Survival and progeny production of three economically important stored product insect species on hulled Kernza®, dehulled Kernza®, and hard red winter wheat

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Fizra Ahmad

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Approved by:

Major Professor
Dr. Bhadriraju Subramanyam

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Abstract

A variety of insect pests are responsible for postharvest losses of cereal grains. Stored product insect pests adversely affect grain quality and quantity of stored commodities. The lesser grain borer, *Rhyzopertha dominica* (Fabricius) (Coleoptera; Bostrichidae); red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae); and rice weevil *Sitophilus oryzae* (Linnaeus), are among the most common, serious and polyphagous stored product insect pests that feed on a variety of stored grains. Kernza®, *Thinopyrum intermedium* (Host) Barkworth & D.R. Dewey, is a low-input perennial cool-season intermediate wheatgrass, grown as a dual-purpose crop for high-quality grazing in the winter and for nutritious whole grain in the summer. No data are available on susceptibility of this novel grain crop to stored-product insect pests, although there are anecdotal reports that the grains from this crop are suffering insect related losses in storage. The aim of this study was to evaluate the survival and progeny production of *R. dominica, T. castaneum,* and *S. oryzae* on hulled Kernza®, dehulled Kernza®, and hard red winter wheat. Laboratory reared populations of these stored grain insect pests were introduced in all three tested grain types to record moisture contents (%), survival rate (%), progeny production, kernel damage, and weight loss. Separate experiments were performed for each insect species using completely randomized design (CRD), with 35 replications and seven observation times (7, 14, 21, 28, 35, 42, and 56 d) per grain type. The 7 to 28 d observation times determined survival of the three insect species on the grain types, while the 35 to 56 d observations were used to collect data on adult progeny production. In each replication, 25 adults of mixed sexes and ages were exposed to each of the grain types (50 g) in 150 ml round plastic containers under laboratory conditions of 28°C and 65% r.h. Adult survival (%) was assessed at 7, 14, 21, and 28 d post-infestation. Adult progeny production, kernel damage, and weight loss assessments were done on samples after 35, 42, and 56 d post-infestation. The moisture content during the duration of the experiment varied but the variation was marginal, despite some significant differences. The survival of all three insect species was significantly and consistently lower on hulled Kernza® compared to dehulled Kernza® and hard red winter wheat. Progeny production and weight loss results for *R. dominica* at 35 to 56 d varied with different grain types, but was generally lowest on hulled Kernza®. In *T. castaneum* experiments, the mean ± SE survival rate 28 d post-infestation period was significantly lower in hulled Kernza® (9.6 ± 2.4%) compared to dehulled Kernza® (100.0%) and hard red winter wheat (99.2 ± 0.8%). Hulled Kernza® exhibited complete suppression of *T. castaneum* progeny production in 35, 42, and 56 d samples. However, the mean ± SE kernel damage and weight loss percentage due to *T. castaneum* infestation was significantly lower i.e., 7.9 ± 0.9 and 1.1 ± 0.5, respectively, in hulled Kernza® compared to the other grain types in 56 d samples. The survival percentages of *S. oryzae* adults in hulled Kernza® decreased from a mean ± SE of 84.8 ± 2.9 at 7 d to 27.2 ± 4.1 at 28 d. Similarly, smaller numbers of adult progenies of *S. oryzae* were produced in hulled and dehulled Kernza® than that of hard red winter wheat. At 28 d post-infestation, no adult progeny of *S. oryzae* was recorded in both hulled and dehulled Kernza®, but a mean ± SE of 21.0 ± 5.4 number of adults were recorded in hard red winter wheat. The mean ± SE weight loss percentage resulting from *S. oryzae* infestation was in the following order after 56 d: hulled Kernza® (2.2 ± 1.1) < dehulled Kernza® (5.5 ± 0.5) < hard red winter wheat (6.5 ± 0.6). In conclusion, hulled Kernza® appears to be an unsuitable commodity for *R. dominica, T. castaneum,* and *S. oryzae* survival and progeny production compared to that of dehulled Kernza®. Therefore, storing Kernza® in its hulled form can be a practical approach to curtail postharvest losses and preserve grain quality, with additional studies

warranted on further understanding the possible reasons for poor performance of the three species on hulled Kernza®.

Table of Contents

List of Figures

List of Tables

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Chapter 1 - Survival and progeny production of lesser grain borer, *Rhyzopertha dominica* (Fabricius), on hulled Kernza®, dehulled Kernza®, and hard red winter wheat

1.1 Abstract

Kernza®, *Thinopyrum intermedium* (Host) Barkworth & D.R. Dewey, is a low-input perennial cool-season intermediate wheatgrass, grown as a dual-purpose crop for high-quality grazing in the winter and for nutritious whole grain in the summer. No data are available on Kernza® grain susceptibility to stored-product insects. Susceptibility of hulled and dehulled Kernza® and hard red winter wheat to lesser grain borer, *Rhyzopertha dominica* (Fabricius) (Coleoptera; Bostrichidae) was evaluated in the laboratory. Twenty-five adults of mixed sexes and ages in replicate samples were exposed to the three grain types at 28 °C and 65% relative humidity. Adult survival (%) was assessed at 7, 14, 21, 28, 35, 42, and 56 d in independent samples. Adult progeny production, kernel damage and weight loss assessment were done in samples only on 35, 42, and 56 d post-infestation. Low survivorship of *R. dominica* was observed on hulled Kernza®. Progeny production and grain damage at 35 to 56 d varied with grain type but was generally lowest on hulled Kernza®. Results suggested that hulled Kernza® appears to be an unsuitable commodity for *R. dominica* development and progeny production compared to dehulled Kernza® and hard red winter wheat. Therefore, prioritizing the storage of Kernza® in its hulled form can be a practical approach to curtail postharvest losses and preserve grain quality.

1.2 Introduction

Kernza® is a registered trade name for the novel perennial grain made from improved lines of intermediate wheatgrass (*Thinopyrum intermedium*) (Host) Barkworth & D. R. Dewey, developed by the scientists at The Land Institute in Salina, Kansas, USA (de Oliveira et al., 2018; DeHaan et al., 2014). It is a low-input, perennial, and cool-season crop that can be grown as a dual-purpose for high-quality grazing in the winter and for nutritious whole grain in the summer (DeHaan et al., 2014). Kernza® plants have a deep and dense root system with roots extending over 10 feet into the soil (Schaap, 2019). This well-established crop provides excellent ground cover to improve soil health with enhanced carbon sequestration and reduced soil erosion (Sprunger et al., 2019). In addition, it takes up fertilizer thus reducing nitrate leaching into the groundwater (Culman et al., 2013). Considering the environmental benefits of sustainable Kernza® farming and its application opportunities in the food/feed industry, it is becoming a popular choice among farmers and food business owners. According to The Land Institute report on Kernza® production in the U.S during 2021, a total of 141 growers cultivated Kernza® in ~4000 acres with Minnesota and Kansas having the most acres planted, with 1,417 and 929.5 acres, respectively (Mohr, 2022). Several research efforts have been made and are in progress to maximize value by improving the agronomic, compositional, and nutritional qualities of Kernza® compared to annual cereal crops (de Oliveira et al., 2018).

A variety of insect pests, most commonly lesser grain borer*, Rhyzopertha dominica* (Fabricius) (Coleoptera; Bostrichidae); rice weevil, *Sitophilus oryzae* (Linnaeus) and angoumois grain moth *Sitotroga cerealella* (Olivier), infest and deteriorate stored cereal grains and other foodstuffs (Yaseen et al., 2019). Like other stored grains, Kernza® may be at risk of insect feeding damage resulting in both qualitative and quantitative losses during its storage, thereby offsetting positive economic returns. The *R. dominica* is one of the major and most destructive stored product insect pests worldwide (Daglish and Nayak, 2018). This pest completes its lifecycle from egg to adult within a short period of twenty-five days. The adult female deposits its eggs on the surface of the kernel and the newly emerged larvae penetrate and start feeding inside the kernel and ultimately completing development until it reaches the adult stage. Once completed feeding, the emerging adult digs out of the kernel, thereby creating a hole beneath. The resultant kernel is then referred to as an insect-damaged kernel for evaluation purposes (Mason and McDonough, 2012). As a result of infestation caused by *R. dominica* feeding, the grain suffers a wide range of direct and indirect losses (Arthur et al., 2020). A recent study has reported that hull attributes i.e., solid, or thicker hulls play a significant role in exhibiting resistance to varieties of rough rice by *R. dominica* infestation (Chanbang et al., 2008). Another study suggests that kernels with cracked or broken hulls are more attracted to *R. dominica* for progeny production (Kavallieratos et al., 2012).

Different control measures including biological, chemical, and physical are used to manage stored grain insect pests (Harush et al., 2021; Rajendran, 2020). As an important component of pest management practices, insecticides have been effectively exploited in storage houses as structural sprays, general surface treatments, and grain protectants (Arthur and Subramanyam, 2012). However, over-reliance and inappropriate use results in environmental pollution and non-target effects (Ansari et al., 2014; Yadav and Devi, 2017) that demand safe and environmentally friendly control measure. Insect pests are responsive to various characteristics of grains, e.g., phenol, ash, protein, amylose, and fat contents, thickness of husk, and kernel hardness (Rizwana et al., 2011). The crop varieties that have fine grains are more susceptible to stored product insect pests than the varieties that have thicker husks (Swamy et al., 2019).

Similarly, it has been reported that a rice cultivar whose grains have the highest hardness but low protein content is less susceptible to *S. cerealella* and *R. dominica* (Swamy et al., 2022). Previously, several studies have reported the susceptibility of various cereal grains, e.g., rice, wheat, maize, sorghum, and barley to *R. dominica* (Arthur et al., 2020; Astuti et al., 2013; Awadalla et al., 2023; Bhargava and Akhter, 2014; Chougourou et al., 2013; D'Isita et al., 2024; Hemmati, 2024; Javanmard et al., 2023; Locatelli et al., 2019; Metwaly et al., 2015; Saad et al., 2018). To date, there is no published data that examines hulled and dehulled Kernza® susceptibility to *R. dominica.*

To our knowledge, there has been little work done and literature published on Kernza®. The studies that have been performed focused on plant attributes, crop management, and grain yields. Thus far, no experiments have been performed and literature cited that have directly determined the susceptibility of Kernza® as a perennial grain crop to stored product insect pests. In this study, we evaluated the susceptibility of Kernza® to *R. dominica* when stored as a hulled and dehulled form of grains. Moreover, various physical parameters such as moisture content, weight loss, and kernel damage of infested Kernza® grains in comparison to wheat were investigated.

1.3 Materials and Methods

1.3.1. Grain commodities

Hulled and dehulled Kernza® grains (variety: TLIC5) from the year 2022 harvest were obtained from the Sustain-A-Grain, a company in McPherson County, Kansas, USA, and shipped to the Department of Grain Science and Industry at Kansas State University, Manhattan, KS, USA. These grains were then held in freezer at ~-20°C for two weeks to kill any live insects present. All the comparisons were made with organic whole grain hard red winter wheat stock (Great

River Organic Milling, Fountain City, Wisconsin, USA) (standard control). Prior to experimentation, both Kernza® and wheat grain samples were removed from the freezer and acclimatized in plastic bags at room conditions (~24.5 °C and ~76% relative humidity (r.h.)) for 1-2 weeks. The grain moisture content was measured by an air oven method (ASAE, 2000). Depending on variability in moisture contents, both Kernza® and wheat grain samples were equilibrated to 13% moisture content (wet basis) in environmental growth chambers maintained at 28°C and 65% r.h. before used in experiments.

