Integrated post-harvest management of stored product insects in sorghum: Effects of kernel properties, temperature, and grain protectant efficacy

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Abstract

Sorghum bicolor, commonly known as sorghum, is one of the five most economically important cereal grains in the world. In the United States, it is used extensively for livestock feed and ethanol production, while globally, it serves as a staple food for >500 million people. Its value lies in part in its exceptional drought tolerance, enabling it to perform in regions where other cereals often fail. This resilience is largely due to sorghum's genetic flexibility, which allows breeders to improve both agronomic performance and grain quality. Through targeted breeding, traits such as drought resistance, kernel hardness, protein content, starch composition, and kernel size can be selected, influencing sorghum's suitability for various end uses. As global temperatures rise and water availability becomes increasingly scarce, sorghum's role as a climate-resilient crop is expected to grow. However, post-harvest storage remains a critical challenge as arthropod pests reduce grain quality and quantity through direct feeding and contamination with frass, exuviae, webbing, and associated microbial growth. Key pest species, *Rhyzopertha dominica*, *Sitophilus oryzae*, *Plodia interpunctella*, and *Prostephanus truncatus*, cause substantial annual losses worldwide. To mitigate these losses during bulk storage, strategies such as sanitation, grain aeration, fumigation and the use of grain protectants are commonly employed. While the efficacy of grain protectants has been well studied in other cereal crops like wheat, rice, and maize, relatively little is known about their performance on sorghum. Integrated Pest Management (IPM) emphasizes the importance of understanding how grain traits, storage conditions, and pest biology interact to influence both stored product susceptibility and grain protectant efficacy. This research applied IPM principles to evaluate post-harvest protection of stored sorghum. First, we assessed the susceptibility of sorghum varieties differing in kernel hardness and protein content to infestation by *R. dominica* and *S. oryzae*. Results showed that both kernel hardness and protein content significantly influenced susceptibility to *S. oryzae*, while only protein content affected susceptibility to *R. dominica*. Second, we examined how storage temperature (22, 27, and 32°C) impacted the efficacy of four commercially available grain protectants (Diacon® IGR, EverGreen®, Gravista®, and Sensat™) against *R. dominica*, *S. oryzae*, and *P. interpunctella*, as well as its influence on pest development. We found that temperature had a significant effect on the grain protectant efficacy, with some formulations exhibiting a decrease or increase in their ability to mitigate infestation

depending on environmental conditions. For instance, we found that insecticide formulations containing pyrethrins or pyrethroids performed better than cooler temperatures in terms of adult mortality and mitigating progeny and further damage, whereas there was an observed decrease in efficacy when temperatures were higher. The opposite trend was observed for the active ingredient spinosad, which saw a higher rate of efficacy at warmer temperatures and less efficacy at cooler temperatures. Overall, species development was faster at higher temperatures regardless of treatment. Third, we evaluated the long-term efficacy of the same grain protectants over a 28-week simulated storage period against *R. dominica* and *S. oryzae*. The grain protectant performance remained consistent over time, with high rates of adult mortality, low progeny, and lower levels of product damage observed for all protectants against *R. dominica* during the course of the experiment. However, the declining moisture content of the stored sorghum over time was a major contributing factor affecting infestation levels. Furthermore, *R. dominica* showed a higher degree of susceptibility to treated sorghum compared to *S. oryzae*. The results of these three studies support the need for the development of a sorghum-specific IPM strategies. The factors of grain varietal traits, grain protectant formulation, and storage environments all play a pivotal role in sorghum's susceptibility to infestation of four primary species that infest bulk grain. Furthermore, this research highlights the importance of understanding the target-species biology and ecology when employing IPM strategies. Ultimately, this research lays the groundwork for understanding the challenges and opportunities for targeted post-harvest IPM practices in the United States and internationally.

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Dedication

I dedicate this thesis and the work that I have done during my time at Kansas State to my loving family and friends. Thank you to my parents for your support, to my friends for always being there, and to my dog, Burton, who is always happy to greet me when I get home.

Chapter 1 - Literature Review

1.1. Introduction

Sorghum bicolor (L., Moench), commonly known as sorghum, great millet, or milo, is one of the top five most important cereal grains globally (Habyarimana et al., 2020). Sorghum's ability to withstand harsh conditions such as drought, heat, and poor soil positions it as a resilient crop that is increasingly recognized for its role in food security, especially in regions facing the challenges of climate change (Abreha et al., 2021; Zheng et al., 2023). In Western countries, it is primarily valued for alternative energy sources and livestock feed, with limited recognition for its potential as a human food source (Umakanth et al., 2018; Khalifa and Elathir, 2023). However, sorghum is an essential food for over 500 million people worldwide, particularly in parts of Africa, Asia, and Latin America, where it is used as a staple in various culinary traditions (Zheng et al., 2023; Mbulwe and Ajayi, 2020). Because of these reasons, sorghum's rising importance globally is reflected in its growing economic impact, with global production valued at around $22.111 billion USD annually as of 2023 (Grand View Research, 2024).

Sorghum is an exceptionally versatile and nutritionally valuable crop, offering a diverse range of applications across multiple sectors, including food production, biofuel generation, and livestock feed (Zheng et al., 2023; Taylor et al., 2006). This versatility is driven by its extensive genetic variation, which influences key agronomic traits such as starch and protein content, allowing breeders to develop specialized cultivars tailored for specific uses (Zheng et al., 2024). For instance, high-starch varieties are ideal for biofuel production, while protein-rich strains serve as an excellent feed source for livestock (Umakanth et al., 2018). Additionally, sorghum's adaptability to diverse climates and growing conditions has contributed to its widespread cultivation across the globe (Abreha et al., 2021; Mundia et al., 2019). Beyond its agricultural applications, sorghum is also a highly nutritious grain that provides numerous health benefits (Khalifa and Eltahir, 2023). Naturally gluten-free, it serves as a safe and nutritious option for individuals with celiac disease or gluten sensitivities (Taylor et al., 2006). It is rich in dietary fiber, promoting digestive health, and contains essential B-vitamins that support energy metabolism (Abreha et al., 2021). Additionally, sorghum is packed with antioxidants, including polyphenols, which help combat oxidative stress and inflammation (Abreha et al., 2021). This

combination of genetic diversity, adaptability, and nutritional value makes sorghum a vital crop for both food security and sustainable agriculture.

