of grain type (df = 2, 108), insecticide concentration (df = 2, 108) and observation time (df = 2, 108) and two-way interaction of grain × insecticide concentration (df = 4, 108) and insecticide concentration × observation time (df = 2, 108) and three-way interaction grain × insecticide concentration × observation type (df = 4, 108) were all significant (F_{range} = 5.08 – 629.39; $P < 0.0001$) (Table 2.1).

The mortality percentage (mean ± SE) of *S. oryzae* adults in control and following exposure to deltamethrin in hulled, dehulled Kernza® and hard red winter wheat for 7, 14, and 21 days are presented in Table 2.2. One-way ANOVA showed that after 7 d, the highest mortality among grain type and insecticide concentration was found in hard red winter wheat treated with 1 ppm deltamethrin followed by 1 ppm deltamethrin treated hulled Kernza® followed by 1 ppm deltamethrin treated dehulled Kernza®. Among control treatments, highest mortality was obtained in hulled Kernza® (F = 89; df = 8, 36; P < .0001). After 14 d, mortality among grain type and insecticide concentration was highest in hard red winter wheat treated with 0.5 and 1 ppm deltamethrin and hulled Kernza® treated with 1 ppm deltamethrin followed by dehulled Kernza® treated with 1 ppm deltamethrin. Lowest insect mortality was found in untreated hulled Kernza® (F = 63.2; df = 8, 36; P < 0.0001). After 21 d, highest mortality of ~ 98% was seen in hard red winter wheat treated with 1 ppm deltamethrin followed by hard red winter wheat treated with 0.5 ppm deltamethrin. More than 70 % mortality of insect was obtained in 1ppm deltamethrin treated dehulled Kernza® and 0.5 and 1 ppm treated hulled Kernza® (F = 67.7; df = 8, 36; P < 0.0001).

2.4.2. Adult progeny of *S. oryzae* exposed to deltamethrin

The main effects of grain type (df = 2, 143), insecticide concentration (df = 2, 143) and observation time (df = 3, 143) and two-way interaction of grain type × insecticide concentration (df = 4, 143); grain type × observation time (df = 6, 143) and insecticide concentration × observation time (df = 6, 143) and three-way interaction grain type × insecticide concentration × observation type (df = 12, 143) were all significant ($F_{,\ range}$ = 4.3 – 406.2); P < 0.0001) (Table 2.3).

The adult progeny number of *R. dominica* adults in control and deltamethrin treated hulled, dehulled Kernza® and hard red winter wheat for 7, 14, 21, and 42 days are presented in Table 2.4. After 7 d, in control highest progeny was observed in heart red winter wheat followed by dehulled Kernza®. The progeny was lowest in hard red winter wheat treated with 1 ppm deltamethrin (F = 97.15; df = 8, 28; P < .0001). The trend was similar after 14d (F = 27.6; df = 8, 28; P < 0.0001). and 21 d (F = 40.2; df = 8, 28; P < 0.0001) exposure. There was no progeny production in hard red winter wheat treated with 1 ppm deltamethrin after 42 days of continuous exposure to insecticide. Low progeny number were also seen in 1 ppm deltamethrin treated dehulled (7.2 ± 2.2) and hulled Kernza® (2.6 ± 1.7). Highest number of progenies were obtained in untreated hard red winter wheat which was statistically similar to progeny number produced in untreated hulled Kernza® which was statistically similar to dehulled Kernza® (F = 119.88; df = 8, 28; P < 0.0001). The progeny produced in all three grains treated with 0.5 ppm deltamethrin was statistically similar and was more than progeny in grains treated with 1 ppm deltamethrin.

2.4.3. Weight loss of grain due to *S. oryzae* exposed to deltamethrin

The main effects of grain type (df = 2, 136), insecticide concentration (df = 2, 136) and observation time (df = 3, 136) and two-way interaction of grain type × insecticide concentration (df = 4, 136); and insecticide concentration × observation time (df = 6, 136) were significant (F, $_{range}$ = 2.74 – 128.05); P = 0.03) The interaction between grain type × insecticide concentration × observation time (F = 1.23; df = 6, 136; P = 0.266) was not significant (Table 2.5).

The weight loss of grain due to *S. oryzae* adults in control and following exposure to deltamethrin in hulled, dehulled Kernza® and Hard red winter wheat for 7, 14, 21 and 42 days are presented in Table 2.6. One-way ANOVA showed that after 7 d, highest weight loss was obtained on untreated hard red winter wheat followed by untreated dehulled Kernza®. All other treatments were statistically similar to it (F = 3.74; df = 8, 36; P = 0.002). After 14 d highest weight loss was seen on untreated dehulled Kernza® which was statistically similar to untreated hard red winter wheat. Less than 1 % weight loss was found on insecticide treated grains (F = 3.74; df = 8, 36; P = 0.266). Similar trend was obtained after 21 d (F = 5.8; df = 8, 35; P < 0.0001). After 42 d of continuous exposure, highest weight loss was obtained on untreated dehulled Kernza® which was statistically similar to untreated hard red winter wheat and hulled

Kernza®. The lowest grain weight loss was found in all grain type treated with deltamethrin 1 ppm (F = 7.22; df = 8, 35; P < 0.0001).

2.4.4. Percentage mortality of *S. oryzae* adults exposed to methoprene

The main effects of grain type (df = 2, 72), insecticide concentration (df = 1, 72) and observation time (df = 1, 72) and two-way interaction of insecticide concentration × observation time (df = 2, 72) and three-way interaction grain type × insecticide concentration × observation type (df = 4, 72) were all significant (F_{range} = 8.9 – 444.9); P < 0.05). Interaction between grain type × insecticide concentration (F = 0.7; df = 2, 72; P = 0.466), grain type × observation time (F = 1.64; df = 4, 72; P = 0.174) was not significant (Table 2.7).

The mortality percentage of *S. oryzae* adults in control and following exposure to Methoprene in hulled, dehulled Kernza® and hard red winter wheat for 7, 14, and 21 days are presented in Table 2.8. In control, higher mortality of insects was observed in hulled Kernza® than dehulled Kernza® and hard red winter wheat and trend was similar in all observation times. After 7d, highest mortality among grain type and insecticide concentration was found on untreated hulled Kernza® which was statistically similar to mortality in methoprene treated hulled Kernza®. Similar trend was found in all observation times. There was no significant difference in mortality of adult insects in untreated and treated grains. High mortality of adult insects was not seen after application of methoprene.