1.3.2. Insect colonies

Cultures of *R. dominica* that have been maintained in the laboratory for more than 20 years in the Department of Grain Science were used for the experiments. The insects were reared in 0.95 liter mason wide-mouth glass jars fitted with wire mesh and filter paper lids containing preconditioned organic soft white winter wheat. The glass jars were held inside growth chambers (Model I-36 VL; Percival Scientific, Perry, Iowa, USA) set at 28°C, 65% r.h., and a photoperiod of 14:10 h (Light: Dark).

1.3.3. Insect survival and progeny production

Twenty-five unsexed adults of *R. dominica* (1 – 3 week mixed ages) were suctioned carefully with the help of a mechanical aspirator from laboratory cultures. The collected adults were introduced into each of the round plastic containers (6.4 cm diameter x 6.9 cm height x 150 ml volume) having 50 g cleaned grains of hulled Kernza®, dehulled Kernza®, and hard red winter wheat. Each grain type held a moisture content of 13-14.5% (wet basis). The plastic containers were covered with perforated lids (10-mm round hole) and a small piece of wire-mesh screens (0.6-mm^2 openings) were glued to the opening of the hole to ease air diffusion. In addition to this, the lids were lined with a 7-cm diameter filter paper. Plastic containers were then kept in an environmental growth chamber set at 28°C, 65% r.h., and a photoperiod of 14:10 h (Light: Dark) to determine adult survival and progeny production. Temperature and relative humidity were monitored during the experiment using HOBO data recorders (Onset Computer, Pocasset, MA). The experiment was performed using Completely Randomized Design (CRD) having seven observation times (7, 14, 21, 28, 35, 42, and 56 d) per grain type. Each combination of grain type and observation time was replicated five times.

Only five plastic containers from samples collected on 7, 14, 21, and 28 d were examined to determine adult beetle survival. Adults of *R. dominica* 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 to assess the number of adults that were alive from the original 25 added insects. Samples from 35, 42, and 56 d were examined to determine adult progeny production. The number of adult progeny produced was counted from each plastic container after subtracting the 25 initially added insects.

1.3.4. Moisture analysis

Moisture content was determined at all observation times by taking 10 g from each grain type. The analysis was conducted in quintuplicates using a hot air oven. The moisture content was expressed as a percentage, on a wet weight (mg) basis, following the standard moisture content determination method of the American Society of Agricultural and Biological Engineers (ASABE) ASAE S352.2 APR1988 (R2017) (ASAE, 2000), with slight modifications in heating time with respect to Kernza® grains i.e., at 130°C for 21 h.

Moisture content (wet basis) % = [(Initial wt. – Final wt.) ÷ Final wt.] x 100

1.3.5. Grain damage and weight loss assessment

The grains from each container were cleaned and passed through a Boerner® divider (Seedburo Equipment Company, Des Plaines, Illinois, USA) five times (Kernza®) and three times (wheat) to obtain a working sample of ~1500 mg hulled and dehulled Kernza® and ~5000 mg wheat for determining un-damaged and insect-damaged kernels in replicate samples. 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).

Insect damaged kernels (%) = $[N_d \div (N_d + N_u)] \times 100$

where,

N_u = Number of undamaged kernels

N_d = Number of damaged kernels

The percentage of grain weight loss was calculated on the basis of counting and weighing damaged and un-damaged kernels from each replicate working samples (Adams and Schulten, 1978; Boxall, 1986) using a digital weighing balance. The weight loss percentage of grains in each replicate samples was calculated using the following formula (Tadesse et al., 2019):

Weight loss (%) = $[(W_uN_d - W_dN_u) \div (W_u \times (N_d + N_u))] \times 100$

where,

W_u = Weight of undamaged kernels

N_u = Number of undamaged kernels

W_d = Weight of damaged kernels

N_d = Number of damaged kernels

1.3.6. Statistical analysis

The data on percentage moisture, adult survival, kernel damage and weight loss were transformed to angular values (Zar, 1984), for analysis using the Statistical Analysis System (SAS) Version 9.4, SAS Institute, Cary, North Carolina, USA. Adult progeny counts were transformed to $\log_{10}(x+1)$, for statistical analysis (Zar, 1984). Two-way analysis of variance (ANOVA) was carried out to determine the effects of grain types, observation times and their interaction effects. One-way analysis of variance (ANOVA) was performed to determine the significant ($P \leq 0.05$) differences among grain types. The means were separated with Ryan-Einot-Gabriel-Welsch (REGWQ) multiple range test at $P \leq 0.05$ and each parameter was replicated five times (SAS Institute, 2012).

1.4 Results

1.4.1. Moisture content of different grain types after *R. dominica* adult infestation

Two-way ANOVA showed that the moisture content among grain types ($F = 21.69$; df = 2, 96; $P < 0.0001$), among observation times ($F = 41.56$; df = 7, 96; $P < 0.0001$), and grain type x observation time interaction ($F = 2.99$; df = 14, 96; $P = 0.0008$) were all significant. One-way ANOVA of moisture analysis showed significant differences in moisture content (%) among different grain types at 0, 21, 28, and 35 d after *R. dominica* infestation ($P < 0.05$). The moisture content (%) among hulled Kernza®, dehulled Kernza®, and hard red winter wheat after 7, 14, 42, and 56 d of infestation with *R. dominica* adults did not differ significantly ($P > 0.05$) (Table 1.1).

1.4.2. Survival percentage of *R. dominica* adults

Two-way ANOVA showed that the adult survival (%) of *R. dominica* between grain types ($F = 516.49$; df = 2, 60; $P < 0.0001$), among observation times ($F = 96.70$; df = 4, 60; $P < 0.0001$), and grain type x observation time interaction ($F = 40.75$; df = 8, 60; $P < 0.0001$) were all significant. The survival percentage (mean ± SE) of *R. dominica* adults following infestation in hulled and dehulled Kernza® and hard red winter wheat for 7, 14, 21, and 28 d are presented in Table 2. One-way ANOVA showed that the means of survival (%) of *R. dominica* at each observation period except zero-day were significantly different among tested grain types ($P < 0.05$). The survival percentage of *R. dominica* in hulled Kernza® at each observation period was significantly shorter compared to dehulled Kernza® and hard red winter wheat ($P < 0.05$). At 28 d of the infestation, survival (%) in hulled Kernza® was significantly reduced to zero (100% adult mortality) than that of the other grain types. The survival (%) of *R. dominica* after adult infestation in dehulled Kernza® and hard red winter wheat was not significantly different at each infestation period ($P > 0.05$) (Table 1.2).

1.4.3. Progeny production, kernel damage (%), and weight loss

Two-way ANOVA showed that the adult progeny production of *R. dominica* between grain types ($F = 74.44$; df = 2, 36; $P < 0.0001$), among observation times ($F = 90.08$; df = 2, 36; $P < 0.0001$), and grain type x observation time interaction ($F = 15.25$; df = 4, 36; $P < 0.0001$) were all significant. One-way ANOVA of progeny production showed that the mean number of progeny production among different grain types after infestation with *R. dominica* adults was significantly different ($P < 0.05$). The number of *R. dominica* (mean ± SE) adult progeny production at 35, 42, and 56 d of infestation in hulled Kernza®, dehulled Kernza®, and hard red winter wheat ranged from 0 to 271.9 ± 53.9 adults. On hulled Kernza®, the adult progeny of *R. dominica* was not observed after 35 d of infestation with *R. dominica* adults, while only 4.6 ± 1.2 number of adult progenies was produced in dehulled Kernza®. After 42 and 56 d of infestation with *R. dominica* adults, hulled Kernza® produced a significantly lower number of adult progenies compared to dehulled Kernza® and hard red winter wheat (Table 1.3).

Two-way ANOVA showed that the kernel damage (%) of *R. dominica* between grain types ($F = 457.83$; df = 2, 35; $P < 0.0001$), among observation times ($F = 433.96$; df = 2, 35; $P < 0.0001$), and grain type x observation time interaction ($F = 35.94$; df = 4, 35; $P < 0.0001$) were all significant. Insect-damaged kernels of hulled Kernza® were significantly lower at each observation period compared to dehulled Kernza® and hard red winter wheat ($P < 0.05$). The mean ± SE percentage of *R. dominica* damaged kernel among all grain types ranged from 6.0 ± 0.7 to 96.3 ± 0.1 for all observation periods. The percentage of kernel damage increased as infestation time increased among all grain types. The kernel damage percentage remained statistically similar between dehulled Kernza® and hard red winter wheat after infestation with *R. dominica* adults. At 56 d of the observation period, the mean ± SE of kernel damage (%) was recorded 27.3 ± 2.4 in hulled Kernza®, 92.7 ± 1.1 in dehulled Kernza®, and 96.3 ± 0.1 in hard red winter wheat. There was an increase in kernel damage (%) with an increase in infestation time to *R. dominica* (Table 1.3).

Two-way ANOVA showed that the weight loss (%) of *R. dominica* between grain types ($F = 45.17$; df = 2, 35; $P < 0.0001$), among observation times ($F = 46.97$; df = 2, 35; $P < 0.0001$), and grain type x observation time interaction ($F = 6.52$; df = 4, 35; $P = 0.0005$) were all significant. The percent weight loss of hulled Kernza®, dehulled Kernza®, and hard red winter wheat were significantly affected by different observation periods to infestation by *R. dominica* ($P < 0.05$); One-way ANOVA). The mean ± SE percent weight loss at 35, 42, and 56 d after infestation with *R. dominica* ranged from 1.6 ± 0.2 to 28.6 ± 2.7 among all grain types. The maximum weight loss in all grain types was recorded after 56 d of storage period. The kernel weight loss increased with an increased storage period after infestation with *R. dominica*. After

35 d of storage period, the mean ± SE weight loss (%) was 1.6 ± 0.2 in hulled Kernza® and it was significantly lower compared to dehulled Kernza® and hard red winter wheat (Table 1.3).

1.5 Discussion

Being a major and most destructive pest, *R. dominica*, cause widespread post-harvest losses of about 40% in stored grains (Guru et al., 2024). Various control strategies i.e., chemical, biological, and physical methods, have been used to manage *R. dominica* infestations, each with varying levels of success (Agrafioti et al., 2023; Wakil et al., 2023). However, frequent and improper use of insecticides led to increased pesticide resistance by this pest in different stored products worldwide (Attia et al., 2020), necessitating the need to explore alternative management practices (Kavallieratos et al., 2020). One promising physical attribute that provides resistance against *R. dominica* attack is the hull character. Numerous studies have reported that stored grains with intact hull offer considerable resistance to insect pest penetration and damage compared to dehulled grains (Chanbang et al., 2008; Kumar, 2020). In this study, comparative analysis of infestation rates in hulled, dehulled Kernza®, and hard red winter wheat suggests that Kernza® with sound hull exhibits significantly lower infestation rates.

Knowledge about the survival rate of stored grain insect pests on different grain varieties provides valuable information for management of these insect pests. This further helps us in storing grains in highly insect-resistant ways, ensuring both grain quality, safety, and economic value. Our results suggest that the survival rates of *R. dominica* varied across hulled, dehulled Kernza® and hard red winter wheat, with hulled Kernza® exhibiting least susceptibility. This study supports the conclusion of several other researchers studying different stored grain insect pests in different grain types (Nietupski et al., 2017; Sinha, 1969; Swamy et al., 2023). Hulled grains have the tendency to provide greater resistance to stored grain insect pests due to their intact outer protective layers than dehulled grains. Moreover, the hull serves as a physical barrier, limiting the availability and attractiveness of the stored grains to insect pests. Another possible reason might be the grains' natural chemical properties, particularly high ash content, in Kernza® offering an antioxidant effect (Locatelli et al., 2022) against insect pest attack as compared to hard red winter wheat. Consequently, storing Kernza® in hulled form can be an effective IPM strategy focusing on conserving stored grains from insect infestations.