Despite the positives related to sorghum, research on this grain lags behind those more widely cultivated, particularly in areas like pest management and post-harvest loss prevention. As interest in sorghum as a food ingredient grows, it is crucial to address these potential post-harvest challenges that could lead to substantial losses. Preventative measures that have shown success in other stable cereal crops must be implemented and/or improved to protect the grain post-harvest, where high amounts of loss due to pest infestations could potentially occur. Effective Integrated Pest Management (IPM) strategies are crucial for safeguarding sorghum grain from stored product infestation throughout the post-harvest supply chain, ensuring its suitability for human consumption (Hagstrum and Anthanassiou, 2019; Athanassiou et al., 2020). IPM is a holistic and science-based approach that integrates multiple control methods, including biological, cultural, mechanical, and chemical strategies, all of which are informed by a deep understanding of the pests involved, their behaviors, their life cycles, and their potential routes for building tolerances and resistance (Hagstrum and Anthanassiou, 2019). By leveraging knowledge of these organisms, IPM enables a more targeted and efficient approach to pest management and control (Morrison et al., 2019). Instead of relying solely on broad-spectrum chemical treatments, IPM emphasizes treatments that are more tailored to the specific pest species, the environmental conditions, and the commodity being treated. In order to bolster IPM strategies and related knowledge specific to sorghum, there is a need to research IPM strategies under a multitude of scenarios to provide recommendation and guidance for pest management professionals to ensure the quality and safety of the grain is maintained throughout the post-harvest supply chain. Despite decades of research on the use of pesticides to prevent infestation on stored grains, there is a lack of research on the use of grain protectants on sorghum as a preventative and control measure. Additional research is needed to understand how pesticide treatments perform on sorghum grain, particularly against the diverse range of stored product pests such as *Rhyzopertha dominica* (F.), lesser grain borer, *Sitophilus* species, *Plodia interpunctella* (Hübner), Indian meal moth, and *Prostephanus truncatus* (Horn), larger grain borer. Additionally, the susceptibility of different sorghum varieties to pest infestation has not been well established, leaving a knowledge gap that hinders the development of effective post-harvest management strategies.

While research on pesticide efficacy in other cereal grains like maize, wheat, and rice can offer some insights and clues, these findings cannot be directly applied to sorghum without further study. Sorghum's unique properties and potential pest interactions warrant more focused research to improve pest control practices for this vital crop. To improve pest management, it is essential to gain a better understanding of the pest organisms at play and the conditions that foster their development and susceptibility to treatments. The aim of this literature review is to address sorghum as an upcoming viable option in the face of climate change when compared to other cereal grains such as maize, wheat, and rice. Furthermore, this review will analyze the current state of IPM practices, particularly the usage of post-harvest grain protectants, while also addressing some of the current challenges and unknowns with current IPM tactics and research.

1. 2. Sorghum grain

1. 2. 1. Sorghum as a field crop

Sorghum is a highly adaptable cereal crop that thrives in challenging environmental conditions, making it a crucial source crop for many regions affected by heat and drought stress. Reaching an average height of around two meters, sorghum flourishes in warm climates with an optimal growth range between 24 – 29°C (OECD, 2017). This heat tolerance allows it to be cultivated in regions where staple crops like wheat and rice often struggle due to rising global temperatures and prolonged heatwaves (Behera et al., 2022). As climate change continues to drive unpredictable weather patterns, sorghum's ability to withstand extreme conditions makes it an increasingly valuable agricultural resource.

A key feature that enables sorghum to survive in arid and semi-arid regions is its deep and extensive root system. These roots can grow about 2-3 cm a day, eventually being able to extract moisture from the ground 1.6 m away from the plant. This root allows the plant to extract moisture from deeper soil layers, providing a significant advantage during prolonged droughts when surface water is scarce. Research suggests that drought-tolerant sorghum genotypes often have a greater number of seminal roots and larger vessel diameters in both nodal and seminal roots, which enhance their ability to uptake water efficiently (Routley et al., 2003). Additionally, sorghum exhibits a waxy cuticle on its leaves that minimizes water loss through transpiration. This trait, combined with its ability to regulate stomatal conductance, helps the plant conserve moisture while maintaining essential metabolic processes (Ali et al., 2023).

Another vital adaptation that enhances sorghum's resilience is its C4 photosynthetic pathway. Unlike C3 plants such as wheat and rice, C4 plants like sorghum and maize are highly efficient in carbon fixation under high temperatures and limited water availability. This photosynthetic mechanism reduces photorespiration and improves water-use efficiency, allowing sorghum to maintain productivity even in drought-prone areas (Zheng et al., 2024; Lisar et al., 2012; Behera et al., 2022). Additionally, some sorghum varieties exhibit an important trait known as "stay-green," which enables its leaves to remain photosynthetically active even under drought stress. This delayed leaf senescence ensures continued grain filling and overall improved yield stability under water-deficient conditions (Borrell et al., 2003; Kamal et al., 2021).

Beyond its structural and physiological adaptations, sorghum also employs biochemical mechanisms to tolerate drought and heat stress. The accumulation of osmoprotectants such as proline and glycine betaine help maintain cell turgor pressure, stabilizing proteins and cellular membranes against dehydration damage (Sivaramakrishnan et al.,1988). Furthermore, research suggests that sorghum regulates its transpiration rates efficiently, making it capable of producing up to 40% more biomass per unit of water compared to maize under non-ideal conditions (Lisar et al., 2012).

Even though it has many advantages, sorghum does face certain limitations that hinder its widespread adoption compared to other cereal grains. One such challenge is its relatively lower yield potential under optimal conditions. While sorghum excels in extreme climates, its grain size and number of grains per plant are typically smaller compared to maize, leading to lower biomass and grain yield per hectare (Craufurd and Peacock, 1993). Additionally, sorghum has a longer growing cycle compared to crops like wheat and some maize varieties, with a maturity period ranging from 90 to 120 d (Bayer Crop Science, 2021). This extended growth duration can be disadvantageous in regions with short growing seasons or unpredictable climate patterns, as early frost or late-season drought may prevent the crop from reaching full maturity (OECD, 2017). Another notable limitation is sorghum's susceptibility to excessive moisture. While the crop is highly drought-resistant, it struggles under prolonged wet conditions, as waterlogging can damage its root system and stunt overall growth. Unlike rice, which thrives in flooded fields, sorghum requires well-drained soil and is not well-suited for regions with heavy or persistent rainfall (Behera et al., 2022).

Despite these challenges, sorghum remains one of the most resilient cereal crops available, offering a crucial solution for food security in drought-prone and high-temperature regions. With ongoing advancements in breeding programs, efforts are being made to develop improved sorghum cultivars with higher yield potential, enhanced drought resistance, and greater adaptability to diverse environmental conditions (Zheng et al., 2024). As climate change continues to pose a significant threat to global agriculture, promoting sorghum cultivation in water-scarce regions could play a pivotal role in ensuring long-term agricultural sustainability and food production stability.

1. 2. 2. Sorghum's versatility

Sorghum is a highly adaptable crop, offering farmers and industries the flexibility to select specific traits that best align with their agricultural and commercial needs (Reddy et al., 2009; Cuevas et al., 2023). Advances in breeding techniques have further refined this adaptability, producing hybrids that enhance yield, nutritional quality, and suitability for various applications (Tuinstra et al., 1998). These breeding efforts focus on key attributes such as grain color, tannin levels, protein and starch composition, and pericarp texture thickness. This ability to tailor traits allows producers to meet the demands of diverse markets, including food production, brewing, biofuels, and biodegradable materials (Taylor et al., 2006).

Unlike maize, wheat, and rice, sorghum thrives in arid and nutrient-poor conditions, making it a crucial crop in regions with limited water resources (Behera et al., 2022). While maize and wheat breeding programs often prioritize yield maximization under optimal growing conditions, sorghum research has concentrated on enhancing resilience, grain composition, and nutritional value (Reddy et al., 2009; Xiang et al., 2019). Sorghum grain's chemical makeup, including starch, protein, lipids, and phenolic compounds plays a crucial role in its functionality across various industries (Xiang et al., 2019).