2.4.5. Adult progeny of *S. oryzae* exposed to methoprene

The main effects of grain type (df = 2, 96), insecticide concentration (df = 1, 96) and observation time (df = 3, 96) and two-way interaction of grain type × insecticide concentration (df = 2, 96); grain type × observation type (df = 6, 96) and insecticide concentration × observation time (df =

3, 96) and three-way interaction grain type × insecticide concentration × observation time (df = 6, 96) were significant (F_{range} = 3.8 – 606.1; $P < 0.0001$) (Table 2.9).

The adult progeny number of *S. oryzae* adults in control hulled, dehulled Kernza® and Hard red winter wheat after 7, 14, 21 and 42 days of infestation are presented in Table 2.10. After 7 d, the lowest progeny was obtained in the Methoprene treated grains and untreated hulled Kernza®. Similar trend was found in all observation times (F_{range} = 54.65 – 121.4; df = 5, 24; $P < 0.05$).

2.4.6. Weight loss of grain due to *S. oryzae* exposed to methoprene

The main effects of grain type (df = 2, 95), insecticide concentration (df = 1, 95) and observation time (df = 3, 95) and two-way interaction of insecticide concentration × observation time (df = 3, 95) were significant (F_{range} = 5.6 – 32.6; $P < 0.05$). The interaction of grain type × insecticide concentration (F = 1.5; df = 2, 95; P = 0.21), grain type × observation time (F = 2.01; df = 6, 95; P = 0.07) and grain type × insecticide concentration × observation time (F = 1.7; df = 6, 95; P = 0.12) are not significant (Table 2.11).

The weight loss of grain due to *S. oryzae* adults in control and following exposure to Methoprene in hulled, dehulled Kernza® and Hard red winter wheat for 7, 14, 21 and 42 d are presented in Table 2.12. The highest grain weight loss was obtained in untreated hard red winter wheat in all observation times. There was significant difference in weight loss among treated and untreated grains in all grain types in all observation periods (F_{range} = 2.9 – 8.5; df = 5, 24; $P <$ 0.05).

2.4.7. Percentage mortality of *S. oryzae* adults exposed to methoprene + deltamethrin

The main effects of grain type (df = 2, 72), insecticide concentration (df = 1, 72) and observation time (df = 2, 72) and two-way interaction of insecticide concentration × observation time (df = 2, 72) and three-way interaction grain type × insecticide concentration × observation time (df = 4, 72) were significant (F_{range} = 8.9 – 447; P < 0.05). The interaction between grain type × insecticide concentration (F = 0.8; df = 2, 72; P = 0.46), grain type × observation time (F = 1.6; df = 4, 72; P = 0.17) are not significant (Table 2.13).

The mortality percentage of *R. dominica* adults in control and following exposure to methoprene + deltamethrin in hulled, dehulled Kernza® and Hard red winter wheat for 7, 14, and 21 days are presented in Table 2.14. After 7 d, highest insect mortality was obtained in insecticide treated hard red winter wheat. The result was statistically similar to treated hulled and dehulled Kernza® (F = 52.4; df = 5, 24; P < 0.001). Among control, highest mortality was found on hulled Kernza®. After 14 d of exposure, highest mortality was seen on treated hard red winter wheat followed by untreated and treated hulled Kernza® respectively (F = 167.4; df = 5, 24; P < 0.001). Similarly, after 21 day of exposure, highest mortality was found in insecticide treated hard red winter wheat and hulled Kernza® followed by treated dehulled Kernza®. Among control, highest mortality was seen on hulled Kernza® followed by dehulled Kernza®. The lowest mortality was obtained in untreated hard red winter wheat (F = 110.8; df = 5, 24; P < 0.001).

2.4.8. Adult progeny of *S. oryzae* exposed to methoprene + deltamethrin

The main effects of grain type (df = 2, 96), insecticide concentration (df = 1, 96) and observation time (df = 3, 96) and two-way interaction of grain type × insecticide concentration (df = 2, 96); grain type × observation time (df = 6, 96) and insecticide concentration × observation time (df =

3, 96) and three-way interaction grain type × insecticide concentration × observation type (df = 6, 96) were all significant (F_{range} = 7.7 – 1316.3; $P < 0.0001$) (Table 2.15).

The adult progeny number of *S. oryzae* adults in control hulled, dehulled Kernza® and hard red winter wheat after 7, 14, 21 and 42 days are presented in Table 2.16. The highest progeny number among both grain type and insecticide concentration were obtained in untreated hard red winter wheat in all observation times followed by untreated dehulled Kernza®. The progeny number were significantly less in insecticide treated grains than in control treatments. Similar result was found in all observation times (F_{range} = 74.5 – 173.8; df = 5, 24; $P < 0.0001$).

2.4.9. Weight loss of grain due to *S. oryzae* exposed to methoprene + deltamethrin

The main effects of grain type (df = 2, 94), insecticide concentration (df = 1, 94) and observation time (df = 3, 94) and two-way interaction of grain type × insecticide concentration (df = 2, 94); grain type × observation time (df = 6, 94) and insecticide concentration × observation time (df = 3, 94) and three-way interaction grain type × insecticide concentration × observation type (df = 6, 94) were all significant (F_{range} = 3.0 – 53.0; $P < 0.05$) (Table 2.17).

The weight loss of grain due to *R. dominica* adults in control and following exposure to methoprene + deltamethrin in hulled, dehulled Kernza® and hard red winter wheat after 7, 14, 21 and 42 d are presented in Table 2.18. There was no significant difference in grain weight loss among grain type and insecticide concentration after 7 (F = 2.8; df = 5, 24; P = 0.03) and 21 (F = 2.8; df = 5, 23; P = 0.03) days. After 14 d, highest grain weight loss was found in untreated hard red winter wheat which was statistically similar to untreated dehulled Kernza® (F = 6.3; df = 5, 24; $P < 0.05$). After 42 d, highest grain weight loss was found in untreated hard red winter wheat followed by untreated dehulled Kernza®, which was statistically similar to all other treatments (F = 13.5; df = 5, 23; P < 0.001).

2.4.10. Percentage mortality of *S. oryzae* adults exposed to spinosad

The main effects of grain type (df = 2, 71), insecticide concentration (df = 1, 71) and observation time (df = 2, 71) and three-way interaction grain type × insecticide concentration × observation type (df = 4, 71) were all significant (F_{range} = 3.15 – 1086.1); P < 0.05). The interaction between grain type × insecticide concentration (F = 296.5; df = 2, 71; P = 0.46), grain type × observation time (F = 2.9; df = 4, 71; P = 0.02) and insecticide concentration × observation time (F = 0.4; df = 2, 71; P = 0.66) are not significant (Table 2.19).