The progeny production of *R. dominica* across hulled, dehulled Kernza® and hard red winter wheat revealed notable differences in insect reproduction. The data suggest that progeny production was significantly lowest in hulled Kernza®, followed by dehulled Kernza®, with the highest progeny production noticed in hard red winter wheat. This aligns with the results of Arthur et al. (2012) which showed physicochemical characteristics of grains play a critical role in the development of insect pests. Hulled grains likely offer significant resistance due to their outer protective layers, which appear to serve as a physical barrier and potentially contain repellent substances, as reported by Astuti et al. (2013) on milled rice varieties. This resistance is further facilitated by the findings of Prasad et al. (2015) and Mwenda et al. (2019), who documented a negative link between kernel hardness and progeny production in sorghum. The greater susceptibility of wheat to *R. dominica* can be linked to its favorable nutritional content, which increases progeny production (Toews et al., 2001). These results prove that hulled grains are highly effective in mitigating insect multiplication, highlighting the importance of selecting resistant grain types for prolonged storage and pest management.

Assessment of grain damage and weight loss among tested grain types due to *R. dominica* showed that hulled Kernza® exhibited the lowest kernel damage and weight loss as compared to dehulled Kernza® and hard red winter wheat. Moreover, progeny production in hulled Kernza®

was consequently lower, leading to lower kernel damage and weight loss. This pattern shows that the physical barrier generated by hull significantly reduces insect entry and resultant damage. Earlier studies support these conclusions, indicating that grains with hard outer coverings and specific biochemical properties tend to experience lower insect infestation and weight loss (Bounechada et al., 2011; Hassan et al., 2023; Messenbet, 2022; Tefera, 2012). The greater susceptibility of wheat, with its softer kernel and higher nutritional content, aligns with study by Bhargava and Akhter (2014), emphasizing the need of grain type in post-harvest pest management. This underlines the importance for choosing grain types with innate resistance traits to reduce losses during storage.

In conclusion, the results from this study highlight the significant advantages of storing grains in their hulled state. Hulled Kernza® demonstrated least susceptibility to *R. dominica*, resulting in lower progeny production, reduced kernel damage, and decreased weight loss compared to dehulled Kernza® and hard red winter wheat. The protective role of the hull is important in maintaining the integrity and nutritional quality of Kernza®. Consequently, prioritizing the storage of Kernza® in its hulled form can be a feasible strategy to minimize postharvest losses and preserve grain quality. This approach aligns with environmentally sound and economically reliable pest management techniques, providing a sustainable solution to the challenges posed by stored grain insect pests. Further research into the specific physicochemical properties of resistance offered by the hull character could boost our understanding and improve post-harvest storage practices, eventually benefiting small-holder farmers and ensuring adequate food availability.

1.6 References

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Table 1.1 Moisture content of different grain types after 0, 7, 14, 21, 28, 35, 42, and 56 days of infestation to *R. dominica* adults.

Grain type	Moisture (% mean ± SE) on day:[a, b]							
	0	7	14	21	28	35	42	56
Hard red winter wheat	14.5 ± 0.3 a	12.9 ± 0.1	12.7 ± 0.1	13.2 ± 0.1 a	13.6 ± 0.1 a	12.7 ± 0.0 b	12.9 ± 0.1	13.2 ± 1.0
Hulled Kernza®	13.7 ± 0.1 b	12.7 ± 0.1	12.6 ± 0.0	12.8 ± 0.1 b	12.9 ± 0.1 b	12.7 ± 0.1 b	12.7 ± 0.1	12.7 ± 0.1
Dehulled Kernza®	14.1 ± 0.1 ab	12.8 ± 0.1	12.8 ± 0.1	13.0 ± 0.1 a	13.7 ± 0.1 a	13.4 ± 0.1 a	12.9 ± 0.2	12.8 ± 0.1
F-value[c]	5.13	0.75	2.10	8.73	14.96	26.45	0.31	2.68
P-value	0.0245*	0.49	0.17	0.0046*	0.0006*	<0.000*	0.74	0.11

[a]Each mean is based on $n = 5$ replicates.

[b]At each observation time, means among grain types followed by different letters are significantly different ($P < 0.05$; by REGWQ test).

[c]At each observation time, the df for the one-way ANOVA *F*-test is 2, 12.

*Significant ($P < 0.05$).

Table 1.2 Survival (%) of *R. dominica* adults after 0, 7, 14, 21, and 28 days of infestation in different grain types.

Grain type	Survival (% mean ± SE) on day:[a, b]				
	0	7	14	21	28
Hard red winter wheat	100.0	91.2 ± 3.4 a	79.2 ± 2.9 a	95.2 ± 2.0 a	95.2 ± 2.0 a
Hulled Kernza®	100.0	18.4 ± 3.5 b	6.4 ± 2.7 b	2.4 ± 1.6 b	0.0 ± 0.0 b
Dehulled Kernza®	100.0	96.0 ± 1.3 a	75.2 ± 3.2 a	97.6 ± 2.4 a	97.6 ± 1.6 a
F-value[c]		79.83	99.73	133.99	237.76
P-value[*]		<.0001	<.0001	<.0001	<.0001

[a]Each mean is based on $n = 5$ replicates.

[b]At each observation time, means among grain types followed by different letters are significantly different ($P < 0.05$; by REGWQ test).

[c]At each observation time, the df for the one-way ANOVA *F*-test is 2, 12.

[*]Significant ($P < 0.05$).

Table 1.3 Number of adult progeny produced, percent kernel damage and weight loss (Mean ± SE) at 35, 42 and 56 days of infestation to *R. dominica*.

Grain type	Observation time (days)	Progeny[a, b]	% Kernel damage[a, b]	% Weight loss[a, b]
Hard red winter wheat	35	73.8 ± 15.6 bc	28.1 ± 2.6 c	6.3 ± 0.7 bc
	42	78.8 ± 13.0 bc	63.9 ± 2.3 b	7.0 ± 1.0 bc
	56	188.4 ± 71.0 ab	96.3 ± 0.1 a	28.6 ± 2.7 a
Hulled Kernza®	35	0.0 ± 0.0 f	6.0 ± 0.7 d	1.6 ± 0.2 d
	42	19.4 ± 2.2 d	7.6 ± 0.6 d	1.7 ± 0.3 d
	56	30.6 ± 9.0 cd	27.3 ± 2.4 c	5.9 ± 0.9 bc
Dehulled Kernza®	35	4.6 ± 1.2 e	20.3 ± 1.2 c	6.4 ± 0.6 bc
	42	144.0 ± 32.9 ab	65.6 ± 5.2 b	4.9 ± 1.3 cd
	56	271.0 ± 53.9 a	92.7 ± 1.1 a	11.6 ± 3.2 b
F-value[c]		48.76	233.15	24.02
P-value[*]		< 0.0001	< 0.0001	< 0.0001

[a]Each mean is based on $n = 5$ replicates.

[b]Means among grain types and observation times followed by different letters are significantly different ($P < 0.05$; by REGWQ test).

[c]At each observation time, the df for the one-way ANOVA F-test is 8, 36 for progeny, and 8, 35 for kernel damage and weight loss.

[*]Significant ($P < 0.05$).

Chapter 2 - Survival and progeny production of red flour beetle, *Tribolium castaneum* (Herbst) and its associated losses in hulled Kernza®, dehulled Kernza®, and hard red winter wheat

2.1 Abstract

A variety of insect pests are responsible for postharvest losses of cereal grains. The red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) is one of the most common, serious, and polyphagous stored product insect pests that feeds on a broad range of stored grain products. The aim of this study was to evaluate the susceptibility performance and population growth potential of *T. castaneum* in hulled, dehulled Kernza® and hard red winter wheat (HRWW). For this purpose, laboratory reared *T. castaneum* was introduced in all three tested grain types to record survival rate (%), progeny production, kernel damage and weight loss at different infestation periods i.e., 7, 14, 21, 28, 35, 42, and 56 d. The mean ± SE survival rate 28 d post-infestation period was recorded significantly lower in hulled Kernza® (9.6 ± 2.4) compared to dehulled Kernza® (100.0) and HRWW (99.2 ± 0.8). Hulled Kernza® exhibited complete suppression of progeny production at 35, 42, and 56 d infestation period. However, the mean ± SE kernel damage and weight loss was recorded significantly lower i.e., 7.9 ± 0.9 and 1.1 ± 0.5, respectively in hulled Kernza® compared to other experimental grain types after 56 d infestation period. Our results suggest that Kernza® stored in its hulled form was proved to be very effective against *T. castaneum* infestation and thus help maintain the grain quality and provide protection against the negative effects of insect pests.

2.2 Introduction

The global population is continually increasing and is predicted to exceed 9 billion people by 2050, which requires nearly 70% more food. To meet this challenge, many countries have revised their policies to advance agricultural production. Alongside improving food production, it is crucial to focus on food security (Godfray et al., 2010; Parfitt et al., 2010). Each year, nearly 1.3 billion tons (one-third of the global production) are lost during postharvest processes. The extent of losses varies between different crops, e.g., on a weight basis, losses are estimated at 44% for vegetables and fruits, 20% for roots crops, and 19% for cereal crops, respectively (Gustavsson et al., 2011). However, in terms of caloric value, stored grain losses of cereal crops constitute a major portion of about 50%. Reducing stored grain losses is essential for strengthening food security and sustainably addressing increasing hunger issues (Kumar and Kalita, 2017).

A variety of insect pests are responsible for postharvest losses of cereal grains. The red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) is one of the most common, serious, and polyphagous stored product insect pests that feeds on a broad range of stored grain products (Ajayi and Rahman, 2006; Đukić et al., 2020). It causes heavy qualitative and quantitative losses to stored grains, dry fruits, and processed foods throughout the world (Appert, 1987; Cui et al., 2020; Hodges et al., 1996; Mangang et al., 2020; Patil et al., 2020). Both the adult and larval stages of *T. castaneum* infest the grain by creating holes inside them and thereby reducing the overall weight and viability of the grain which leads to poor yield in grain market (Mariadoss and Umamaheswari, 2020). *Tribolium castaneum* secretes thirteen types of carcinogenic quinones that may also cause dermatitis, allergies, and eye disorder in humans

(Howard, 1987; Hubert et al., 2018). Hence, its status in damaging stored grain products cannot be neglected (Ali et al., 2009).

Though two-third of the cultivated land is dominated by annual crops in worldwide grain production (Leff et al., 2004), there are some negative consequences associated with such cropping systems i.e., soil erosion, water pollution, high input utilization, and loss of biodiversity (Cox et al., 2006; Crews et al., 2018; Wan, 2018; Zhang et al., 2023). Perennial crops, on the other hand, offer sustainable ecological benefits. These include promoting soil health by preventing soil erosion and nutrient leaching, thereby maximizing nutrient uptake, and increasing biodiversity (Chapman et al., 2022; Chen et al., 2022; Kaye and Quemada, 2017). Kernza®, is a first perennial grain crop in the world, developed from improved breeding lines of Intermediate wheatgrass (IWG) (*Thinopyrum intermedium* (Host) Barkworth & D.R. Dewey) at The Land Institute of Salina, Kansas in the United States (de Oliveira et al., 2018; DeHaan et al., 2014). The deep and extensive root system of Kernza® serves as an excellent ground cover year-round. The flour obtained from Kernza® grain can be used in a wide variety of snacks and bakery products such as pasta, pancakes, cookies, cereals, and breads (Broom, 2019). Being sweet and nutty in flavor, Kernza® grain serves as a key ingredient in the production of alcoholic beverages, especially beer and wine (Cureton et al., 2023). Additionally, being a dual-purpose crop for both grain and forage makes it highly promising to farmers (Crews and DeHaan, 2015).