The color of sorghum grains, which range from white and yellow to red and brown, affecting both nutritional value and industrial applications (Awika, 2011; Xiang et al., 2019). Tannins, a group of polyphenolic compounds found in certain sorghum varieties, those with a pigmented testa layer, provide antioxidant properties and can sometimes even act as a means of natural pest resistance (Banerjee and Roychoudhury, 2022). When consumed, tannins within the grain can also inhibit protein digestion by binding to proteins (Macáková et al., 2014). Non-tannin (those without a pigmented testa layer) are preferred for human consumption and animal

feed due to their enhanced nutrient availability (Stefoska-Needham et al., 2024), whereas tannin sorghum is sought after for brewing, bio-based materials, and specialty food products (Pereira and Hawkes, 2022). Modern analytical methods, such as attenuated total reflectance, Fourier transform infrared spectroscopy (ATR-FTIR) spectroscopy, have improved the efficiency of distinguishing between tannin and non-tannin varieties, aiding in better grain selection for specific uses (Lin et al., 2020).

Grains with harder pericarps are better suited for milling, and long-term storage due to their durability and resistance shipping damage and according to some studies done on other cereal grains, it could be less susceptible to pests (Awika et al., 2005). Conversely, softer pericarps are more desirable for direct food processing and livestock feed due to their higher digestibility (Taylor et al., 2006). However, softer grains pose challenges in post-harvest handling, as they are more susceptible to breakage, thus necessitating careful management (Dlamini et al., 2007).

Sorghum's protein content varies between 6% and 20%, influenced by both genetic and environmental factors (Cuevas et al., 2023). Sorghum's primary storage proteins, known as kafirins, include α-, β-, γ-, and δ-kafirins, significantly impact digestibility (Stefoska-Needham et al., 2024). γ-kafirins contribute to protein cross-linking, reducing digestibility in wet-cooked sorghum (Maclean et al., 1981). Traditionally, sorghum has been regarded as less digestible compared to other grains, but recent advancements in processing techniques such as extrusion, fermentation, and enzymatic treatments have enhanced protein availability (Stefoska-Needham et al., 2016; Taylor and Duodu, 2015). Non-tannin sorghum is especially beneficial in animal feed due to its higher digestible protein and energy content (Herstad, 1979). Additionally, high-protein sorghum hybrids are increasingly used in gluten-free human food products, such as flour, cereals, and baked goods (Awika, 2011).

High-protein sorghum strains generally exhibit lower starch levels, which can impact their effectiveness in ethanol production and processed foods (Wu et al., 2007). Sorghum starch consists of amylose and amylopectin, with varying ratios affecting digestibility and functionality (Haziman et al., 2025). While sorghum starch in grain or flour is less digestible than that in maize flour, this characteristic is beneficial in low-glycemic food products and modified starch applications (Haziman et al., 2025). Consequently, selecting the right sorghum variety based on market needs is essential for maximizing its economic and functional potential.

Historically, sorghum has been a staple food in Africa and Asia, and its role in global food systems continues to grow (Awika, 2011). Available as whole grain, flour, or processed products, sorghum is especially valued for its gluten-free properties, making it an excellent option for individuals with celiac disease or gluten intolerance (Frankowski et al., 2022). In baking, sorghum flour is commonly blended with other flours to improve texture and structure in bread, cakes, and cookies (Apostol et al., 2020). However, due to its unique protein composition, sorghum-based baked goods often require additional ingredients to enhance functionality (Taylor and Duodu, 2015). Beyond its use in food, sorghum starch is a key component in brewing, serving as a major source of malts for beer production, especially in regions where barley is less accessible (Dlamini et al., 2007).

Sorghum is also a significant component of livestock feed, particularly for cattle, poultry, and swine, particularly non-tannin varieties (Ronda et al., 2019). High-protein sorghum hybrids, bred for improved kafirin composition, offer enhanced protein availability, making them ideal for poultry and swine diets (Herstad, 1979). Additionally, sorghum distillers' dried grains with solubles (DDGS), a byproduct of ethanol production, serves as a valuable feed ingredient, contributing additional fiber and protein to enhance overall feed quality (Taylor et al., 2006). It offers similar digestible energy and protein levels to maize while providing the added advantage of greater resilience in suboptimal growing conditions (Duodo et al., 2002).

Beyond its applications in food and feed, sorghum is gaining recognition as a key resource in biofuel production (Audilakshmi and Swarnalatha, 2019). Certain sorghum varieties, such as sweet sorghum, are particularly advantageous for biofuel production due to their high fermentable sugar content in both grains and stalk juice (Kasegn et al., 2023). Additionally, high-starch sorghum varieties are optimized for ethanol yield, making them a preferred choice for large-scale biofuel operations (Taylor et al., 2006). Research into enhancing starch composition and enzymatic conversion efficiency further supports sorghum’s growing role as a sustainable bioenergy crop (Zheng et al., 2023). With its high starch content, sorghum is an efficient grain for ethanol, providing a renewable energy source that reduces reliance on fossil fuels (Wang et al., 2008). Additionally, there is increasing interest in sorghum being used in the production of bioplastics and eco-friendly coatings, showcasing its potential in sustainable industrial applications (Taylor et al., 2006). As industries seek renewable alternatives, sorghum’s role in

the bioeconomy continues to expand, reinforcing its importance as a versatile and economically valuable crop.

Sorghum remains a vital crop worldwide, with Africa and Asia leading in both production and consumption. In 2023, Africa produced 58 million metric tons, with the demand slightly exceeded supply at 58.35 million metric tons (FAO, 2017). Meanwhile, Asia produced 15.07 million metric tons but remains heavily dependent on imports, with consumption soaring to 28.38 million metric tons (FAO, 2017). India continues to cultivate sorghum as a dietary staple, while China primarily uses it for animal feed (OECD, 2017). East Asia alone imported 13.21 million metric tons, highlighting the region's reliance on external sources (FAO, 2017). Pakistan has also expanded cultivation in dryland areas to support both food and fodder needs (OECD, 2017).

North America, particularly the United States (U.S.), is a major force in global sorghum production. The region produced 20.46 million metric tons in 2023, with consumption at 15.78 million metric tons (FAO, 2017). The U.S. sorghum belt stretches across Kansas, Texas, Oklahoma, and Nebraska, and Kansas is the number one producer of sorghum accounting for nearly 60% of the national production (USDA, 2023). The U.S. market is valued at over $1.5 billion USD annually, driven by both domestic demand and exports (Dalton and Hodjo, 2020).

In 2023, global sorghum production reached 118.9 million metric tons, nearly matching total consumption at 118.5 million metric tons (FAO, 2023). As demand for a secure resource crop in the face of increasing global temperatures and drought occurrences grows, sorghum remains a resilient and valuable crop commodity (Behera et al., 2022). However, climate change presents new challenges, particularly in major growing regions like the sorghum belt, where increasing temperatures and worsening water scarcity threaten the viability of many crops. With projections indicating more frequent heatwaves and reduced rainfall, sorghum's natural drought tolerance and heat resistance make it increasingly critical (Behera et al., 2022).