The mortality percentage of *S. oryzae* adults in control and following exposure to Spinosad in hulled, dehulled Kernza® and hard red winter wheat for 7, 14, and 21 days are presented in Table 2.20. One-way ANOVA showed that in after 7 d, highest mortality was found in untreated hulled Kernza® which was statistically similar to insecticide treated hulled Kernza® and hard red winter wheat. The lowest insect mortality was found in untreated hard red winter wheat (F = 140.8; df = 5, 24; P < 0.001). After 14 d, 100% mortality was observed in all grain type treated with Spinosad and untreated hulled Kernza® (F = 263.1; df = 5, 23; P < 0.001). Similar trend was observed after 21 d (F = 167.1; df = 5, 24; P < 0.001).

2.4.11. Adult progeny of *S. oryzae* exposed to spinosad

The main effects of grain type (df = 2, 96), insecticide concentration (df = 1, 96) and observation time (df = 3, 96) and two-way interaction of grain type × insecticide concentration (df = 2, 96); grain type × observation time (df = 6, 96) and insecticide concentration × observation time (df =

3, 96) and three-way interaction grain type × insecticide concentration × observation type (df = 6, 96) were all significant (F_{range} = 24.9 – 1073.1); $P < 0.0001$) (Table 2.21).

Progeny numbers of *S. oryzae* adults in control and following exposure to spinosad in hulled, dehulled Kernza® and hard red winter wheat after 7, 14, 21 and 42 days are presented in Table 2.22. After 7d of exposure, the highest progeny number was found in untreated hard red winter wheat followed by dehulled Kernza®. The lowest progeny number was found in spinosad treated hulled Kernza® (F = 105.2; df = 5, 24; $P < 0.001$). After 14 d of exposure, highest progeny was seen on untreated hard red winter wheat followed by untreated dehulled Kernza®. Low progeny was found in the grains treated with spinosad. However, no progeny was found in both treated and untreated hulled Kernza® (F = 90.9; df = 5, 24; $P < 0.001$). Similar trend was observed after 21 d (F = 121.1; df = 5, 24; $P < 0.001$). After 42 d of continuous infestation, highest progeny number was obtained in hard red winter wheat followed by dehulled Kernza®. The lowest progeny was found in insecticide treated hard red winter wheat (F = 135.9; df = 5, 24; $P < 0.0001$).

2.4.12. Weight loss of grain due to *S. oryzae* exposed to spinosad

The main effects of grain type (df = 2, 96), insecticide concentration (df = 1, 96) and observation time (df = 3, 96) and two-way interaction of grain type × insecticide concentration (df = 2, 96); grain type × observation time (df = 6, 96) and insecticide concentration × observation time (df = 3, 96) and three-way interaction grain type × insecticide concentration × observation type (df = 6, 96) were all significant (F_{range} = 4.8 – 27.6); $P < 0.05$) (Table 2.23).

The weight loss of grain due to *S. oryzae* adults in control and following exposure to spinosad in hulled, dehulled Kernza® and hard red winter wheat for 7, 14, 21, and 42 d are presented in Table 2.24. After 7 d of exposure, highest weight loss (~ 0.3%) was found in untreated dehulled Kernza®. Similar result was obtained after 21 d. After 14 d, highest grain weight loss was obtained in hard red winter wheat. After 42 d highest weight loss was found in untreated hard red winter wheat and there was no progeny produced in insecticide treated grains ($F = 7.39$; df = 5, 24; $P = 0.0003$). Weight loss increased as there was increase in observation time. In spinosad treated grains no weight loss was seen in hard red winter wheat and dehulled Kernza®.

2.5 Discussion

Deltamethrin is a pyrethroid insecticide widely approved for use in stored grain protection across many countries. It is also effective in controlling pests that infest stored products and offers extended protection, lasting for months or longer (Samson et al., 1990). Deltamethrin can poison insects by penetrating their cuticle or through ingestion. It is highly effective in controlling a wide range of pests and is considered the most powerful of the synthetic pyrethroids. The technical-grade version of deltamethrin has a purity exceeding 98% and is chemically stable. Deltamethrin's mode of action is thought to be mainly central in action, or at least originate in higher nerve centers of the brain. Death of insects seems to be due to irreversible damage to the nervous system occurring when poisoning lasts more than a few hours (Rashid et al., 2009)

In 2012, the United States Environmental Protection Agency (EPA) approved deltamethrin, marketed as Centynal by Wellmark International (Schaumburg, Illinois, USA), for use on barley, corn, oats, popcorn, rice, rye, sorghum, and wheat intended for storage, as well as for treating empty storage facilities designated for these grains. Centynal is currently the sole formulation of deltamethrin available in the U.S. for treating both empty grain storage structures and stored grain.(Sehgal and Subramanyam, 2014).

The current research examined the activity of 0.5 and 1 ppm deltamethrin insecticide concentration in Kernza® grains with compared to wheat against *S. oryzae*. The study concluded that high insect mortality was obtained in Kernza® grains and wheat after application of both insecticide concentration of deltamethrin in all observation periods. In dehulled Kernza® the adult mortality was ~ 50% which was higher than control but not as 1ppm insecticide concentration. Among control, high insect mortality was seen on hulled Kernza®. This might be due to the presence of hulls.

The study by (Trostanetsky et al., 2023) concluded that when deltamethrin insecticide concentration of 0.2 to 2 ppm was applied to stored wheat grain and dust, insect mortality increased according to application Insecticide concentration. The study conducted by (Quellhorst et al., 2023), found that there was less mortality of insects when only some layer of grains were treated with deltamethrin. Numerous past studies have reported that hulled grains prevent insect pest penetration and damage (Chanbang et al., 2008; Kumar et al., 2020). Significant progeny reduction and less grain weight loss was found in wheat treated with 1 ppm insecticide concentration in compared to 0.5 ppm insecticide concentration and control. In Kernza® grains the progeny were higher in treated grains as compared to wheat. According study by (Athanassiou et al., 2009), chlorpyriphos-methyl + deltamethrin was more effective against psocid species in wheat and rice.