Beside all these advantages, Kernza® and its products are also at risk of insect feeding damage during storage like other cereal grains. The level of infestation vary greatly depending upon characteristics of grains, e.g., phenol, ash, protein, amylose, and fat contents, thickness of husk, and kernel hardness (Rizwana et al., 2011). The crop varieties that have fine grains are more susceptible to stored grain insect pests than the varieties that have thicker husks (Swamy et al., 2019). In a recent study, Memon et al. (2024) studied morphometric and life history parameters of *T. castaneum* on barley, rice, cowpea, corn, wheat, and sorghum flour and reported the fastest development period on wheat flour. Previously, various studies reported the susceptibility of stored grain products, e.g., flour of rice, cowpea, corn, millet, wheat, sorghum, yam, and cassava to *T. castaneum* (Ajayi and Rahman, 2006; Awadalla et al., 2023; Gerken and Campbell, 2020; Swamy et al., 2020; Turaki et al., 2007). The findings of these studies would help us to manage insect pests and maintain the quality of these products under storage.

Several studies claim that storing grain in hulled form provides resistance against insect infestation, thereby minimizing dependence on chemical control, allowing long-term storage, and reducing stored grain losses. As per the authors' knowledge, the impact of hulled and dehulled Kernza® on progeny production and survival rate of *T. castaneum* has not been previously studied. Therefore, the aim of this study was to evaluate the susceptibility performance and population growth potential of *T. castaneum* in hulled, dehulled Kernza® and hard red winter wheat. Various physical parameters like moisture content, kernel damage, weight loss percentages were also investigated following *T. castaneum* infestation.

2.3 Materials and Methods

2.3.1. Grain commodities

Hulled and dehulled Kernza® grains (variety: TLIC5) from the year 2022 harvest were obtained from the Sustain-A-Grain, a company in McPherson County, Kansas, USA, and shipped to the Department of Grain Science and Industry at Kansas State University, Manhattan, KS, USA. These grains were then held in freezer at ~-20°C for two weeks to kill any live insects present. All the comparisons were made with organic whole grain hard red winter wheat stock (Great River Organic Milling, Fountain City, Wisconsin, USA) (standard control). Prior to experimentation, both Kernza® and wheat grain samples were removed from the freezer and acclimatized in plastic bags at room conditions (~24.5 °C and ~76% relative humidity (r.h.)) for 1-2 weeks. The grain moisture content was measured by an air oven method (ASAE, 2000). Depending on variability in moisture contents, both Kernza® and wheat grain samples were equilibrated to 13% moisture content (wet basis) in environmental growth chambers maintained at 28°C and 65% r.h. before used in experiments.

2.3.2. Insect colonies

Cultures of *T. castaneum* that have been maintained in the laboratory for more than 20 years in the Department of Grain Science were used for the experiments. The insects were reared in 0.95 liter mason wide-mouth glass jars fitted with wire mesh and filter paper lids containing 95% organic whole wheat bread flour and 5% (by weight) brewer's yeast. The glass jars were held inside growth chambers (Model I-36 VL; Percival Scientific, Perry, Iowa, USA) set at 28°C, 65% r.h., and a photoperiod of 14:10 h (Light: Dark).

2.3.3. Insect survival and progeny production

Twenty-five unsexed adults of *T. castaneum* (1 – 3 week mixed ages) were suctioned carefully with the help of a mechanical aspirator from laboratory cultures. The collected adults were introduced into each of the round plastic containers (6.4 cm diameter x 6.9 cm height x 150 ml volume) having 50 g cleaned grains of hulled Kernza®, dehulled Kernza®, and hard red winter wheat. Each grain type held a moisture content of 13-14.5% (wet basis). The plastic containers were covered with perforated lids (10-mm round hole) and a small piece of wire-mesh screens (0.6-mm^2 openings) were glued to the opening of the hole to ease air diffusion. In addition to this, the lids were lined with a 7-cm diameter filter paper. Plastic containers were then kept in an environmental growth chamber set at 28°C, 65% r.h., and a photoperiod of 14:10 h (Light: Dark) to determine adult survival and progeny production. Temperature and relative humidity were monitored during the experiment using HOBO data recorders (Onset Computer, Pocasset, MA). The experiment was performed using Completely Randomized Design (CRD) having seven observation times (7, 14, 21, 28, 35, 42, and 56 d) per grain type. Each combination of grain type and observation time was replicated five times.

Only five plastic containers from samples collected on 7, 14, 21, and 28 d were examined to determine adult beetle survival. Adults of *T. castaneum* 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 to assess the number of adults that were alive from the original 25 added insects. Samples from 35, 42, and 56 d were examined to determine adult progeny production. The number of adult progeny produced was counted from each plastic container after subtracting the 25 initially added insects.

2.3.4. Moisture analysis

Moisture content was determined at all observation times by taking 10 g from each grain type. The analysis was conducted in quintuplicates using a hot air oven. The moisture content was expressed as a percentage, on a wet weight (mg) basis, following the standard moisture content determination method of the American Society of Agricultural and Biological Engineers (ASABE) ASAE S352.2 APR1988 (R2017) (ASAE, 2000), with slight modifications in heating time with respect to Kernza® grains i.e., at 130°C for 21 h.

Moisture content (wet basis) % = [(Initial wt. – Final wt.) ÷ Final wt.] x 100

2.3.5. Grain damage and weight loss assessment

The grains from each container were cleaned and passed through a Boerner® divider (Seedburo Equipment Company, Des Plaines, Illinois, USA) five times (Kernza®) and three times (wheat) to obtain a working sample of ~1500 mg hulled and dehulled Kernza® and ~5000 mg wheat for determining un-damaged and insect-damaged kernels in replicate samples. 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).

Insect damaged kernels (%) = $[N_d \div (N_d + N_u)] \times 100$

where,

N_u = Number of undamaged kernels

N_d = Number of damaged kernels

The percentage of grain weight loss was calculated on the basis of counting and weighing damaged and un-damaged kernels from each replicate working samples (Adams and Schulten, 1978; Boxall, 1986) using a digital weighing balance. The weight loss percentage of grains in each replicate samples was calculated using the following formula (Tadesse et al., 2019):

Weight loss (%) = $[(W_u N_d - W_d N_u) \div (W_u \times (N_d + N_u))] \times 100$

where,

W_u = Weight of undamaged kernels

N_u = Number of undamaged kernels

W_d = Weight of damaged kernels

N_d = Number of damaged kernels

2.3.6. Statistical analysis

The data on percentage moisture, adult survival, kernel damage and weight loss were transformed to angular values, (Zar, 1984) for analysis using the Statistical Analysis System (SAS) Version 9.4, SAS Institute, Cary, North Carolina, USA. Adult progeny counts were transformed to $\log_{10}(x+1)$, for statistical analysis (Zar, 1984). Two-way analysis of variance (ANOVA) was carried out to determine the effects of grain types, observation times and their interaction effects. One-way analysis of variance (ANOVA) was performed to determine the significant ($P \leq 0.05$) differences among grain types. The means were separated with Ryan-Einot-Gabriel-Welsch (REGWQ) multiple range test at $P \leq 0.05$ and each parameter was replicated five times (SAS Institute, 2012).

2.4 Results

2.4.1. Moisture percentage

Two-way ANOVA showed that the moisture content among grain types ($F = 188.42$; df = 2, 96; $P < 0.0001$), among observation times ($F = 17.73$; df = 7, 96; $P < 0.0001$), and grain type x observation time interaction ($F = 3.82$; df = 14, 96; $P = 0.0008$) were all significant. The moisture percentages among grains of hard red winter wheat, hulled Kernza® and dehulled Kernza® at each observation period after infestation of *T. castaneum* adults were significantly different ($P < 0.05$). The mean ± SE moisture percentage in grains of hulled Kernza® ranged from 13.2 ± 0.1 to 13.7 ± 0.1 at each observation period. One-way ANOVA showed that moisture (%) in hulled Kernza® after 7, 14, 21, 28, 35, 42, and 56 d of infestation with *T. castaneum* adults was significantly lower compared to hard red winter wheat and dehulled Kernza® ($P < 0.05$). The lowest mean ± SE moisture (%) was recorded i.e., 13.2 ± 0.1 in hulled Kernza® after 7 and 42 d of storage period and the highest was recorded 14.8 in dehulled Kernza® after 28 d of *T.*

castaneum infestation. The moisture (%) in each grain type remained nearly unchanged throughout whole storage periods after infestation with *T. castaneum* adults (Table 2.1).

2.4.2. Survival (%) of *T. castaneum*

Two-way ANOVA showed that the adult survival (%) of *T. castaneum* between grain types (F = 454.65; df = 2, 60; P < 0.0001), among observation times (F = 43.06; df = 4, 60; P < 0.0001), and grain type x observation time interaction (F = 30.23; df = 8, 60; P < 0.0001) were all significant. The survival percentage of *T. castaneum* adults infestation in hulled Kernza® for 7, 14, 21, and 28 d was significantly lower compared to dehulled Kernza® and hard red winter wheat (P < 0.05). The mean ± SE survival of *T. castaneum* adults in all grain types ranged from 9.6 ± 2.4 to 100 %. Mortality of all adults was not recorded at any exposure period in all grain types. However, the minimum mean ± SE survival % was recorded 9.6 ± 2.4 in hulled Kernza® at 28 d after infestation of *T. castaneum* adults. The survival % decreased in hulled Kernza® with an increased storage period. The survival (%) of *T. castaneum* infestation in dehulled Kernza® and hard red winter wheat was not significantly different at each observation period (P > 0.05). (Table 2.2).

2.4.3. Progeny of *T. castaneum*, kernel damage, and weight loss

Two-way ANOVA showed that the adult progeny production of *T. castaneum* between grain types (F = 211.61; df = 2, 36; P < 0.0001), among observation times (F = 27.72; df = 2, 36; P < 0.0001), and grain type x observation time interaction (F = 6.96; df = 4, 36; P = 0.0003) were all significant. One-way ANOVA showed that the mean number of next generation adults of *T. castaneum* among different grain types after various storage periods was significantly different (F = 63.31; df = 8, 36; P < 0.0001). The mean ± SE number of *T. castaneum* adult progeny production at 35, 42, and 56 d of infestation in hulled Kernza®, dehulled Kernza®, and hard red winter wheat ranged from 0 to 120 ± 15.1 adults. Next generation adults were not observed at any observation period in hulled Kernza®. Dehulled Kernza® produced significantly higher number of adult progenies after *T. castaneum* adult's infestation at all observation periods compared to hard red winter wheat. There was an increase in the number of adult progenies produced with an increase in infestation time of *T. castaneum* in both dehulled Kernza® and hard red winter wheat. On the 56 d after infestation, the mean ± SE number of *T. castaneum* adult progeny was recorded i.e., 0, 41.6 ± 13.9, and 120 ± 15.1 in hulled Kernza®, hard red winter wheat, and dehulled Kernza®, respectively (Table 2.3).

Two-way ANOVA showed that the kernel damage (%) of *T. castaneum* between grain types (F = 514.77; df = 2, 36; $P < 0.0001$), among observation times (F = 78.02; df = 2, 36; $P < 0.0001$), and grain type x observation time interaction (F = 6.93; df = 4, 36; P = 0.0003) were all significant. The mean ± SE kernel damage (%) after infestation of *T. castaneum* adults was significantly different among grain types and ranged from 2.4 ± 0.2 to 70.0 ± 2.1 damage kernels (F =151.66; df = 8, 36; P <.0001; One-way ANOVA). The kernel damage increased as storage time increased in all grain types. The kernel damage (%) in hulled Kernza® after infestation of *T. castaneum* for 35, 42, and 56 d was significantly lower compared to dehulled Kernza® and hard red winter wheat. After 42 d of infestation with *T. castaneum*, the trend of kernel damage (%) among all grain types was as follows: hulled Kernza® < dehulled Kernza® < hard red winter wheat. The mean ± SE kernel damage (%) was recorded i.e., 7.9 ± 0.9 in hulled Kernza®, 70.0 ± 2.1 in dehulled Kernza®, and 69.8 ± 1.2 in hard red winter wheat after 56 d of storage (Table 2.3).