1. 2. 3. Harvest and post-harvest storage

Sorghum is typically harvested in late summer or early autumn, depending on the growing region, with the harvest period typically occurring between August and October (Day et al., 2011). The crop is often ready to harvest once the grain reaches physiological maturity, with the kernels firming up and drying down to a moisture level that is safe for storage, typically around 13−15% (Day et al., 2011).

Sorghum, like other grains, is stored in large silos or bins that regulate temperature, humidity, and airflow to maintain its quality and prevent deterioration during storage (Day et al., 2011). The ideal storage conditions are between 10 – 15°C with 60–70% relative humidity, which prevents moisture absorption that could lead to mold or spoilage. Aeration systems within the silos ensure consistent airflow, preventing heat buildup and hot spots that could degrade the grain and promote microbial and fungal growth (Ziegler et al., 2021). Regular monitoring of temperature and humidity throughout the grain mass is essential to maintain optimal conditions, as fluctuations can affect the grain's quality, texture, color, and nutritional value (Ziegler et al., 2021). Proper management of these factors ensures sorghum remains in good condition for processing or sale.

1.3 Common stored product pests

Stored product pest insects are a significant concern during the post-harvest storage of sorghum (Kalaisekar et al., 2017). These pests are attracted to cereal grains that are not properly sealed or that have higher moisture content, and they cause damage to the grain during storage (Hagstrum et al., 2012). Infestations during post-harvest storage can lead to both quantitative loss of grain and qualitative degradation of the grain as pests consume the grain and create conditions that promote further spoilage (Kalaisekar et al., 2017). Effective pest management, such as ensuring proper sealing of storage facilities and monitoring signs of infestation via traps or lures, is crucial to reduce the risk of pest-related damage and maintain the overall quality of the stored sorghum. Despite this, the study of stored product pests on sorghum remains underexplored compared to other staple grains like maize, wheat, or rice. Given sorghum's growing significance in global markets, it is essential to gain a deeper understanding of pest behavior, infestation dynamics, and their specific impacts on sorghum storage. Tailored pest management strategies are needed to address the unique characteristics of sorghum to ensure more efficient protection of the crop during storage and transport.

Pest identification plays a vital role in IPM since different pest species require different strategies, and understanding which species are present helps ensure that interventions are both effective and efficient. Failure to correctly identify pests may lead to ineffective control measures, resulting in increased damage to stored products and the need to retreat (Hagstrum et al., 2012). Once the species are identified, treatment can begin in a targeted manner, which is fundamental to the success of an IPM strategy.

Different stored product pests have unique life cycles, behaviors, and feeding patterns, all of which influence the severity of damage they cause and the best approach to control them. For example, larvae of internal feeders such as *R. dominica* and *S. oryzae* bore into individual kernels and develop inside the grain kernel, thus weakening the internal structure of the kernel and making infestations difficult to detect and control until significant damage has already occurred (Hagstrum et al., 1999; Campbell and Arbogast, 2004). These insects are classified as primary feeders since they consume whole kernels of grain and develop and feed inside the kernel. Eggs of these species are either laid directly into the kernel or outside of the kernel whereby the newly hatched neonate larvae will bore into the kernel. The larvae will continue to feed, progressing throughout their pupal stage before finally emerging as an adult by chewing their way out of the grain kernel and leaving behind a clear exit hole (Hagstrum et al., 1999). Because the bulk of their lifecycle is spent developing within kernels, they can often emerge as adults in large numbers, posing a significant risk in the shipping and receiving of large grain shipments, as infestations may be well established before they are identified, potentially leading to widespread contamination (Hagstrum et al., 1999).

In contrast to internal feeders, external feeders primarily consume broken kernels, processed foods, and grain dust, often thriving in areas where mechanical damage has already compromised the grain (Hagstrum et al., 1999). These insects, classified as secondary feeders and develop outside of the grain kernel (Hagstrum et al., 2012). While secondary feeder infestations are generally easier to detect than those of internal feeders, infestations can still lead to reduced grain quality and greater overall losses during storage (Campbell et al., 2004).

1. 3. 1. Rhyzopertha dominica

Rhyzopertha dominica, commonly known as the lesser grain borer and a member of the Bostrichidae family, is classified as a primary internal feeder due to its larval development occurring entirely within intact kernels of grains such as sorghum, wheat, rice, and maize (Majeed et al., 2015; Suleiman et al., 2020; Hemmati, 2024). Although it is well-established that *R. dominica* can infest sorghum, evidence suggests that its performance varies significantly among cultivars. For example, Hemmati (2024) demonstrated that certain sorghum cultivars supported rapid development and high fecundity, while others significantly suppressed larval growth, digestive enzyme activity, and reproductive output due to higher polyphenolic content. Nevertheless, despite clear susceptibility of some sorghum varieties, most published research on

R. dominica has historically focused on more globally traded commodities such as wheat and rice, leaving sorghum relatively underexplored in the context of stored grain pest ecology and management.

Under optimal environments, temperatures between 27 – 34°C and relative humidity between 60 – 75%, females can lay between 300 and 500 eggs over their lifetime, either loosely scattered among stored grain or directly affixed to individual kernels (Baldassari et al., 2005; Majeed et al., 2015; Suleiman et al., 2020). After oviposition, the triungulin (first instar) larvae hatch and actively move through the grain mass to locate and bore into suitable kernels. Once inside, they transition into a scarabaeiform form and complete four larval instars before pupating (Suleiman et al., 2020; Baldassari et al., 2005). Development from egg to adult ranges from 25 – 30 days, whereas on other commodities such as rice or maize, the duration of the larval stage lasts around 35 – 40 days, respectively. Furthermore, the commodity type, as found by Ren et al. (2023), also influences the overall fecundity of this species, as they found that female *R. dominica* laid an average of 246 eggs when on wheat, vs only 123 –150 eggs on average when exposed to maize or rice, respectively. In general, pupation for this species generally lasts 4–6 days at 27°C and 60 – 75% r.h., after which the adult remains in the kernel for several more days to complete cuticle sclerotization. The adults emerge from the kernel by chewing a characteristic round exit hole through the grain wall (Hemmati, 2024; Ren et al., 2023).

The destructive potential of *R. dominica* in stored agricultural systems is substantial, both in terms of direct kernel damage and broader economic loss. As internal feeders, larvae hollow out kernels from within, consuming the nutrient-rich endosperm and germ, which results in significant product weight loss, decreased grain quality, and reduced germination potential (Hemmati, 2024; Suleiman et al., 2020). Infestation leads to a characteristic pattern of circular exit holes in the grain, accompanied by frass and broken kernels, reducing both processing efficiency and market value. Teixeira et al. (2021) reported *R. dominica* infestations in packaged sorghum sold in retail stores, confirming the pest's capacity to persist through distribution and marketing chains. In high-infestation scenarios, grain may be rendered unsuitable for human consumption or sale due to contamination and microbial growth. These factors underscore *R. dominica's* reputation as one of the most economically destructive pests of stored cereal grains worldwide.