Insect Growth Regulators (IGRs) primarily disrupt three physiological functions: the growth and development of immature insects, the initiation of metamorphosis, or the synthesis of chitin in their exoskeleton (Oberlander and Silhacek, 2000; Wijayaratne et al., 2018). Juvenile hormone (JH) is released by the corpora allata, a pair of endocrine glands located in the heads of insects (Riddiford, 1994; Smith, 1985). Synthetic analogues of juvenile hormone (JH) have been created, and unlike the natural hormone, these compounds are resistant to enzymatic breakdown within the insect body (Riddiford, 1994). Methoprene was the first juvenile hormone analogue (JHA) to be developed and has a structure that most closely resembles the natural juvenile hormone (JH). When juvenile hormone (JH) or juvenile hormone analogues (JHAs) are applied externally to insects, they disrupt the normal regulation of JH levels in the body, causing various morphological and physiological changes compared to a healthy insect (Bai et al., 2010; Parthasarathy et al., 2010; Wissinger and Grosch, 1975; Zeng et al., 1997). Synthetic juvenile hormone analogues (JHAs), like methoprene, block the metamorphosis of juvenile insects into adults when applied, but they typically do not kill adult insects (Oberlander and Silhacek, 2000). Methoprene can eliminate larvae and pupae, halt their development, and/or delay their progression to the next life stage (Sutton et al., 2011; Wijayaratne and Fields, 2010).

Our study found that, no significant mortality was associated with the application of methoprene after 42 d of exposure to adult *S. oryzae* in hard red winter wheat. The mortality in Kernza® was also low even after application of insecticide. Methoprene do not kill the insects when they are externally applied but halt their development in the next generation (Oberlander and Silhacek, 2000). Methoprene significantly decreased progeny number in hard red winter wheat in all observation period but failed to reduce progeny in hulled and dehulled Kernza® after 42 d of exposure to insecticide. The toxicity of Insect Growth Regulators (IGRs) depends on the particular IGR, the insect species, and/or the specific type of grain (Athanassiou et al., 2011a; Athanassiou et al., 2011b; Daglish and Samson, 1991; Wijayaratne et al., 2018). Likewise, the grain weight loss by insects were higher in control than methoprene treated grains. Methoprene treated wheat kernels, *T. castaneum* emerged as adults but further could not produce offspring (Wijayaratne et al., 2012)

In our study with mixture of methoprene + deltamethrin we found high insect mortality was obtained in all grain type in all observation period and there was no progeny production and no grain weight loss in dehulled Kernza® and hard red winter wheat in all observation times. Higher mortality of insects in hulled Kernza® was observed in control treatments. Progeny production was lower in insecticide treated Kernza® grains but not as low as in treated wheat. Similar, trend was also observed in grain weight loss after 42 d of exposure. The toxicity of insect growth regulators also depends specific type of grain (Athanassiou et al., 2011a; Athanassiou et al., 2011b; Daglish and Samson, 1991; Wijayaratne et al., 2018).

Spinosad, an insecticide developed by Dow AgroSciences (Zionsville, Indiana, USA), is produced through the fermentation of a naturally occurring soil actinomycete, *Saccharopolyspora spinosa* Mertz and Yao (Bacteria: Actinobacteridae). The formulation of spinosad contains two active insecticidal components, spinosyns A and D, in a final product ratio of approximately 85% to 15% (Mertz and Yao, 1990). Spinosyns act on the nicotinic acetylcholine and gamma-aminobutyric acid (GABA) receptor sites in the insect nervous system. So far, no cross-resistance with other known insecticides has been observed (Kirst, 2010). Currently, Spinosad is registered in many countries as a grain protectant at a maximum labeled use Insecticide concentration of 1 ppm (1 mg ai/kg of grain) and with the Maximum Residue Level (MRL) or tolerance on grains set at 1 or 1.5 ppm (Hertlein et al., 2011). Spinosad residues remain very stable on grains stored in bins, providing protection for periods ranging from 6 months to 2 years. Various factors influencing the overall effectiveness of spinosad include the insect pest species, pest life stage, grain type, grain variety, and formulation type (Hertlein et al., 2011).

In our study, we found that 1 ppm spinosad caused 100 % mortality of *S. oryzae* in all grain type in all observation times. Our result is supported by experiment done by (Fang et al., 2002), where 1 ppm spinosad was effective against *S. oryzae* in durum wheat. Spinosad was also seen to be effective in Kernza® grains which are smaller in size than wheat grain. Past studies showed that Spinosad is more effective on the smaller grains like wheat and barley compared to larger grains such as maize (Ksoura et al., 2022).

In conclusion, deltamethrin at 0.5 and 1 ppm gave >50% *S. oryzae* adult mortality after 14 and 21 d resulting in <10 adult progeny production and <0.5% grain weight loss in all grain types. Methoprene at 1.25 ppm did not cause significant mortality of *S. oryzae* but caused low progeny numbers in dehulled Kernza® and hard red winter wheat. Adult mortality was 60-100 %, progeny production was < 21 and grain weight loss was 0.2 to 0.7 % in methoprene + deltamethrin treatment in all grain types. Spinosad at 1 ppm caused >87 % adult mortality of *S. oryzae* resulting in progeny production less than 6 and there was no grain weight loss. *S. oryzae* adult mortality was consistently higher in hulled Kernza® in control treatments for all insecticides.

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Table 2.1 Three-way ANOVA statistics for mortality of *S. oryzae* exposed to deltamethrin.

Source	df	Mean Square	*F* -value	*P*-value*
Grain type	2	5632.82	62.28	< 0.0001
Insecticide concentration	2	56020.65	629.39	< 0.0001
Observation time	2	2131.58	23.57	< 0.0001
Grain type × Insecticide concentration	4	2830.07	31.29	< 0.0001
Grain type × Observation time	4	40.60	0.45	0.77
Insecticide concentration × Observation time	2	702.82	7.77	< 0.0001
Grain type × Insecticide concentration × Observation time	4	459.71	5.08	< 0.0001
Error	108	90.44		

* Significant ($P < 0.05$)

Table 2.2 Mortality of *S. oryzae* adults exposed for 7, 14, and 21 d to untreated and deltamethrin treated dehulled Kernza®, hulled Kernza®, and hard red winter wheat.