Two-way ANOVA showed that the weight loss (%) of *T. castaneum* between grain types (F = 27.04; df = 2, 36; $P < 0.0001$), among observation times (F = 3.33; df = 2, 36; P = 0.0472), and grain type x observation time interaction ($F = 4.76$; df = 4, 36; $P = 0.0035$) were all significant. The weight loss percentages among hulled Kernza®, dehulled Kernza®, and hard red winter wheat was significantly different after infestation of *T. castaneum* ($F = 9.97$; df = 8, 36; $P < .0001$; One-way ANOVA). The mean ± SE weight loss (%) at 35, 42, and 56 d after infestation of *T. castaneum* ranged from 0.3 ± 0.1 to 5.3 ± 1.1 among all grain types. There were no significant differences in weight loss (%) among dehulled Kernza® and hard red winter wheat at 35 and 56 d after infestation of *T. castaneum*. While after 42 d storage period, the decrease in kernel weight of hulled and dehulled Kernza® were similar to each other but significantly lower than that of the hard red winter wheat grains. After 56 d of the storage period, the mean ± SE weight loss (%) of hulled Kernza® was 1.1 ± 0.5 and significantly lower compared to other experimental grain types. The weight loss among hulled Kernza® or hard red winter wheat was not significantly affected by storage durations (Table 2.3).

2.5 Discussion

The remarkable value of Kernza® as a perennial grain crop in agro-ecosystem cannot be overemphasized, yet its storage is susceptible to infestations by stored product insect pests such as *T. castaneum*. This pest is highly disastrous, mainly in processed cereal commodities, causing widespread losses in grain both qualitatively and quantitatively (Campbell and Arbogast, 2004; Padın et al., 2002). Various effective control methods to manage *T. castaneum* populations include chemical use (Manivannan and Subramanyam, 2023), integrated pest management (IPM) practices (Wakil et al., 2023), and biological control (Javed et al., 2020). The physical properties of grains, particularly the integrity of the hull, are crucial for offering natural resistance against insect pest attacks; hulled grains provide significantly better protection in comparison to dehulled varieties (Antunes et al., 2016; Breese, 1963; Cogburn et al., 1983; Gałęcki et al., 2019). In our study, we performed laboratory-controlled experiments to evaluate the impact of hulled, dehulled Kernza® and hard red winter wheat on *T. castaneum* infestation rates and grain damage. Our results concluded that hull character of Kernza® significantly reduces susceptibility to *T. castaneum* infestation.

Our study shows significant differences in the survival and progeny production rates of *T. castaneum*, across tested grain types, with lowest survival and no progeny production noticed in hulled Kernza®. Regardless of being a secondary pest, *T. castaneum* still exhibited higher survival and progeny production rates in dehulled Kernza® and hard red winter wheat. This conclusion aligns with previous studies that have demonstrated reduced survival rates of stored product insects in hulled grains. For instance, studies have proved that the presence of hull in stored grains can interfere with the feeding and development of stored grain insect pests such as wheat weevil, *Sitophilus granarius* and *Rhyzopertha dominica* in oats (Sinha, 1969), *Sitotroga cerealella* in maize (Demissie et al., 2019), and flour mill beetle *Cryptolestes turcicus* in barley (Sinha, 1971). In our case, hulled Kernza® proved to be an effective physical barrier that restricted *R. dominica* access to the nutrient-rich endosperm, thereby providing greater resistance compared to dehulled Kernza® and hard red winter wheat. This resistance mechanism is in trend with the research findings of Russell and Cogburn (1977) and Cogburn et al. (1983), who documented similar results with different rice varieties showing varied susceptibility to *R. dominica*. Moreover, the physical characteristics of hulled grains, such as increased kernel hardness, have been linked with lower insect fitness and progeny emergence, as cited by Doggett (1958) and Davey and Elcoate (1962). The survival rate in our research was significantly lower in hulled Kernza®, followed by dehulled Kernza® and hard red winter wheat, which supports the idea that the structural integrity offered by hull plays a critical role in reducing the survival and reproduction of *T. castaneum*. Therefore, our results contribute to a growing body of evidence that illustrates the importance of hull in enhancing the resistance of stored product grains against insect pest infestations.

The present study demonstrated significant differences in physical properties such as moisture content, kernel damage, and weight loss as a result of *T. castaneum* infestation on three tested grain types. Increased moisture content was noted in dehulled Kernza® and hard red winter wheat over time, presumably due to increased respiration and metabolic activities during infestation, as mentioned by Arthur et al. (2007). This correlation of increased moisture content with the level of infestation aligns with various studies, suggesting that higher moisture levels could potentially attract more beetles (Asemu et al., 2020; Sinha and Sinha, 1992; Stathers et al., 2020). Kernel damage and weight loss percentages were more prominent in dehulled and hard red winter wheat, supporting studies that illustrate the protective role of hull against pest introduction and feeding (Bodroža-Solarov et al., 2010; Chanbang et al., 2008; Solarov and Filipčev, 2020). These results highlight the importance of kernels' physical characteristics in determining susceptibility to pest infestation and resultant losses, manifesting that the integrity of the grain husk plays a vital role in reducing damage and maintaining grain quality during storage.

In summary, our research demonstrated that Kernza® stored in its hulled form was proved to be very effective against *T. castaneum* infestation. However, despite being a secondary pest, *T. castaneum* still has the potential to feed on dehulled Kernza® leading to a notable increase in progeny production, kernel damage, and weight loss. Therefore, to efficiently manage *T. castaneum* infestations and reduce postharvest losses, it is suggested that Kernza® be stored in its hulled form. This practice can help maintain the grain quality and provide protection against the negative effects of insect pests.

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Table 2.1 Moisture content of different grain types after 0, 7, 14, 21, 28, 35, 42, and 56 days of infestation to *T. castaneum* adults.

Grain type	Moisture (% mean ± SE) on day:[a, b]							
	0	7	14	21	28	35	42	56
Hard red winter wheat	14.5 ± 0.3 a	13.8 ± 0.1 a	14.1 ± 0.1 a	14.3 ± 0.1 a	14.3 ± 0.1 b	14.2 ± 0.1 b	14.1 ± 0.1 a	14.4 ± 0.1 a
Hulled Kernza®	13.7 ± 0.1 b	13.2 ± 0.1 b	13.4 ± 0.0 c	13.6 ± 0.1 b	13.5 ± 0.1 c	13.5 ± 0.1 c	13.2 ± 0.1 b	13.5 ± 0.1 b
Dehulled Kernza®	14.1 ± 0.1 ab	13.6 ± 0.1 a	13.9 ± 0.0 b	14.3 ± 0.1 a	14.8 ± 0.0 a	14.6 ± 0.1 a	14.1 ± 0.1 a	14.5 ± 0.1 a
F-value[c]	5.13	20.63	45.33	17.03	88.76	33.26	67.71	54.93
P-value[*]	0.0245	0.0001	< 0.0001	0.0003	< 0.0001	< 0.0001	< 0.0001	< 0.0001

[a]Each mean is based on $n = 5$ replicates.

[b]At each observation time, means among grain types followed by different letters are significantly different ($P < 0.05$; by REGWQ test).

[c]At each observation time, the df for the one-way ANOVA *F*-test is 2, 12.

[*]Significant ($P < 0.05$).

Table 2.2 Survival (%) of *T. castaneum* adults after 0, 7, 14, 21, and 28 days of infestation in different grain types.

Grain type	Survival (% mean ± SE) on day:[a, b]				
	0	7	14	21	28
Hard red winter wheat	100.0	100.0 ± 0.0 a	96.0 ± 1.8 a	98.4 ± 1.0 a	99.2 ± 0.8 a
Hulled Kernza®	100.0	31.2 ± 6.0 b	20.0 ± 4.6 b	14.4 ± 6.4 b	9.6 ± 2.4 b
Dehulled Kernza®	100.0	99.2 ± 0.8 a	96.0 ± 2.5 a	97.6 ± 1.6 a	100.0 ± 0.0 a
F-value[c]		162.33	68.96	72.21	444.06
P-value[*]		< 0.0001	< 0.0001	< 0.0001	< 0.0001

[a]Each mean is based on $n = 5$ replicates.

[b]At each observation time, means among grain types followed by different letters are significantly different ($P < 0.05$; by REGWQ test).

[c]At each observation time, the df for the one-way ANOVA *F*-test is 2, 12.

[*]Significant ($P < 0.05$).

Table 2.3 Number of adult progeny produced, percent kernel damage and weight loss (Mean ± SE) at 35, 42 and 56 days of infestation to *T. castaneum* in different grain types.

Grain type	Observation time (days)	Progeny[a, b]	% Kernel damage[a, b]	% Weight loss[a, b]
Hard red winter wheat	35	4.6 ± 1.8 c	40.1 ± 4.3 c	2.1 ± 0.7 abc
	42	22.4 ± 5.1 b	55.6 ± 2.1 b	5.3 ± 1.1 a
	56	41.6 ± 13.9 b	69.8 ± 1.2 a	5.1 ± 1.0 a
Hulled Kernza®	35	0.0 ± 0.0 d	2.4 ± 0.2 e	0.5 ± 0.1 c
	42	0.0 ± 0.0 d	4.7 ± 0.8 de	0.3 ± 0.1 c
	56	0.0 ± 0.0 d	7.9 ± 0.9 d	1.1 ± 0.5 c
Dehulled Kernza®	35	15.8 ± 3.4 b	34.9 ± 1.2 c	4.3 ± 0.8 a
	42	93.0 ± 8.9 a	43.1 ± 4.7 c	1.6 ± 0.8 bc
	56	120.0 ± 15.1 a	70.0 ± 2.1 a	4.1 ± 0.8 ab
F-value[c]		63.31	151.66	9.97
P-value[*]		< 0.0001	< 0.0001	< 0.0001

[a]Each mean is based on $n = 5$ replicates.

[b]Means among grain types and observation times followed by different letters are significantly different ($P < 0.05$; by REGWQ test).

[c]At each observation time, the df for the one-way ANOVA F-test is 8, 36.

[*]Significant ($P < 0.05$).

Chapter 3 - Determination of susceptibility of hulled Kernza®, dehulled Kernza®, and hard red winter wheat to *Sitophilus oryzae* (Linnaeus) (Coleoptera; Curculionidae)

3.1 Abstract

Stored grain insect pests adversely affect grain quality and its nutritional value during storage. The rice weevil *Sitophilus oryzae* (Linnaeus), is among the most common insect pests infesting stored grains worldwide. The present experiment was planned to determine the resistance of hulled Kernza® to *S. oryzae* compared to dehulled Kernza® and hard red winter wheat. Kernza® is a novel grain develop from intermediate wheatgrass, *Thinopyrum intermedium* (Host) and there is no report on the susceptibility of Kernza® grains to *S. oryzae*. For this purpose, a laboratory reared population of *S. oryzae* was used and experiment was performed using completely randomized design (CRD), having thirty-five replications and seven observation times (7, 14, 21, 28, 35, 42, 56 d) for each grain type. The results showed that survival % of *S. oryzae* was significantly low in hulled Kernza® compared to dehulled Kernza® and hard red winter wheat. The survival percentages decrease from 84.8 ± 2.9 to 27.2 ± 4.1 in hulled Kernza® at 7 and 28 d of infestation, respectively. Similarly, a smaller number of adult progenies was produced in hulled and dehulled Kernza® than that of hard red winter wheat. At 28 d post-infestation, no adult progeny was recorded in both hulled and dehulled Kernza®, but 21.0 ± 5.4 number of adults were recorded in hard red winter wheat. The mean ± SE weight loss percentage was in the following order after 56 d of infestation; hulled Kernza® (2.2 ± 1.1) < dehulled Kernza® (5.5 ± 0.5) < hard red winter wheat (6.5 ± 0.6). The findings of current study suggest that hulled Kernza® is more resistant to rice weevil than dehulled Kernza® and widely cultivated hard red winter wheat which suggest that this less known perennial grain might be a better option to replace traditional wheat and Kernza® should be stored in hulled form to prevent insect infestation.