A distinctive trait of *R. dominica* is its ability to infest more than just stored grains. It has been observed feeding on or sheltering in non-grain products such as pharmaceuticals, leather, books, and wooden materials within storage facilities, including pallets, bins, and grain-handling equipment (Majeed et al., 2015; Suleiman et al., 2020; Ren et al., 2023). This behavior allows *R. dominica* to persist in storage environments even after grain has been removed or treated, thereby increasing the risk of reinfestation (Suleiman et al., 2020). Additionally, adult beetles produce a distinctive odor, often described as pungent or musty, which can contaminate grain and reduce its marketability and acceptability for human consumption (Majeed et al., 2015).

Another factor contributing to the challenge of controlling *R. dominica* is its strong flight capability. Adults are capable of dispersing over considerable distances, which facilitates the spread of infestations between silos, warehouses, and transport vehicles (Suleiman et al., 2020; Majeed et al., 2015). This mobility, coupled with the beetle's resilience in various microhabitats, necessitates the implementation of robust IPM strategies. Effective approaches include continuous monitoring through traps, grain sampling, and pheromone lures, sanitation, using aeration systems to regulate grain temperature and moisture, and cultivating pest-resistant grain varieties such as those high in phenolic compounds (Hemmati, 2024; Majeed et al., 2015).

1. 3. 2. Sitophilus spp.

The *Sitophilus* genus, belonging to the family Curculionidae, comprises several primary internal feeders that threaten stored grain products globally. Among them, *S. oryzae* is one of the most destructive, especially in tropical and subtropical regions (Rita Devi et al., 2017). Members of this genus are highly adapted to storage environments and are capable of infesting a broad range of cereal grains including rice, maize, wheat, sorghum, and barley (Ojo et al., 2016). Their ability to develop entirely within intact kernels allows them to evade early detection and rapidly multiply, resulting in substantial post-harvest losses if not properly managed (Diab et al., 2025).

Although the destructive capacity of *Sitophilus* spp. is well-documented for major staple crops, relatively few studies have explored their infestation potential or developmental performance on sorghum grain. Nevertheless, observations of *S. oryzae* and *S. granarius* developing on sorghum under laboratory conditions indicate that infestations are possible, especially in susceptible cultivars or under optimal environmental conditions (Prasad et al., 2015; Diab et al., 2025). This lack of focused research on sorghum presents a knowledge gap that is

increasingly important to address, given sorghum's expanding role in global food and feed systems.

Adult *Sitophilus* spp. measure between 2 – 4 mm in length, with elongated snouts and elbowed antennae (Rita Devi et al., 2017). As primary pests, they infest whole grain kernels where the female weevil bores a hole into the grain to oviposit a single egg. After hatching, the scarabaeiform larvae develop entirely within the grain, consuming the endosperm until pupation. Adults later emerge by boring through the grain surface, leaving behind distinct exit holes indicative of infestation (Majd-Marani et al., 2023). The complete life cycle from egg to adult typically spans 32 – 48 days in environmental conditions that span 27 – 32ºC and 60 – 70% r.h. (Lu et al., 2024). Larval development ranges from 25 – 32 days, while pupation lasts approximately 7 to 10 days under ambient laboratory conditions (Sachin et al., 2023). Development is heavily influenced by temperature and humidity; optimal reproductive and developmental performance occurs at 28 ± 1°C and 70 ± 5% relative humidity, where fecundity can exceed 300 eggs per female (Majd-Marani et al., 2023). Conversely, development is prolonged, and survival reduced under suboptimal conditions or when feeding on resistant cultivars.

The damage caused by *Sitophilus* spp. is both quantitative and qualitative. Larvae feed on the internal starch reserves of the grain, while adults add to surface abrasions and introduce frass, pupal skins, and insect fragments that reduce market value and product hygiene (Diab et al., 2025). Contamination is often accompanied by increased susceptibility to fungal colonization, especially by *Aspergillus* and *Penicillium* spp., which may result in mycotoxin production that poses risks to human and animal health (Majd-Marani et al., 2023). In extreme cases, grain damage has been reported at 92 – 98% in untreated maize and rice under favorable storage conditions (Sachin et al., 2023). These effects are exacerbated in tropical regions where environmental conditions allow for continuous development and generation turnover.

Given the biological adaptability, high reproductive potential, and cryptic life cycle of *Sitophilus* spp., their potential impact on sorghum grain should not be underestimated. Preliminary findings indicate that under certain conditions, sorghum may still be susceptible to significant damage, particularly if post-harvest management and storage conditions are not optimized (Teixeira et al., 2021; Diab et al., 2025). The expanding cultivation and storage of sorghum in regions vulnerable to Sitophilus infestation underscore the need for further

investigation into host resistance traits, pest biology on this commodity, and IPM strategies tailored specifically to sorghum-based systems.

1. 3. 3. Plodia interpunctella

Plodia interpunctella, commonly known as the Indian meal moth, is one of the most globally distributed and destructive pests of stored products. As a member of the Pyralidae family, this species affects a wide range of commodities including cereals, nuts, dried fruits, and processed foods, with both its larval feeding and contamination capacity presenting serious challenges in food storage and processing environments (Mohandass et al., 2007). Its ability to infest a broad spectrum of food products has made it a major concern in both household and industrial storage settings (Mohandass et al., 2007).

The full life cycle of *P. interpunctella* spans between 30 – 60 days under ideal conditions. These conditions, which are also optimal reproduction and larval development conditions, are between 25 – 30°C and relative humidity between 60 – 70% (Defilippo et al., 2019). Under such conditions, populations can grow rapidly, with overlapping generations leading to persistent and escalating infestations throughout storage seasons.

Adult moths are small, typically 1.1 – 1.3 cm in wingspan and are identifiable by their reddish-brown forewings that contrast sharply with their pale-gray hindwings. Though adults do not feed, their role in reproduction initiates severe infestations. Females can lay 100 – 400 eggs over several days, with oviposition occurring directly on or near suitable food substrates (Defilippo et al., 2019). Upon hatching, the highly mobile larvae begin feeding immediately, producing silk that results in visible webbing and clumped food material. This webbing not only taints the infested product but also contributes to microbial growth and the accumulation of frass and larval debris, making the commodity unsuitable for consumption (Subramanyam 2018). In addition to direct feeding damage, the contamination caused by *P. interpunctella* can result in product rejection in commercial markets, increased mold incidence, and regulatory concerns in food safety (Subramanyam 2018). The larvae actively seek pupation sites by exiting infested materials and searching for crevices or container edges, often making detection and control difficult (Hagstrum and Philips, 2012). Monitoring programs often rely on pheromone-baited traps, which are essential for early detection and population assessment (Hagstrum and Philips, 2012).

1. 3. 4. Prostephanus truncatus

Prostephanus truncatus, commonly referred to as the larger grain borer, is a destructive pest of stored cereals, particularly maize (Hodges, 1986). A member of the Bostrichidae family, *P. truncatus* is of growing international concern due to its aggressive feeding behavior, resistance to control measures, and increasing potential for geographic expansion (Quellhorst et al., 2021). Originally native to Central America and Mexico, *P. truncatus* was introduced to Africa in the late 1970s and has since spread rapidly across many Sub-Saharan countries due to infested grain shipments and inadequate quarantine control (Hodges, 1986). Its introduction to new regions has had severe consequences, particularly in maize-dependent agricultural systems. Although *P. truncatus* has not yet established widespread populations in temperate areas like the U.S., warming climate conditions could facilitate future invasions. Its ability to thrive in warmer regions has already raised red flags regarding its potential establishment in new territories (Quellhorst et al., 2021).