Grain type	Insecticide concentration (ppm)	% Mean ± SE mortality at[a]:		
		7 d	14 d	21 d
Dehulled Kernza®	0	1.2 ± 1.2 f	11.6 ± 2.0 e	10.8 ± 1.2 f
Dehulled Kernza®	0.5	37.2 ± 5.6 de	52.0 ± 8.9 d	51.2 ± 8.4 d
Dehulled Kernza®	1.0	59.6 ± 9.1 c	80.4 ± 3.4 bc	72.4 ± 6.2 c
Hulled Kernza®	0	27.6 ± 2.5 e	49.6 ± 5.3 d	27.2 ± 2.2 e
Hulled Kernza®	0.5	50.0 ± 4.4 cd	62.4 ± 3.5 cd	83.2 ± 3.9 bc
Hulled Kernza®	1.0	87.6 ± 3.0 b	82.8 ± 2.7 a	79.6 ± 3.9 bc
Hard red winter wheat	0	1.6 ± 0.7 f	3.2 ± 0.8 e	9.2 ± 1.4 f
Hard red winter wheat	0.5	56.8 ± 3.4 cd	93.6 ± 4.3 a	88.8 ± 3.1 b
Hard red winter wheat	1.0	100 ± 0.0 a	99.6 ± 0.4 a	98.8 ± 0.4 a

[a]Means among grain type and insecticide concentration at each observation time followed by different letters are significantly different ($P < 0.05$; by REGWQ) test.

Table 2.3 Three-way ANOVA statistics for adult progeny production of *S. oryzae* exposed to deltamethrin.

Source	df	Mean Square	*F*- value	*P*- value*
Grain	2	4.6	68.48	< 0.0001
Insecticide concentration	2	27.6	406.2	< 0.0001
Observation time	3	0.7	11.2	< 0.0001
Grain type × Insecticide concentration	4	5.1	74.7	< 0.0001
Grain type × Observation time	6	0.5	7.9	< 0.0001
Insecticide concentration × Observation time	6	0.8	11.7	< 0.0001
Grain type × Insecticide concentration × Observation time	12	0.3	4.3	< 0.0001
Error	143	0.068		

* Significant ($P < 0.05$)

Table 2.4 Adult progeny production of *S. oryzae* exposed for 7, 14, 21, and 42 d to untreated and deltamethrin treated dehulled Kernza®, hulled Kernza®, and hard red winter wheat.

Grain type	Insecticide concentration (ppm)	Mean ± SE adult progeny production at[a]:			
		7 d	14 d	21 d	42 d
Dehulled Kernza®	0	139.6 ± 19.6 b	152.6 ± 13.8 a	171.4 ± 17.8 b	269.4 ± 33.4 b
Dehulled Kernza®	0.5	67.0 ± 9.1 cd	57.2 ± 7.9 ab	133.4 ± 18.9 b	82.6 ± 25.7 d
Dehulled Kernza®	1.0	41.6 ± 5.7 d	73.8 ± 9.2 ab	69.8 ± 10.2 cb	7.2 ± 2.2 de
Hulled Kernza®	0	59.6 ± 10.2 cd	66.6 ± 13.9 ab	64.0 ± 8.8 bc	90.4 ± 5.9 c
Hulled Kernza®	0.5	49.6 ± 4.1 cd	34.2 ± 12.7 b	26.8 ± 6.0 cd	65.4 ± 13.0 d
Hulled Kernza®	1.0	17.0 ± 1.6 e	17.8 ± 3.6 b	11.6 ± 2.2 d	2.6 ± 1.7 ef
Hard red winter wheat	0	510.0 ± 21.6 a	152.6 ± 13.8 a	610.2 ± 53.3 a	702.8 ± 92.1 a
Hard red winter wheat	0.5	90.6 ± 9.5 bc	24.2 ± 14.8 c	68.8 ± 27.8 cb	6.2 ± 1.5 d
Hard red winter wheat	1.0	0.8 ± 0.5 f	0.0 ± 0.0 c	1.4 ± 0.9 e	0.0 ± 0.0 f

[a]Means among grain type and insecticide concentration at each observation time followed by different letters are significantly different ($P < 0.05$; by REGWQ) test.

Table 2.5 Three-way ANOVA statistics for weight loss of dehulled Kernza®, hulled Kernza®, and hard red winter wheat due to *S. oryzae* after exposure to deltamethrin.

Source	df	Mean Square	*F* -value	*P*-value*
Grain type	2	0.0239	9.23	0.0002
Insecticide concentration	2	0.3326	128.05	< 0.0001
Observation time	3	0.0577	22.24	< 0.0001
Grain type × Insecticide concentration	4	0.0071	2.74	0.0310
Grain type × Observation time	6	0.0047	1.81	0.1010
Insecticide concentration × Observation time	6	0.0162	6.26	< 0.0001
Grain type× Insecticide concentration × Observation time	12	0.0032	1.23	0.2665
Error	136	0.0025		

* Significant ($P < 0.05$)

Table 2.6 Grain weight loss of untreated and deltamethrin treated dehulled Kernza®, hulled Kernza®, and hard red winter wheat after exposure to *S. oryzae* adults for 7, 14, 21, and 42 d.

Grain type	Insecticide concentration (ppm)	% Mean ± SE weight loss at[a]:			
		7 d	14 d	21 d	42 d
Dehulled Kernza®	0	1.3 ± 0.4 b	6.1 ± 2.8 a	4.1 ± 1.6 a	10.6 ± 2.7 a
Dehulled Kernza®	0.5	0.9 ± 0.27 b	0.4 ± 0.5 b	2.5 ± 0.4 abc	2.9 ± 0.7 bcd
Dehulled Kernza®	1.0	0.0 ± 0.0 b	0.8 ± 0.2 b	0.9 ± 0.3 bc	0.9 ± 0.3 d
Hulled Kernza®	0	0.5 ± 0.1 b	1.1 ± 0.3 b	3.6 ± 0.7 ab	7.5 ± 1.4 abc
Hulled Kernza®	0.5	0.8 ± 0.4 b	0.6 ± 0.1 b	0.5 ± 0.1 c	2.3 ± 0.5 cd
Hulled Kernza®	1.0	0.2 ± 0.1 b	0.1 ± 0.0 b	0.5 ± 0.2 c	0.2 ± 0.1 d
Hard red winter wheat	0	4.3 ± 1.9 a	2.9 ± 0.8 ab	4.1 ± 0.6 a	8.9 ± 3.3 ab
Hard red winter wheat	0.5	0.3 ± 0.2 b	0.4 ± 0.2 b	1.0 ± 0.2 bc	0.5 ± 0.1 d
Hard red winter wheat	1.0	0.8 ± 0.1 b	0.1 ± 0.1 b	0.4 ± 0.2 c	0.3 ± 0.1 d

[a]Means among grain type and insecticide concentration at each observation time followed by different letters are significantly different ($P < 0.05$; by REGWQ) test.