3.2 Introduction

The rice weevil, *Sitophilus oryzae* (Linnaeus) (Coleoptera; Curculionidae) is among the most commonly encountered insects infesting stored grains worldwide (Gowda et al., 2019; Hong et al., 2018) . It is a noxious and primary pest of economic importance, causing damage to various types of stored grain cereals and their products global (Benzi et al., 2009). It has been accounted to inflict damage of about 10-65% and 80% during short-term and long-term storage conditions, respectively (Park et al., 2004). As an internal feeder, the adult weevil bores into the kernel, feeding primarily on the endosperm and thereby decreasing the carbohydrate content of the grain, whereas, the larvae feed preferably on the seed germ, resulting in reducing the nutritional value of the grain (Jayakumar et al., 2017). Moreover, the damaged or broken kernels as a result of feeding by *S. oryzae* larvae are more susceptible to attack by external feeders which further deteriorates the grain and ultimately, leading to huge economic losses (Jadhav, 2006). High population densities of *S. oryzae* can be effortlessly established due to its short developmental time causing widespread infestation (Aitken, 1975). A few latest studies documenting susceptibility of stored grains to *S. oryzae* have been published (Doherty et al., 2023; Kalsa et al., 2019; Yadav et al., 2018).

Kernza® is a novel perennial grain made from improved lines of intermediate wheatgrass (*Thinopyrum intermedium*) (Host) Barkworth & D. R. Dewey, holds resemblance to wheat but produces longer and slender seed head (spikes) containing more seeds than an annual wheat head (Culman et al., 2013; de Oliveira et al., 2018; DeHaan et al., 2014). It is a much smaller grain (20-50% the size of wheat) and has a higher weight ratio of hull to kernel (~30% hull to ~70% kernel) than other grains (AURI, 2022). On an average, the per acre yield of Kernza® is about a third of traditional wheat (Robbins, 2019). Kernza® grain varies somewhat with growing and storage conditions. On an average, it has 19.2 grams of protein per 100 grams (2x higher compared to wheat) and 18 grams of dietary fiber per 100 grams (8x higher compared to wheat). Kernza® grain contains gluten but it is deficient in high molecular weight glutenin (gluten components) compared to wheat gluten (AURI, 2022). It is currently used in beer, cereal, pasta and baked commodities such as crackers, breads, and waffles (Muckey, 2019). Despite the growing interest in Kernza® grain production, the susceptibility of Kernza® grain to stored-product insect in storage conditions is unknown.

Stored grain insect pests, not only adversely affect grain quality and its nutritional value during storage but also contaminate the grain with their body fragments and metabolic by-products resulting in huge economic impact of over $300 trillion annually in storage losses and control costs associated with prevention and treatment activities in the United States (Hagstrum et al., 2012; Perkin et al., 2016). Like other stored grains, Kernza® is also at risk of insect feeding damage resulting in both qualitative and quantitative losses during its storage. Knowledge about the development of stored insect pests, their survival rate, and progeny production on hulled and dehulled Kernza® grain could play a role in providing protection from stored-product insects. Mehta and Kumar (2021) studied relative susceptibility of seven wheat cultivars and categorized wheat varieties into three types: less susceptible, moderately susceptible, and highly susceptible to *S. oryzae*. They suggested that farmers should avoid storing those varieties that are highly susceptible and more affected by the rice weevil. Similarly, Doherty et al. (2023) and Okpile et al. (2021) evaluated the susceptibility of various rice (*Oryzae sativa*) varieties to *S. oryzae* by recording the developmental time, adult damage, grain weight loss, and progeny production of rice weevil. There are several papers published reporting feeding damage and progeny production of *S. oryzae* in sorghum (Prasad et al., 2015) and wheat (McGaughey et al., 1990;

Saad et al., 2018). However, to the best of the author's knowledge, the susceptibility of hulled and dehulled Kernza® grains to *S. oryzae* has been studied for the first time globally.

This experiment designed to investigate the susceptibility of both hulled and dehulled Kernza® to rice weevil, *S. oryzae*. We measured the kernel damage and weight losses incurred by *S. oryzae* in both Kernza® grains and compared them with those observed in hard red winter wheat. Additionally, moisture contents of grains, survival percentages, and progeny production after different time intervals is also recorded. Based on authors understanding, it is important to highlight that such measurements and comparisons on Kernza® grains have not been previously reported. These findings shed light on the expected outcome of hull presence or absence on its susceptibility to *S. oryzae* and associated losses, providing useful insights for the insect pest management of stored grains.

3.3 Materials and Methods

3.3.1. Grain commodities

Hulled and dehulled Kernza® grains (variety: TLIC5) from the year 2022 harvest were obtained from the Sustain-A-Grain, a company in McPherson County, Kansas, USA, and shipped to the Department of Grain Science and Industry at Kansas State University, Manhattan, KS, USA. These grains were then held in freezer at ∼-20°C for two weeks to kill any live insects present. All the comparisons were made with organic whole grain hard red winter wheat stock (Great River Organic Milling, Fountain City, Wisconsin, USA) (standard control). Prior to experimentation, both Kernza® and wheat grain samples were removed from the freezer and acclimatized in plastic bags at room conditions (~24.5 °C and ~76% relative humidity (r.h.)) for 1-2 weeks. The grain moisture content was measured by an air oven method (ASAE, 2000). Depending on variability in moisture contents, both Kernza® and wheat grain samples were equilibrated to 13% moisture content (wet basis) in environmental growth chambers maintained at 28°C and 65% r.h. before used in experiments.

3.3.2. Insect colonies

Cultures of *S. oryzae* that have been maintained in the laboratory for more than 20 years in the Department of Grain Science were used for the experiments. The insects were reared in 0.95 liter mason wide-mouth 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 A, Perry, Iowa, USA) set at 28°C, 65% r.h., and a photoperiod of 14:10 h (Light: Dark).

3.3.3. Insect survival and progeny production

Twenty-five unsexed adults of *S. oryzae* (1 – 3 week mixed ages) were suctioned carefully with the help of a mechanical aspirator from laboratory cultures. The collected adults were introduced into each of the round plastic containers (6.4 cm diameter x 6.9 cm height x 150 ml volume) having 50 g cleaned grains of hulled Kernza®, dehulled Kernza®, and hard red winter wheat. Each grain type held a moisture content of 13-14.5% (wet basis). The plastic containers were covered with perforated lids (10-mm round hole) and a small piece of wire-mesh screens (0.6-mm^2 openings) were glued to the opening of the hole to ease air diffusion. In addition to this, the lids were lined with a 7-cm diameter filter paper. Plastic containers were then kept in an environmental growth chamber set at 28°C, 65% r.h., and a photoperiod of 14:10 h (Light: Dark) to determine adult survival and progeny production. Temperature and relative humidity were monitored during the experiment using HOBO data recorders (Onset Computer, Pocasset, MA). The experiment was performed using Completely Randomized Design (CRD) having seven observation times (7, 14, 21, 28, 35, 42, and 56 d) per grain type. Each combination of grain type and observation time was replicated five times.

Only five plastic containers from samples collected on 7, 14, 21, and 28 d were examined to determine adult beetle survival. Adults of *S. oryzae* 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 to assess the number of adults that were alive from the original 25 added insects. Samples from 28, 35, 42, and 56 d were examined to determine adult progeny production. The number of adult progenies produced were counted from each plastic container after subtracting the 25 initially added insects.

3.3.4. Moisture analysis

Moisture content was determined at all observation times by taking 10 g from each grain type. The analysis was conducted in quintuplicates using a hot air oven. The moisture content was expressed as a percentage, on a wet weight (mg) basis, following the standard moisture content determination method of the American Society of Agricultural and Biological Engineers (ASABE) ASAE S352.2 APR1988 (R2017) (ASAE, 2000), with slight modifications in heating time with respect to Kernza® grains i.e., at 130°C for 21 h.

Moisture content (wet basis) % = [(Initial wt. – Final wt.) ÷ Final wt.] x 100

3.3.5. Grain damage and weight loss assessment

The grains from each container were cleaned and passed through a Boerner® divider (Seedburo Equipment Company, Des Plaines, Illinois, USA) five times (Kernza®) and three times (wheat) to obtain a working sample of ~1500 mg hulled and dehulled Kernza® and ~5000 mg wheat for determining un-damaged and insect-damaged kernels in replicate samples. 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).

Insect damaged kernels (%) = $[N_d \div (N_d + N_u)] \times 100$

where,

N_u = Number of undamaged kernels

N_d = Number of damaged kernels

The percentage of grain weight loss was calculated on the basis of counting and weighing damaged and un-damaged kernels from each replicate working samples (Adams and Schulten, 1978; Boxall, 1986) using a digital weighing balance. The weight loss percentage of grains in each replicate samples was calculated using the following formula (Tadesse et al., 2019):

Weight loss (%) = $[(W_uN_d - W_dN_u) \div (W_u \times (N_d + N_u))] \times 100$

where,

W_u = Weight of undamaged kernels

N_u = Number of undamaged kernels

W_d = Weight of damaged kernels

N_d = Number of damaged kernels

3.3.6. Statistical analysis

The data on percentage moisture, adult survival, kernel damage and weight loss were transformed to angular values, (Zar, 1984), for analysis using the Statistical Analysis System (SAS) Version 9.4, SAS Institute, Cary, North Carolina, USA. Adult progeny counts were transformed to $\log_{10}(x+1)$, for statistical analysis (Zar, 1984). Two-way analysis of variance

(ANOVA) was carried out to determine the effects of grain types, observation times and their interaction effects. One-way analysis of variance (ANOVA) was performed to determine the significant ($P \leq 0.05$) differences among grain types. The means were separated with Ryan-Einot-Gabriel-Welsch (REGWQ) multiple range test at $P \leq 0.05$ and each parameter was replicated five times (SAS Institute, 2012).

The non-linear model, $y^2 = a + bx$, was fitted to the percentage survival data of *S. oryzae* on hulled Kernza®, dehulled Kernza® and hard red winter wheat across different observation times using Table Curve2D software, Version 5.01.01 (Anonymous, 1996). The values for parameters *a* and *b* were determined by fitting equations to the data on *S. oryzae* adult survival relative to both grain types and observation times. Each possible pairwise comparison between the grain types and their respective observation times was performed by comparing individual models with a pooled model (Draper and Smith, 1998). Any two models were considered statistically significant from one another ($P \leq 0.05$) when the *F*-test showed that individual models differed from the pooled model. The graph on percentage survival was plotted using SigmaPlot 12.5 software (Systat, Software, Inc., San Jose, California, USA).

3.4 Results

3.4.1. Moisture percentage

Two-way ANOVA showed that the moisture content among grain types ($F = 50.26$; df = 2, 96; $P < 0.0001$), among observation times ($F = 11.64$; df = 7, 96; $P < 0.0001$), and grain type x observation time interaction ($F = 3.39$; df = 14, 96; $P = 0.0002$) were all significant. The moisture contents among grains of hard red winter wheat, hulled Kernza® and dehulled Kernza® at each observation period after infestation of *S. oryzae* adults were significantly different ($P < 0.05$). In hard red winter wheat, the mean ± SE moisture percentage was increased continuously from 13.7 ± 0.1 to 21 ± 2.5 at 7 and 56 d of observation, respectively. One-way ANOVA showed that moisture (%) in hulled and dehulled Kernza® after 7, 14, 21, 28, 35, and 56 d of infestation with *S. oryzae* adults was significantly lower than that of the hard red winter wheat ($P < 0.05$). The lowest mean ± SE moisture (%) was recorded 12.2 ± 0.1 in hulled Kernza® after 21 d of storage period and the highest was recorded 21 ± 2.5 in hard red winter wheat after 56 d of *S. oryzae* infestation (Table 3.1).