The adult beetle, measuring approximately 3.5 – 5 mm in length, possesses strong mandibles and a cylindrical body adapted for burrowing. As a primary feeder, *P. truncatus* causes direct losses in both weight and nutritional value as both larvae and adults bore deep into grain kernels, generating extensive internal damage and producing large amounts of frass and fine powder, which rapidly degrades grain quality (Hill et al., 2002). Compounding its threat is its ability to infest and survive in wooden storage bins, pallets, and infrastructure, much like *R. dominica*, thus serving as a persistent source of reinfestation even when grain is removed (Tyler and Hodges, 2002).

Reproductive output in *P. truncatus* is moderately low compared to other stored product pests such as *R. dominica*, with females laying 50 – 100 eggs during their lifespan. Its full life cycle ranges from 25 – 55 days depending on temperature and humidity (Shire, 1979; Hodges et al., 1986). Optimal development occurs between 25 – 32°C and 70 – 80% relative humidity, conditions frequently encountered in grain storage facilities in tropical and subtropical regions (Papadimitriou et al., 2024; Quellhorst et al., 2020). Like the other insect species mentioned thus far, under favorable conditions, P. *truncatus* can complete multiple generations per year, leading to exponential population growth and overwhelming infestations if not monitored and managed effectively.

The potential establishment of *P. truncatus* in the U.S. remains a serious threat, particularly in southern and coastal grain-producing regions where warming climates and high

humidity could permit year-round reproduction. Given the pest's history of international spread via grain trade and its ability to survive in shipping materials and structures, strict quarantine measures are essential (Hill et al., 2002). Without such precautions, unintentional introductions into vulnerable ecosystems could result in extensive post-harvest losses and economic disruption.

1. 4. Pest infestation and management protocol

Stored product pests are insect species that target grains and other stored food products throughout the post-harvest supply chain. Unlike field pests, these insects thrive in the controlled environments of storage facilities, shipping containers, distribution centers, and processing facilities, causing extensive product loss through direct feeding, contamination, and the introduction of spoilage agents such as mold (Kalaisekar et al., 2017). Their ability to persist undetected in storage environments makes them particularly destructive, leading to visible damage and less obvious declines in product integrity (Reddy, 1991). This problem is more severe in regions with inadequate storage infrastructure, where poor sealing, high temperatures, and humidity increase the risk of infestation (Guo et al., 2010).

The transportation of grains further complicates pest control, as stored product insects can travel unnoticed on grain bags, processing equipment, and inside transport containers, potentially contaminating multiple elements withing the post-harvest supply chain (Roesli et al., 2003; Arthur et al., 2014). These insects may be introduced through infested loads or may already reside in poorly sanitized machinery and transportation vehicles (Campbell et al., 2002; Subramanyam et al., 2001). If left unchecked, they can establish populations in warehouses, grain storage facilities, or in commercial environments. Routine inspection of trucks, storage containers, and receiving systems paired with standardized sanitation protocols can prevent widespread infestations by interrupting these hidden dispersal pathways (Arbogast et al., 2000; Semeao et al., 2013).

To strengthen IPM strategies in post-harvest grain logistics, industry professionals have begun implementing protective technologies along with traditional sanitation techniques. In-transit fumigation using phosphine under sealed conditions has been effective in reducing pest populations during long-distance shipping (Kalaisekar et al., 2017). Additionally, chemically treated packaging materials, including methoprene-integrated films and insect-resistant polymers, act as potential passive barriers to larval development and adult emergence during extended storage (Klein and Burkholder, 1984; Mullen et al., 2012). For instance, methoprene-

treated polyethylene terephthalate / polyethylene (PET/PE) liners have been shown to suppress adult emergence in some stored product pests such as *Trogoderma variabile* (Ballion), warehouse beetle, and *Tribolium castaneum*, without affecting product quality (Scheff et al., 2017). Packaging structure matters too with thicker films, odor barriers, and reinforced seams implemented in hopes to minimize the ability of pest entry and survival (Wohlgemuth, 1979; Marsh and Bugusu, 2007). The success of these strategies, however, hinges on consistent implementation. Training personnel to identify high-risk zones, properly seal packages, and follow IPM protocols throughout the storage and transit chain post-harvest could help limit the success and spread of pests through the integration of sanitation, structural inspection, protective packaging, and selective fumigation.

1. 4. 1. Trapping and monitoring

Trapping is a foundational tool in any IPM program stored product pest management and is valued for its use in detecting and monitoring the presence, spread, and the population dynamics of insects within grain bins, warehouses, and processing facilities (Mueller and VanRyckeghem, 2012; Dowdy and Mullen, 1998). These traps provide insights into pest identity, abundance, and distribution, which helps pest control operators (PCOs), and facility managers make informed decisions about the timing and type of tactics to be employed for treatment or intervention. Common trap types include pheromone traps, pitfall traps, probe traps, and light traps, each suited to different pest behaviors and storage environments. When deployed strategically and monitored consistently, traps confirm the presence of infestation, identify high-risk areas, and measure treatment effectiveness over time. High trap captures may signal the need for additional inspection or control, while continued monitoring can reveal reinfestation trends or overlooked populations of pests (Mueller, 2003). As part of a broader IPM program, trapping is a proactive surveillance strategy essential to maintaining grain quality and regulatory compliance.

Pheromone traps operate by releasing synthesized chemical cues that mimic natural insect communication. These cues, often sex or aggregation pheromones, target specific behaviors like mating or foraging. For example, male lepidopterans such as *P. interpunctella* are highly responsive to female-emitted sex pheromones and are efficiently captured in sticky wing traps baited with these lures (Burkholder, 1984; Mueller and VanRyckeghem, 2012). Similarly, aggregation pheromones are used to draw both sexes toward traps containing these chemical lures (Burkholder and Ma, 1985; Mueller and VanRyckeghem, 2012). Beyond monitoring,

pheromones can also posses disruptive functions, such as mating disruption. High concentrations of female sex pheromones through a space and disrupt the male's ability to local the female and successfully mate. This technique is currently utilized for P. *interpunctella* and Trogoderma *spp.* (Mueller, 2004).

Pitfall traps, commonly deployed at ground level or within grain bins, are often baited with a combination of food attractants and pheromones. These traps are particularly effective for capturing crawling insects (Mullen, 1992). In grain bins, bulk grain probe traps are inserted several centimeters below the grain surface to intercept mobile insects that migrate through the grain masses. These traps exploit the tendency of many stored grain pests to move through spaces in search of food or mates. Probe traps are particularly useful for detecting early infestations of internal feeders such as *Sitophilus* spp. and *R. dominica*, which often remain hidden deep within bulk commodities until populations grow large enough to cause visible damage (Mueller and VanRyckeghem, 2012; Hagstrum et al., 1990). Because of their passive design and sensitivity to low-level activity, probe traps are a valuable complement to surface-level monitoring tools and are frequently used to trigger interventions before grain quality is compromised.