Table 2.7 Three-way ANOVA statistics for mortality of *S. oryzae* exposed to methoprene.

Source	df	Mean Square	*F* -value	*P*-value*
Grain type	2	5.32	444.9	< 0.0001
Insecticide concentration	1	0.23	19.57	< 0.0001
Observation time	2	0.75	62.71	< 0.0001
Grain type × Insecticide concentration	2	0.009	0.77	0.4669
Grain type × Observation time	4	0.019	1.64	0.1748
Insecticide concentration × Observation time	2	0.108	9.08	0.0003
Grain type × Insecticide concentration × Observation time	4	0.106	8.91	< 0.0001
Error	72	0.0119		

* Significant ($P < 0.05$)

Table 2.8 Mortality of *S. oryzae* adults exposed for 7, 14, and 21 d to untreated and methoprene treated dehulled Kernza®, hulled Kernza®, and hard red winter wheat.

Grain types	Insecticide concentration (ppm)	% Mean ± SE mortality at[a]:		
		7 d	14 d	21 d
Dehulled Kernza®	0	4.4 ± 0.7 bc	18.8 ± 5.0 b	24.4 ± 4.9 d
Dehulled Kernza®	1.25	12.0 ± 3.1 b	13.2 ± 2.9 b	55.2 ± 3.5 c
Hulled Kernza®	0	67.2 ± 4.8 a	84.4 ± 4.5 a	74.4 ± 2.4 b
Hulled Kernza®	1.25	72.8 ± 4.6 a	74.0 ± 5.5 a	97.2 ± 1.2 a
Hard red winter wheat	0	2.4 ± 0.9 c	6.0 ± 1.4 b	27.2 ± 3.2 d
Hard red winter wheat	1.25	8.8 ± 3.0 bc	14.0 ± 4.0 b	20.2 ± 2.2 d

[a]Means among grain type and insecticide concentration at each observation time followed by different letters are significantly different ($P < 0.05$; by REGWQ) test.

Table 2.9 Three-way ANOVA statistics for adult progeny production of *S. oryzae* exposed to methoprene.

Source	df	Mean Square	*F*- value	*P*- value*
Grain type	2	15.2	314.4	< 0.0001
Insecticide concentration	1	29.3	606.1	< 0.0001
Observation time	3	3.3	67.5	< 0.0001
Grain type × Insecticide concentration	2	4.2	86.3	< 0.0001
Grain type × Observation time	6	0.7	13.9	< 0.0001
Insecticide concentration × Observation time	3	0.9	18.0	< 0.0001
Grain type × Insecticide concentration × Observation time	6	0.18	3.8	< 0.0001
Error	96	0.048		

* Significant ($P < 0.05$)

Table 2.10 Adult progeny production of *S. oryzae* exposed for 7, 14, 21, and 42 d to untreated and methoprene treated dehulled Kernza®, hulled Kernza®, and hard red winter wheat.

Grain type	Insecticide concentration (ppm)	Mean ± SE adult progeny production at[a]:			
		7 d	14 d	21 d	42 d
Dehulled Kernza®	0	47.0 ± 6.3 b	60.8 ± 14.9 b	221.4 ± 38.9 b	88.8 ± 12.5 b
Dehulled Kernza®	1.25	1.6 ± 0.9 c	2.0 ± 0.8 d	6.6 ± 1.3 d	65.0 ± 11.2 b
Hulled Kernza®	0	1.6 ± 0.8 c	3.4 ± 0.7 d	4.4 ± 1.8 d	18.0 ± 4.2 c
Hulled Kernza®	1.25	0.0 ± 0.0 c	1.6 ± 0.8 d	0.2 ± 0.2 cd	15.0 ± 1.8 c
Hard red winter wheat	0	215.6 ± 49.5 a	540.2 ± 64.2 a	488.4 ± 36.0 a	300.6 ± 43.6 a
Hard red winter wheat	1.25	2.8 ± 1.6 c	13.8 ± 2.8 c	10.8 ± 0.6 c	14.8 ± 1.8 c

[a]Means among grain type and insecticide concentration at each observation time followed by different letters are significantly different ($P < 0.05$; by REGWQ) test.

Table 2.11 Three-way ANOVA statistics for weight loss of dehulled Kernza®, hulled Kernza® and hard red winter wheat due to *S. oryzae* after exposure to methoprene.

Source	df	Mean Square	*F* -value	*P*-value*
Grain type	2	123.87	32.66	< 0.0001
Insecticide concentration	1	77.014	20.31	< 0.0001
Observation time	3	28.436	7.50	0.0001
Grain type × Insecticide concentration	2	5.9829	1.58	0.2118
Grain type × Observation time	6	7.6121	2.01	0.0722
Insecticide concentration × Observation time	3	21.540	5.68	0.0013
Grain type × Insecticide concentration × Observation time	6	6.5822	1.74	0.1211
Error	95	3.7926		

* Significant ($P < 0.05$)

Table 2.12 Grain weight loss of untreated and methoprene treated dehulled Kernza®, hulled Kernza®, and hard red winter wheat after exposure to *S. oryzae* adults for 7, 14, 21, and 42 d.

Grain type	Insecticide concentration (ppm)	% Mean ± SE weight loss at[a]:			
		7 d	14 d	21 d	42 d
Dehulled Kernza®	0	0.9 ± 0.1 b	1.5 ± 0.7 bc	2.2 ± 0.8 ab	2.4 ± 0.6 b
Dehulled Kernza®	1.25	0.1 ± 0.0 b	0.1 ± 0.1 c	0.1 ± 0.1 b	0.1 ± 0.3 b
Hulled Kernza®	0	0.1 ± 0.1 b	0.3 ± 0.2 c	0.9 ± 0.2 ab	2.9 ± 0.7 b
Hulled Kernza®	1.25	0.0 ± 0.0 b	0.4 ± 0.1 c	0.0 ± 0.0 b	0.5 ± 0.2 b
Hard red winter wheat	0	2.6 ± 0.5 a	3.3 ± 0.9 ab	3.9 ± 2.0 a	10.3 ± 2.9 a
Hard red winter wheat	1.25	1.3 ± 0.8 ab	4.1 ± 0.7 a	2.4 ± 0.4 ab	2.8 ± 0.5 b

[a]Means among grain type and insecticide concentration at each observation time followed by different letters are significantly different ($P < 0.05$; by REGWQ) test.