3.4.2. Survival (%) of *S. oryzae*

Two-way ANOVA showed that the adult survival (%) of *S. oryzae* between grain types (F = 45.05; df = 2, 60; $P < 0.0001$), among observation times (F = 65.67; df = 4, 60; $P < 0.0001$), and grain type x observation time interaction (F = 4.51; df = 8, 60; P = 0.0002) were all significant. The survival percentage (mean ± SE) of *S. oryzae* adults infestation in hulled Kernza® for 7, 14, 21, and 28 d was significantly lower compared to dehulled Kernza® and hard red winter wheat (P < 0.05; df = 2, 12; one-way ANOVA). The mean ± SE survival of *S. oryzae* adults after infestation in hulled Kernza®, dehulled Kernza®, and hard red winter wheat ranged from 27.2 ± 4.1 to 100 %. The complete mortality of adults was not recorded at any observation period in all grain types. However, the minimum survival % (mean ± SE) was recorded 27.2 ± 4.1 in hulled Kernza® at 28 d after infestation of *S. oryzae* adults. The survival (%) of *S. oryzae* infestation in dehulled Kernza® and hard red winter wheat was not significantly different at each observation period ($P > 0.05$). The survival % decreased in all grain types with an increased observation period (Table 3.2).

The model comparisons of *S. oryzae* adult survival responses between hard red winter wheat and hulled Kernza® (F = 43.36; df = 2, 6; P = 0.0003), hulled Kernza® and dehulled Kernza® (F =

32.07; df = 2, 6; $P = 0.0006$) were significant; while the survival response among hard red winter wheat and dehulled Kernza® ($F = 4.62$; df = 2, 6; $P = 0.0610$) is non-significant (Table 3.4).

The decrease in the percentage survival of *S. oryzae* in hulled Kernza® at increasing infestation time was non-linear. The relationship between survival data and observation days in hard red winter wheat ($r^2 = 0.99$), hulled Kernza® ($r^2 = 0.94$), and dehulled Kernza® ($r^2 = 0.98$) was satisfactory as described by fitted regression model (Fig. 3.1). Comparison of non-linear model fitted for *S. oryzae* survival (%) was significantly lower for hulled Kernza® compared to dehulled Kernza® and hard red winter wheat after 7 days ($F = 7.18$; df = 2, 12; $P = 0.009$), 14 ($F = 11.44$; df = 2, 12; $P = 0.002$), 21 ($F = 5.94$; df = 2, 12; $P = 0.016$), and 28 days ($F = 25.13$; df = 2, 12; $P < 0.0001$) of infestation. These results indicated that hulled Kernza® is more protective against *S. oryzae* adults compared to dehulled Kernza® and hard red winter wheat.

3.4.3. Adult progeny production, kernel damage, and weight loss percentage

Two-way ANOVA showed that the adult progeny production of *S. oryzae* between grain types ($F = 149.30$; df = 2, 48; $P < 0.0001$), among observation times ($F = 269.84$; df = 3, 48; $P < 0.0001$), and grain type x observation time interaction ($F = 8.55$; df = 6, 48; $P < 0.0001$) were all significant. The results of one-way ANOVA showed that the mean number of adult progeny production among different grain types after infestation of *S. oryzae* adults was significantly different ($F = 105.40$; df = 11, 48; $P < 0.0001$). The number of *S. oryzae* (mean ± SE) adult progeny production at 28, 35, 42, and 56 d of infestation in hulled Kernza®, dehulled Kernza®, and hard red winter wheat ranged from 0 to 523.6 ± 139.5 adults. After 28 d of infestation with *S. oryzae* adults, the adult progeny of *S. oryzae* were not observed in hulled and dehulled Kernza® while 21.0 ± 5.4 adult progeny was recorded in hard red winter wheat. After 35, 42, and 56 d of infestation with *S. oryzae* adults, hulled and dehulled Kernza® produced a significantly lower number of adult progenies compared to hard red winter wheat. There was an increase in the number of adult progenies produced with an increase in infestation time to *S. oryzae* (Table 3.3).

Two-way ANOVA showed that the kernel damage (%) of *S. oryzae* between grain types ($F = 71.42$; df = 3, 47; $P < 0.0001$), and among observation times ($F = 112.20$; df = 3, 47; $P < 0.0001$) were significant, while the interaction between grain type and observation time ($F = 2.05$; df = 6, 47; $P = 0.0777$) was non-significant. The (mean ± SE) percentage of *S. oryzae* damaged kernel significantly differed in hulled Kernza® compared to hard red winter wheat at each observation period and ranged from 1.0 ± 0.3 to 71.9 ± 3.0% damage kernels ($F = 51.97$; df = 11, 48; $P <.0001$; One-way ANOVA). The percentage of kernel damage increased as the infested period increased in all grain types. At 56 d of the observation period, the mean ± SE of kernel damage (%) was recorded at 31.9 ± 0.4 in hulled Kernza®, 39.2 ± 0.4 in dehulled Kernza®, and 71.9 ± 3.0 in hard red winter wheat. The trend of kernel damage (%) among all grain types at 35 d post infestation was as follows: hulled Kernza® < dehulled Kernza® < hard red winter wheat (Table 3.3).

Two-way ANOVA showed that the weight loss (%) of *S. oryzae* between grain types ($F = 42.58$; df = 2, 48; $P < 0.0001$), as well as among observation times ($F = 60.67$; df = 3, 48; $P < 0.0001$) were significant, while the interaction between grain type and observation time ($F = 1.42$; df = 6, 48; $P = 0.2260$) was non-significant. The percent weight loss resulted from *S. oryzae* infestation among hulled Kernza®, dehulled Kernza®, and hard red winter wheat differed at different post-infestation periods ($F = 25.06$; df = 11, 48; $P <.0001$; one-way ANOVA). The mean ± SE percent weight loss at 28, 35, 42, and 56 d after infestation with *S. oryzae* ranged from 0.5 ± 0.1 to 7.0 ± 0.3 among all grain types. There were no significant differences in weight loss (%) among tested grain types at 42 d post-infestation with *S. oryzae*. After 56 d of the storage period, the mean ± SE weight loss (%) of hulled Kernza® was 3.3 ± 0.3 and statistically similar with dehulled Kernza® (4.2 ± 0.3) but significantly lower compared to hard red winter wheat (7.0 ± 0.3). The weight loss (%) among dehulled Kernza® and hard red winter wheat did not differ significantly at 35 and 42 d storage durations with *S. oryzae*. Interestingly, regardless of grain type, the percent weight loss increased with an increase in the number of *S. oryzae* progeny (Table 3.3).

3.5 Discussion

Kernza® is a perennial, cool season, and intermediate wheat grass that is commercially available as its grains are used in making beer, bread, pasta, and crackers (Broom, 2019; Lanker et al., 2020). According to an estimate, stored grain losses can exceed 20-30% with a cost of $4 billion per year and most of the losses are caused by insects. If left uncontrolled, insect infestation can cause up to 80% losses in a few months (Harein and Meronuck, 1995; Mhlanga et al., 2010; Pimentel, 2002). Stored grains are infested by variety of insect pests, e.g., *Rhyzopertha dominica* (F.), *Sitotroga cerealella* (Olivier), *Sitophilus zeamais* Motschulsky, and *Tribolium castaneum* (Herbst), but the rice weevil, *S. oryzae* is the most important pest. On an average, 24-40% of grain loss is caused by rice weevil within six months and the damage may reach up to 50% (Bell and Posamentier, 1998; Dal Bello et al., 2000; Helbig, 1995; Ladang et al., 2008). The use of resistant grain varieties for stored grain pests can be effective and economical as compared to chemical control, as this would not be harmful to consumers and environment. Moreover, it would neither cause resistance in pests nor require any additional cost (Ratnadass et al., 1994; Zakka et al., 2015). Certain characteristics of grains include the tightness of the hull, the hardness of kernel, and chemical constituents of the kernel have been shown to impart a certain degree of resistance against insects of stored grains (Breese, 1960; Chanbang et al., 2008;

Cogburn, 1974; Cogburn et al., 1983; Juliano, 1981). The present study investigated the resistance of hulled Kernza® grains in comparison to hard red winter wheat and dehulled Kernza®, against the rice weevil. The results showed significant differences in moisture content, survival rate, adult progeny production, kernel damage, and weight loss % among the tested grain types when infested with *S. oryzae* adults.

The moisture content of grains is one of the most important factor of insect infestation in stored grains (Fields and Korunic, 2000; Likhayo et al., 2018). The current study found significant differences in moisture content among hard red winter wheat, hulled Kernza, and dehulled Kernza® following infestation by *S. oryzae*. Over a period of 56 days, hard red winter wheat exhibited a continuous increase in moisture content from 13.7% to 21%. A study by Christensen and Hodson (1960) also found similar trend where moisture content of wheat grains infested by granary weevils increased from 13% to 16%. The increase in moisture support previous researches indicating that moisture content in stored grains can rise due to metabolic activities of insects and respiration of the grains themselves (Bartosik et al., 2008; Bartosik et al., 2012; Navarro and Calderon, 1982). This increase in moisture content of grain is important because it can make the environment more favorable for *S. oryzae* and other pests and potentially leading to greater spoilage and loss (Fields and Korunic, 2000; Neethirajan et al., 2007). In contrast, hulled and dehulled Kernza® showed significantly lower moisture content compared to hard red winter wheat, and the lowest recorded moisture content in hulled Kernza® was 12.2% after 21 days of storage which indicated lowest infestation. Similar studies on rice also show that hull can preclude entry of rice weevil and other pests (Antunes et al., 2016; Cogburn, 1974). The differential moisture content in hulled versus dehulled Kernza® indicated that the presence of the hull may act as a barrier for infestation (Cogburn, 1974). The lower moisture content in grains can impede the development and survival of stored grain pests (Fields and Korunic, 2000; Flinn et al., 2003; Khan et al., 2016; Neethirajan et al., 2007; Van Alfen, 2014).

The different survival rates of *S. oryzae* adults among different grain types highlights the impact of hull presence on the development and survival of the weevil. Hulled Kernza® consistently demonstrated lower survival rates of *S. oryzae* compared to dehulled Kernza® and hard red winter wheat across different infestation periods. The lowest survival rate observed was 27.2% in hulled Kernza® at 28 days post-infestation. This supports the finding by Antunes et al. (2016) who showed that development of *S. zeamais* was affected by hulled variety of rice, and similar trend was observed in rice weevil in another study by Boles and Pomeranz (1979). This observation is in line with the general understanding that physical characteristics of grains, such as the presence of hulls, can influence pest survivability (Fields and Muir, 2018; Mason and McDonough, 2012; Throne, 1994). The hull may provide a less favorable environment by limiting access to nutrients and protection against desiccation. There was no significant difference in survival rate between dehulled Kernza® and hard red winter wheat at any observation period indicating that the removal of the hull might reduce the protection of grains as seen in hulled Kernza®.