Light traps, which utilize UV or blue light wavelengths, are useful for capturing nocturnal flying insects like *P. interpunctella and Ephestia spp*. These traps exploit the phototactic behavior of moths and other flying pests, which are naturally drawn to specific light spectra (Wirtz et al., 1980). While not species-specific, light traps offer a practical, non-chemical option for ongoing monitoring in food processing plants, packaging facilities, and storage areas (Pinniger, 2021). Their visibility-based attraction makes them effective for gauging adult moth activity, and their consistent use can support risk assessment and inform exclusion or sanitation efforts within an IPM framework.

1. 4. 2. Fumigation

Fumigation involves the introduction of gaseous pesticides, most commonly phosphine (PH_3) or sulfuryl fluoride (SF), into a sealed environment to eliminate stored product pests, including those concealed deep within grain masses or structures (Fields and White, 2002; Walse et al., 2022). Once released, the fumigant disperses passively through the spaces between kernels and into cracks and crevices, enabling it to reach all insect life stages. Phosphine is particularly valued in grain systems for its low sorption by grain, meaning that only a small portion of the

fumigant is absorbed onto grain surfaces during treatment. This allows more of the active gas to remain in the airspace, increasing its efficacy against pests throughout the storage structure (Ducom, 2022; Bell et al., 2021). In addition to its use in bulk grain storage, fumigation is also widely applied in shipping containers, transit holds, and warehouse facilities, where infestations can originate or spread during storage and transport (Yan et al., 2025). In these settings, fumigants are injected through designated ports into sealed containers or palletized cargo stacks and monitored throughout treatment.

Application methods vary depending on the fumigant used and the facility design. Phosphine is commonly applied via tablets, pellets, sachets, or as compressed gas, with delivery either in static form or through recirculation, which helps distribute gas evenly throughout a sealed structure or bin (Walse et al., 2022). Recirculation is especially effective in deep grain masses, helping reduce fumigation time and ensure uniform exposure. Sulfuryl fluoride, another commercial fumigant, is used where phosphine resistance is present or rapid action is required; it is applied via pressurized cylinders and acts more quickly but lacks deep penetration into grain kernels (Yan et al., 2025; Ducom, 2022).

The effectiveness of fumigation is governed by the concentration-time (Ct) product, which defines the required dose over time to achieve pest mortality. This is strongly influenced by environmental conditions, especially temperature and relative humidity, as well as species-specific tolerance levels and the presence of resistant pest populations (Walse et al., 2022). Resistance to phosphine has been documented in key pests such as *T. castaneum* and *R. dominica*, which can survive sublethal concentrations, requiring adjusted dosages and longer exposure windows (Nayak et al., 2020; Yan et al., 2025).

While fumigation is highly effective and capable of reaching inaccessible pests and treating large volumes of stored commodities, it has several notable drawbacks. It is labor- and cost-intensive, requiring trained applicators, air-tight conditions, and specialized monitoring equipment. Most fumigants are toxic to humans and non-target organisms, necessitating careful aeration and gas clearance verification before re-entry (Walse et al., 2022). Additionally, fumigation is generally considered a reactive strategy, best suited for remedial control rather than continuous prevention. It does not offer long-term residual protection and cannot prevent reinfestation without follow-up sanitation or exclusion measures (Campbell et al., 2012; Yan et al., 2025). However, when used as part of a larger IPM program, fumigation remains a critical

intervention tool for controlling entrenched infestations and safeguarding food quality in high-throughput or high-risk grain storage environments.

1. 4. 3. Contact insecticides

Contact insecticides are typically applied to floor surfaces, walls, empty storage bins, and outside walls, specifically targeting areas where pests are most likely to move, hide, or breed (Campbell et al., 2012; Rajarushi et al., 2024). Applications are commonly made prior to grain loading, after cleaning, or during facility shutdown periods, forming a protective barrier to reduce the likelihood of reinfestation. Unlike fumigants, which act as gases and penetrate bulk grain, residual insecticides remain bound to treated surfaces and kill insects primarily through contact during normal movement across those surfaces (Arthur, 2009; Athanassiou and Arthur, 2018). These treatments are preventive and are often employed between storage cycles or following facility sanitation, offering weeks to months of residual protection depending on the material substrate and insecticide formulation. Active ingredients used in contact insecticides include pyrethroids such as deltamethrin and lambda-cyhalothrin, organophosphates such as pirimiphos-methyl, and newer compounds like chlorfenapyr or spinosad (Saglam et al., 2022; Vassilakos and Athanassiou, 2015). These are typically applied as emulsifiable concentrates (ECs), which spread well on non-porous surfaces; suspension concentrates (SCs), which provide good surface adhesion; or wettable powders (WPs), which offer broad coverage but may degrade more quickly on some substrates.

Surface type plays a critical role in treatment efficacy. Non-porous materials such as sealed concrete or painted metal allow more of the active ingredient to remain on the surface where pests can contact it. In contrast, porous surfaces like untreated concrete or wood can absorb insecticides, reducing efficacy (Jankov et al., 2013; Kavallieratos et al., 2017). These surfaces may require higher application rates or more frequent reapplications to maintain protective activity (Tawfik et al., 2021).

When integrated with sanitation, monitoring, and other control methods, surface insecticide applications provide an important residual defense within an IPM program. While not a replacement for fumigation or grain treatments, they help suppress pest activity between major interventions and contribute to long-term protection of stored commodities (Arthur, 2008; Campbell et al., 2012).

1. 4. 4. Grain protectants

Grain protectants are liquid or dust insecticides applied directly to bulk commodities such as wheat, maize, rice, and sorghum to provide long-term residual control of stored product pests (Arthur, 1996; Subramanyam and Hagstrum, 1996). Unlike fumigants, grain protectants form a thin film of insecticide on the surface of each grain kernel, providing continuous protection during the storage period (Arthur, 1996; Arthur, 2019). Protectants are typically applied as grain is loaded into storage, using spray nozzles positioned on grain handling equipment such as conveyors, augers, or belt systems to ensure uniform coverage across the grain mass (Arthur, 2019; Daglish et al., 2018). In commercial practice, grain protectants are most commonly applied immediately after harvest during the first intake into bins or silos, or when grain is transferred between bins during aeration cycles or blending. This timing is critical to intercept pest colonization during early storage, when grain is most vulnerable to invasion and prior to significant pest buildup (Arthur, 2019; Subramanyam and Hagstrum, 1996).

Grain protectants are most commonly formulated as emulsifiable concentrates (ECs) or suspension concentrates (SCs), which are applied as water-based sprays during grain movement (Arthur, 2019; Subramanyam and Hagstrum, 1996). ECs typically offer good penetration and spreading on grain surfaces, while SCs provide improved physical stability and lower volatility (Arthur, 2000). Some common active ingredients include insect growth regulators (IGR), natural pyrethrins and synthetic pyrethroids, and spinosyns. Each active ingredient differs in mode of action, residual persistence, and tolerance to environmental factors, making product selection a key consideration for successful long-term protection (Arthur, 2019; Subramanyam and Hagstrum, 1996).