Table 2.13 Three-way ANOVA statistics for mortality of *S. oryzae* exposed to methoprene + deltamethrin (1.25 + 0.5 ppm).

Source	df	Mean Square	*F* -value	*P*-value
Grain type	2	5.32	447.0	< 0.0001
Insecticide concentration	1	0.23	19.3	< 0.0001
Observation time	2	0.75	62.7	< 0.0001
Grain type × Insecticide concentration	2	0.01	0.8	0.466
Grain type × Observation time	4	0.02	1.6	0.17
Insecticide concentration × Observation time	2	0.11	9.1	0.0003
Grain type × Insecticide concentration × Observation time	4	0.11	8.9	< 0.0001
Error	72	0.0119		

[*] Significant ($P < 0.05$)

Table 2.14 Mortality of *S. oryzae* adults exposed for 7, 14, and 21 d to untreated and methoprene + deltamethrin (1.25 + 0.5 ppm) dehulled Kernza®, hulled Kernza®, and hard red winter wheat.

Grain type	Insecticide concentration (ppm)	% Mean ± SE mortality at[a]:		
		7 d	14 d	21 d
Dehulled Kernza®	0	13.2 ± 4.1 c	10.0 ± 1.1 d	24.4 ± 4.9 d
Dehulled Kernza®	1.25 + 0.5	82.0 ± 4.8 a	66.4 ± 5.8 c	87.6 ± 4.9 b
Hulled Kernza®	0	61.2 ± 6.8 b	81.6 ± 2.3 b	73.2 ± 2.2 c
Hulled Kernza®	1.25 + 0.5	82.4 ± 2.9 a	74.8 ± 2.1 bc	99.6 ± 0.4 a
Hard red winter wheat	0	8.4 ± 2.2 c	11.2 ± 2.4 d	8.8 ± 1.8 e
Hard red winter wheat	1.25 + 0.5	90.4 ± 2.2 a	99.6 ± 0.4 a	100.0 ± 0.0 a

[a]Means among grain type and insecticide concentration at each observation time followed by different letters are significantly different ($P < 0.05$; by REGWQ) test.

Table 2.15 Three-way ANOVA statistics for adult progeny production of *S. oryzae* exposed to methoprene + deltamethrin (1.25 + 0.5 ppm).

Source	df	Mean Square	*F*- value	*P*- value
Grain	2	5.2	129.7	< 0.0001
Insecticide concentration	1	52.7	1316.3	< 0.0001
Observation time	3	2.1	52.5	< 0.0001
Grain type × Insecticide concentration	2	5.3	133.2	< 0.0001
Grain type × Observation time	6	0.7	13.9	< 0.0001
Insecticide concentration × Observation time	3	0.5	11.4	< 0.0001
Grain type × Insecticide concentration × Observation time	6	0.3	7.7	< 0.0001
Error	96	0.04		

[*] Significant ($P < 0.05$)

Table 2.16 Adult progeny production of *S. oryzae* exposed for 7, 14, 21, and 42 d to untreated and methoprene + deltamethrin (1.25 + 0.5 ppm) treated dehulled Kernza®, hulled Kernza®, and hard red winter wheat.

Grain type	Insecticide concentration (ppm)	Mean ± SE adult progeny production at[a]:			
		7 d	14 d	21 d	42 d
Dehulled Kernza®	0	27.0 ± 2.8 b	37.6 ± 4.2 b	70.2 ± 13.8 b	76.2 ± 6.8 b
Dehulled Kernza®	1.25 + 0.5	1.0 ± 0.7 d	0.0 ± 0.0 d	2.4 ± 0.7 cd	21.2 ± 2.8 d
Hulled Kernza®	0	1.8 ± 0.8 cd	3.4 ± 0.5 c	27.0 ± 3.6 c	41.6 ± 7.5 c
Hulled Kernza®	1.25 + 0.5	0.0 ± 0.0 d	0.2 ± 0.2 d	0.6 ± 0.6 d	14.4 ± 4.5 d
Hard red winter wheat	0	295.4 ± 50.0 a	360.0 ± 77.9 a	228.2 ± 29.0 a	287.6 ± 23.9 a
Hard red winter wheat	1.25 + 0.5	2.2 ± 0.6 c	2.2 ± 0.7 c	0.2 ± 0.2 cd	0.8 ± 0.8 e

[a]Means among grain type and insecticide concentration at each observation time followed by different letters are significantly different ($P < 0.05$; by REGWQ) test.

Table 2.17 Three-way ANOVA statistics for weight loss of dehulled Kernza®, hulled Kernza® and hard red winter wheat due to *S. oryzae* after exposure to methoprene + deltamethrin (1.25 + 0.5 ppm).

Source	df	Mean Square	*F*- value	*P*-value
Grain type	2	49.836	12.78	< 0.0001
Insecticide concentration	1	206.74	53.03	< 0.0001
Observation time	3	30.689	7.870	< 0.0001
Grain type × Insecticide concentration	2	56.133	14.40	< 0.0001
Grain type × Observation time	6	11.923	3.060	0.0089
Insecticide concentration × Observation time	3	25.716	6.600	0.0004
Grain type × Insecticide concentration × Observation time	6	12.221	3.130	0.0076
Error	94	3.8983		

* Significant ($P < 0.05$)

Table 2.18 Grain weight loss of untreated and methoprene + deltamethrin (1.25 + 0.5 ppm) treated dehulled Kernza®, hulled Kernza®, and hard red winter wheat after exposure to *S. oryzae* adults for 7, 14, 21, and 42 d.

Grain type	Insecticide concentration (ppm)	% Mean ± SE weight loss at[a]:			
		7 d	14 d	21 d	42 d
Dehulled Kernza®	0	2.4 ± 1.5	1.5 ± 0.7 ab	2.2 ± 0.8	2.4 ± 0.6 b
Dehulled Kernza®	1.25 + 0.5	0.9 ± 0.5	0.2 ± 0.2 b	0.1 ± 0.6	0.7 ± 0.2 b
Hulled Kernza®	0	0.5 ± 0.0	0.3 ± 0.2 b	0.9 ± 0.2	2.9 ± 0.7 b
Hulled Kernza®	1.25 + 0.5	0.0 ± 0.0	0.1 ± 0.1 b	0.6 ± 0.6	0.3 ± 0.1 b
Hard red winter wheat	0	2.6 ± 0.5	3.3 ± 0.9 a	3.9 ± 2.0	12.0 ± 2.8 a
Hard red winter wheat	1.25 + 0.5	0.0 ± 0.0	0.2 ± 0.2 b	0.0 ± 0.0	0.2 ± 0.1 b

[a]Means among grain type and insecticide concentration at each observation time followed by different letters are significantly different ($P < 0.05$; by REGWQ) test.