The number of adult progenies produced by adults of *S. oryzae* on different types of grains were significantly different ($P < 0.0001$). After 28 days of infestation with *S. oryzae* adults, complete suppression of progeny was observed in both hulled and dehulled Kernza®, whereas the adult progeny of *S. oryzae* was observed in hard red winter wheat (21.0 ± 5.4 adults). Similar to our study, Khamis et al. (2011) also reported emergence of *S. oryzae* adult progeny after 26 days of infestation in hard red winter wheat. The significantly lower progeny numbers in Kernza® suggest that it is a less suitable host for *S. oryzae*, potentially due to its lower moisture content and possibly less favorable nutritional profile. A similar study on rice weevil indicated that more number of rice weevil were produced on dehulled rice (Boles and Pomeranz, 1979).

Kernel damage and weight loss followed similar trends. The hard red winter wheat showed the highest levels of damage and weight loss after 56 days. The kernel damage in hulled Kernza® was significantly lower than in dehulled Kernza® and hard red winter wheat, which is consistent with the findings that showed more damage and grain weight loss in dehulled variety of rice (Khaliq et al., 2013; Sharma and James, 2023). This indicates that physical barriers such as hulls can reduce pest damage. The weight loss percentages also differed significantly among the different types of grains at the 56 day interval ($P < 0.0001$). Hulled Kernza® showed the lowest weight loss, this highlights that Kernza® with the presence of hull has higher level of resistance against rice weevil than other varieties. The current finding support study by Sharma and James (2023) which noted that grains with protective hulls or coatings experienced lower overall weight loss caused by rice weevil infestations.

The findings of the current study suggest that Kernza®, especially the hulled Kernza®, is more resistant to rice weevil than the widely cultivated hard red winter wheat. Hulled Kernza® grains showed less infestation, low moisture content, less survival rate and the fewer adult progeny production, as well as low kernel damage and weight loss which suggest that this less known perennial grain might be a better option to replace traditional wheat. The Kernza® grains showed some level of resistance against the rice weevil due to the presence of hull and possible metabolites found on the grains. However, additional studies are required to determine precisely why such resistance was observed. The future research could involve the identification and characterization of the other physical and chemical factors like defensive metabolites and composition of protective hull that is responsible for the decreased survival, reproduction, and feeding activities of *S. oryzae* in Kernza® grains. Additionally, it is essential to consider the resistance of Kernza® grains against other stored product insect pests because different insects may show different levels of resistance to the chemical and physical defense mechanisms of grains. Further research involving other stored product insect pests, such as confused flour beetle (*T. confusum*) and the Angoumois grain moth (*S. cerealella*) should be conducted to get a broader perspective regarding the pest resistance of this new grain, Kernza®.

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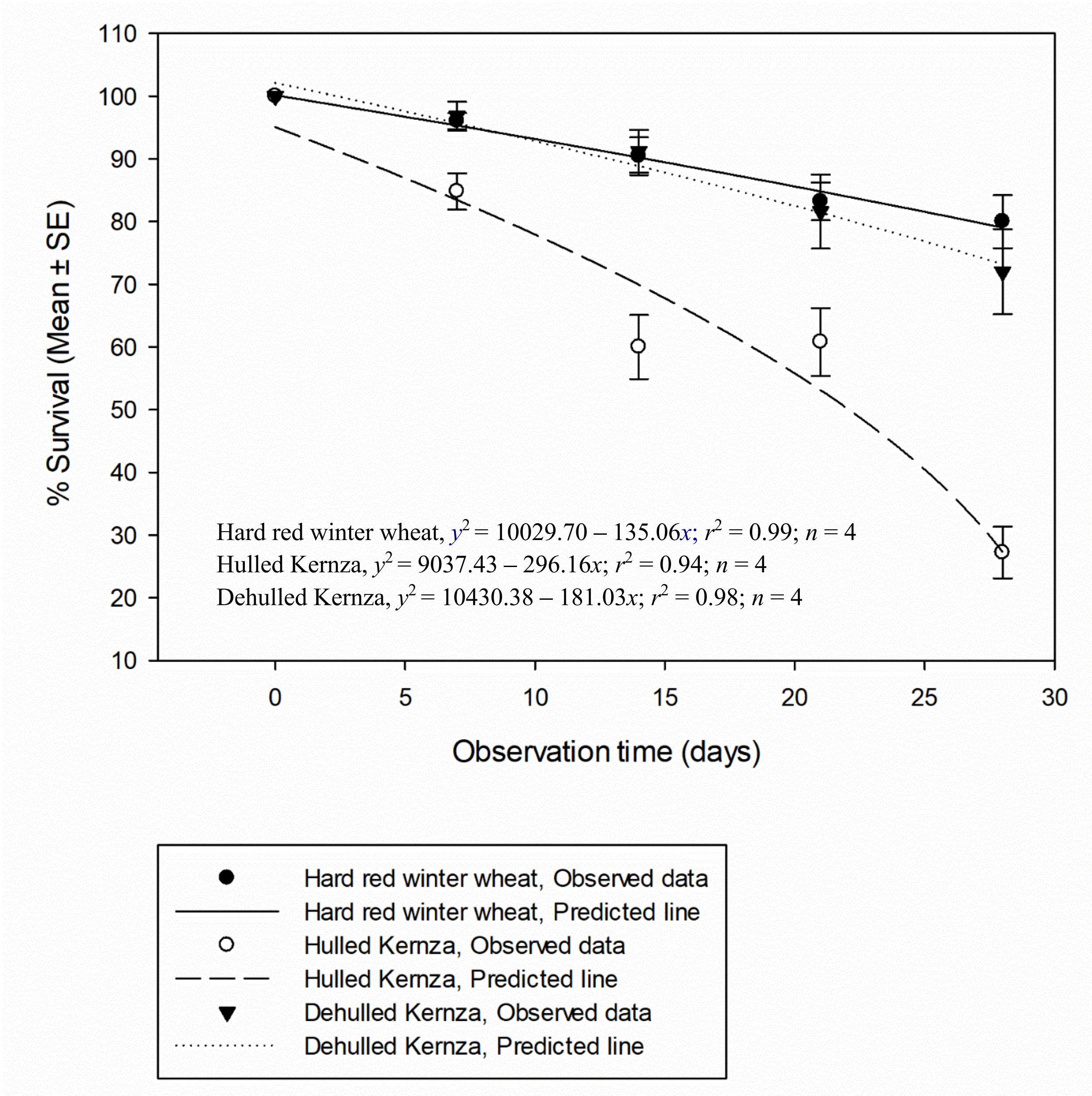

Figure 3.1. Percentage survival of *S. oryzae* adults after 0, 7, 14, 21, and 28 days of infestation to hulled Kernza®, dehulled Kernza® and hard red winter wheat.

Table 3.1 Moisture content of different grain types after 0, 7, 14, 21, 28, 35, 42, and 56 days of infestation to *S. oryzae* adults.

Grain type	Moisture (% mean ± SE) on day:[a, b]							
	0	7	14	21	28	35	42	56
Hard red winter wheat	14.5 ± 0.3 a	13.7 ± 0.1 a	14.3 ± 0.1 a	15.2 ± 0.3 a	17.0 ± 0.5 a	17.0 ± 1.0 a	19.0 ± 1.6 a	21.0 ± 2.5 a
Hulled Kernza®	13.7 ± 0.1 b	12.8 ± 0.1 b	12.3 ± 0.1 c	12.2 ± 0.1 b	13.3 ± 0.2 b	13.2 ± 0.1 b	15.8 ± 1.2 ab	14.9 ± 0.2 b
Dehulled Kernza®	14.1 ± 0.1 ab	12.9 ± 0.1 b	12.9 ± 0.1 b	12.7 ± 0.2 b	14.1 ± 0.2 b	14.7 ± 0.2 b	13.4 ± 0.2 b	13.8 ± 0.1 b
F-value[c]	5.13	20.37	93.75	62.88	31.09	10.50	5.81	7.98
P-value[*]	0.0245	0.0001	< 0.0001	< 0.0001	< 0.0001	0.0023	0.0172	0.0062

[a]Each mean is based on $n = 5$ replicates.

[b]At each observation time, means among grain types followed by different letters are significantly different ($P < 0.05$; by REGWQ test).

[c]At each observation time, the df for the one-way ANOVA *F*-test is 2, 12.

[*]Significant ($P < 0.05$).

Table 3.2 Survival (%) of *S. oryzae* adults after 0, 7, 14, 21, and 28 days of infestation in hulled Kernza®, dehulled Kernza® and hard red winter wheat.

Grain type	Survival (% mean ± SE) on day:[a, b]				
	0	7	14	21	28
Hard red winter wheat	100.0 ± 0.0	96.0 ± 1.3 a	90.4 ± 3.0 a	83.2 ± 3.0 a	80.0 ± 4.2 a
Hulled Kernza®	100.0 ± 0.0	84.8 ± 2.9 b	60.0 ± 5.1 b	60.8 ± 5.4 b	27.2 ± 4.1 b
Dehulled Kernza®	100.0 ± 0.0	96.8 ± 2.3 a	91.2 ± 3.4 a	81.6 ± 5.9 a	72.0 ± 6.8 a
F-value[c]		7.18	11.44	5.94	25.13
P-value[*]		0.009	0.002	0.016	< 0.0001

[a]Each mean is based on $n = 5$ replicates.

[b]At each observation time, means among grain types followed by different letters are significantly different ($P < 0.05$; by REGWQ test).

[c]At each observation time, the df for the one-way ANOVA *F*-test is 2, 12.

[*]Significant ($P < 0.05$).

Table 3.3 Number of adult progeny produced, percent kernel damage and weight loss (Mean ± SE) at 28, 35, 42 and 56 days of infestation to *S. oryzae* in hulled Kernza®, dehulled Kernza® and hard red winter wheat.

Grain type	Observation time (days)	Progeny[a, b]	% Kernel damage[a, b]	% Weight loss[a, b]
Hard red winter wheat	28	21.0 ± 5.4 d	17.8 ± 1.7 ef	2.2 ± 0.1 dc
	35	307.8 ± 34.7 a	36.0 ± 3.4 bc	3.8 ± 0.1 bc
	42	472.4 ± 99.5 a	49.3 ± 8.8 b	4.5 ± 1.1 b
	56	523.6 ± 139.5 a	71.9 ± 3.0 a	7.0 ± 0.3 a
Hulled Kernza®	28	0.0 ± 0.0 e	1.0 ± 0.3 h	0.5 ± 0.1 f
	35	10.8 ± 2.2 d	7.8 ± 1.0 g	1.4 ± 0.2 de
	42	69.4 ± 3.7 c	22.2 ± 1.5 de	2.9 ± 0.3 bc
	56	124.6 ± 17.1 bc	31.9 ± 0.4 cd	3.3 ± 0.3 bc
Dehulled Kernza®	28	0.0 ± 0.0 e	8.9 ± 0.6 fg	0.8 ± 0.2 ef
	35	15.8 ± 4.3 d	17.8 ± 1.1 ef	2.3 ± 0.1 cd
	42	105.4 ± 11.4 bc	28.3 ± 1.7 cde	3.4 ± 0.3 bc
	56	190.6 ± 22.4 ab	39.2 ± 0.4 bc	4.2 ± 0.3 b
F-value[c]		105.40	51.97	25.06
P-value[*]		< 0.0001	< 0.0001	< 0.0001

[a]Each mean is based on $n = 5$ replicates.

[b]Means among grain types and observation times followed by different letters are significantly different ($P < 0.05$; by REGWQ test).

[c]At each observation time, the df for the one-way ANOVA F-test is 11, 48.

[*]Significant ($P < 0.05$).

Table 3.4 Pairwise comparison of non-linear models fitted to adult survival percentage of *S. oryzae* over time between three tested grain types.

Comparison	*F*-value	df	*P*-value
Hard red winter wheat vs Hulled Kernza®	43.36	2, 6	0.0003*
Hard red winter wheat vs Dehulled Kernza®	4.62	2, 6	0.0610
Hulled Kernza® vs Dehulled Kernza®	32.07	2, 6	0.0006*

*Significant ($P < 0.05$).