Table 2.19 Three-way ANOVA statistics for mortality of *S. oryzae* exposed to spinosad.

Source	df	Mean Square	*F* -value	*P*-value*
Grain type	2	4.3	332.5	< 0.0001
Insecticide concentration	1	14.1	1086.1	< 0.0001
Observation time	2	0.2	18.5	< 0.0001
Grain type × Insecticide concentration	2	3.8	296.5	0.466
Grain type × Observation time	4	0.03	2.9	0.02
Insecticide concentration × Observation time	2	0.005	0.4	0.66
Grain type × Insecticide concentration × Observation time	4	0.04	3.15	0.019
Error	71	0.0129		

* Significant ($P < 0.05$)

Table 2.20 Mortality of *S. oryzae* adults exposed for 7, 14 and 21 d to untreated and spinosad treated dehulled Kernza®, hulled Kernza®, and hard red winter wheat.

Grain type	Insecticide concentration (ppm)	% Mean ± SE mortality at[a]:		
		7 d	14 d	21 d
Dehulled Kernza®	0	2.4 ± 1.6 c	10.4 ± 6.4 b	8.0 ± 3.6 c
Dehulled Kernza®	1	87.2 ± 3.6 b	100.0 ± 0.0 a	100.0 ± 0.0 a
Hulled Kernza®	0	100.0 ± 0.0 a	100.0 ± 0.0 a	100.0 ± 0.0 a
Hulled Kernza®	1	97.6 ± 0.9 a	100.0 ± 0.0 a	100.0 ± 0.0 a
Hard red winter wheat	0	8.0 ± 4.5 c	14.4 ± 2.0 b	26.4 ± 6.4 b
Hard red winter wheat	1	98.4 ± 1.6 a	100.0 ± 0.0 a	100.0 ± 0.0 a

[a]Means among grain type and insecticide concentration at each observation time followed by different letters are significantly different ($P < 0.05$; by REGWQ) test.

Table 2.21 Three-way ANOVA statistics for adult progeny production of *S. oryzae* exposed to spinosad.

Source	df	Mean Square	*F*- value	*P*- value*
Grain type	2	17.5	597.62	< 0.0001
Insecticide concentration	1	20.9	711.7	< 0.0001
Observation time	3	1.1	8.1	< 0.0001
Grain type × Insecticide concentration	2	4.7	163	< 0.0001
Grain type × Observation time	6	0.5	15.7	< 0.0001
Insecticide concentration × Observation time	3	1.9	74.7	< 0.0001
Grain type × Insecticide concentration × Observation time	6	0.6	19.1	< 0.0001
Error	96	0.029		

* Significant ($P < 0.05$)

Table 2.22 Adult progeny production of *S. oryzae* exposed for 7, 14, 21, and 42 d to untreated and spinosad treated dehulled Kernza®, hulled Kernza®, and hard red winter wheat.

Grain type	Insecticide concentration (ppm)	Mean ± SE adult progeny production at[a]:			
		7 d	14 d	21 d	42 d
Dehulled Kernza®	0	37.2 ± 0.8 b	21.4 ± 2.4 b	35.0 ± 6.6 b	67.4 ± 8.5 b
Dehulled Kernza®	1	21.4 ± 2.5 cd	1.6 ± 0.5 c	6.0 ± 1.6 c	5.4 ± 0.2 c
Hulled Kernza®	0	1.0 ± 0.5 de	0.0 ± 0.0 d	0.0 ± 0.0 d	2.8 ± 0.8 d
Hulled Kernza®	1	0.0 ± 0.0 e	0.0 ± 0.0 d	0.0 ± 0.0 d	1.6 ± 0.5 cd
Hard red winter wheat	0	116.2 ± 8.8 a	50.0 ± 8.6 a	324 ± 64.2 a	241.1 ± 29.5 a
Hard red winter wheat	1	3.6 ± 0.6 c	19.8 ± 3.2 b	6.4 ± 1.9 c	0.0 ± 0.0 e

[a]Means among grain type and insecticide concentration at each observation time followed by different letters are significantly different ($P < 0.05$; by REGWQ) test.

Table 2.23 Three-way ANOVA statistics for weight loss of dehulled Kernza®, hulled Kernza®, and hard red winter wheat due to *S. oryzae* after exposure to spinosad.

Source	df	Mean Square	*F*- value	*P*-value
Grain type	2	20.495	9.16	0.0002
Insecticide concentration	1	61.749	27.61	< 0.0001
Observation time	3	25.584	11.44	< 0.0001
Grain type × Insecticide concentration	2	20.495	9.16	<.0002
Grain type × Observation time	6	10.861	4.86	0.0002
Insecticide concentration × Observation time	3	25.584	11.4	< 0.0001
Grain type × Insecticide concentration × Observation time	6	10.861	4.86	0.0002
Error	96	2.236		

* Significant ($P < 0.05$)

Table 2.24 Grain weight loss of untreated and spinosad treated dehulled Kernza®, hulled Kernza®, and hard red winter wheat after exposure to *S. oryzae* adults for 7, 14, 21, and 42 d.

Grain type	Insecticide concentration (ppm)	% Mean ± SE weight loss at[a]:			
		7 d	14 d	21 d	42 d
Dehulled Kernza®	0	0.3 ± 0.1 a	0.9 ± 0.4 b	0.4 ± 0.1 a	0.9 ± 0.2 b
Dehulled Kernza®	1	0.0 ± 0.0 b	0.0 ± 0.0 c	0.0 ± 0.0 b	0.0 ± 0.0 b
Hulled Kernza®	0	0.0 ± 0.0 b	0.0 ± 0.0 c	0.0 ± 0.0 b	2.2 ± 0.7 b
Hulled Kernza®	1	0.0 ± 0.0 b	0.0 ± 0.0 c	0.0 ± 0.0 b	0.0 ± 0.0 b
Hard red winter wheat	0	0.1 ± 0.1 ab	2.5 ± 0.2 a	0.5 ± 0.1 a	9.2 ± 3.1 a
Hard red winter wheat	1	0.0 ± 0.0 b	0.0 ± 0.0 c	0.0 ± 0.0 b	0.0 ± 0.0 b

[a]Means among grain type and insecticide concentration at each observation time followed by different letters are significantly different ($P < 0.05$; by REGWQ) test.