The efficacy of grain protectants is influenced by multiple abiotic factors, and these effects can vary greatly depending on the specific active ingredient used (Arthur, 2000a; Subramanyam et al., 1996). Temperature plays a critical role with higher temperatures accelerating the degradation of certain active ingredients. Relative humidity and grain moisture content also impact protectant performance, as elevated moisture can increase hydrolysis or sorption of some compounds, reducing bioavailability (Arthur, 2000; Samson et al., 1990). In addition, ambient humidity within the storage environment itself can influence protectant persistence and may promote microbial growth or grain respiration, indirectly affecting chemical stability (Arthur, 2000a). Exposure to light, particularly ultraviolet (UV) radiation, can also degrade some active ingredients, especially in surface layers of grain exposed near bin openings

or in transparent storage environments (Arthur, 2000a). Additionally, grain type affects absorption and surface coverage; grains with rougher textures or higher oil content may reduce the effective concentration of the insecticide on the kernel surface (Arthur, 2000). Numerous studies have evaluated protectant efficacy on grains such as wheat, maize, and rice, under various temperature and storage conditions (Arthur, 2019; Kavallieratos et al., 2012). However, comparatively little work has been published on the performance of protectants specifically on sorghum grain, which presents unique surface and chemical characteristics that may influence insecticide performance. Understanding these grain-specific interactions remains an important area for future research, particularly as sorghum continues to expand in commercial storage systems.

Grain protectants offer several advantages: they provide long-term residual protection, are relatively cost-effective compared to fumigation, and do not require the sealing of storage structures (Arthur, 1996; Subramanyam and Hagstrum, 1996). However, they are preventive and established infestations or hidden infestations often require fumigation or other interventions (Arthur, 2019). In addition, the potential for pest resistance and regulatory restrictions on maximum residue levels (MRLs) in export markets necessitate careful product selection, application accuracy, and chemical rotation to preserve efficacy and marketability (Haddi et al., 2018; Daglish et al., 2018; Athanassiou and Arthur, 2018).

1. 5. Common active ingredients in grain protectants

In IPM systems, the primary goal is to minimize pest damage with minimal disruption to the environment and human health and maintain the quality and safety of the grain (Subramanyam and Hagstrum, 1996; Arthur, 2019). The use of grain protectants in stored product settings needs to be efficacious to the target pests but also have low toxicity to facility workers, consumers, and non-target species (Athanassiou and Arthur, 2018; Haddi et al., 2018). At the same time, the development of more environmentally friendly and human-safe insecticides is an ongoing goal in pest management (Athanassiou et al., 2021; Arthur, 2019). While traditional insecticides may still be widely used, there is increasing interest in alternative methods that focus on minimal risk to human health and the environment.

1. 5. 1. (S)-Methoprene

Methoprene is an insect growth regulator widely used in pest management for controlling insects that infest stored products, insects that vector diseases, and fly control for livestock

(Oberlander et al., 1997; Athanassiou et al., 2011). Developed by the Zoecon Corporation in 1972, this synthetic compound mimics the natural juvenile hormone (JH) found in insects, disrupting their normal development and preventing them from reaching maturity (Henrick, 2007). Methoprene functions as a juvenile hormone analogue, disrupting the hormonal balance required for molting and metamorphosis in insects (Tunaz and Uygun, 2004; Oberlander et al., 1997). Due to its targeted mode of action, methoprene is regarded as an environmentally safer alternative with minimal risk to mammals and non-target organisms (U.S. EPA, 2001; Oberlander et al., 1997).

Methoprene can be internalized via several potential pathways such as absorption, ingestion, inhalation through the spiracles, or absorption through the cuticle (Henrick, 2007; Oberlander et al., 1997). When applied as a grain protectant, insects feeding on or moving within the treated commodities make contact with the active ingredient. Once inside the insect's body, methoprene binds to juvenile hormone receptors, signaling the insect to remain in its immature state. By mimicking juvenile hormone and interfering with ecdysone-regulated processes, affected larvae become trapped in their juvenile phase and are unable to develop into fully functional adults (Tunaz and Uygun, 2004).

Although methoprene is primarily designed to disrupt immature insect stages, adults exposed to the compound can also experience sublethal effects that can significantly impair population sustainability (Athanassiou et al., 2011). Additionally, even if previously exposed adult pests emerge from treated grain, they may often display physical deformities such as crumpled wings and abnormal cuticle formation (Oberlander et al., 1997). Other sublethal adult effects reported include reduced mating success, altered pheromone production, and decreased adult longevity following surface or dietary exposure to methoprene residues (Oberlander et al., 1997). These indirect adult impacts complement the larvicidal effects of methoprene, contributing to long-term pest population decline across multiple generations. Because it specifically targets insect endocrine systems.

Methoprene is available in multiple formulations tailored for different applications, including surface treatments for storage structures, aerosol sprays for food-processing areas, and direct grain treatments to safeguard bulk-stored grains (Arthur, 2019; Athanassiou et al., 2011; Oberlander et al., 1997). Since its primary mode of action targets immature life stages, methoprene is frequently combined with other active ingredients, such as pyrethroids or

diatomaceous earth.to create a product with multiple modes of action to target adult and juvenile stages of the insect (Athanassiou et al., 2011). This integrative approach not only enhances overall control but may also aid in delaying resistance development, a growing concern associated with long-term reliance on single-mode chemical treatments (Oberlander et al., 1997; Athanassiou and Arthur, 2018).

The residual efficacy of methoprene depends strongly on formulation type, application method, and environmental factors such as temperature, humidity, and light exposure (Arthur, 2019; Oberlander et al., 1997). As a grain protectant, methoprene has been shown to remain effective for 4–6 months under typical storage conditions (Athanassiou et al., 2012). Similarly, Oberlander et al. (1997) reported that methoprene applied to metal and concrete surfaces remained active for up to 2–3 months against *R. dominica*, depending on surface porosity and environmental exposure. Notably, UV exposure and high humidity accelerate the degradation of methoprene, emphasizing the importance of application timing and environmental monitoring in treated facilities (Arthur, 2019; Tunaz and Uygun, 2004). However, in a study by Author (2016), it was found that Methoprene applied to wheat, brown rice, and maize resulted in complete suppression of *R. dominica* and *T. castaneum* progeny for two years yet was not as effective at suppressing pest insects such as *Sitotroga cerealella* (Oliv) Angoumois grain moth .

One key advantage of methoprene is its benign degradation profile, meaning it breaks down into non-toxic byproducts such as methoxyphenols and fatty acid esters, which are naturally present in many food products and pose no food safety risk (Henrick, 2007; U.S. EPA, 2001). These byproducts do not accumulate concerning levels in treated grain and are readily metabolized or removed during milling and processing (Henrick, 2007). Unlike neurotoxic pesticides that affect the central nervous system, this compound targets only insect developmental pathways, specifically interfering with metamorphosis, making it largely harmless to mammals and other vertebrates (Henrick, 2007; Oberlander et al., 1997). Insects rely on hormonal pathways—juvenile hormone signaling and ecdysteroid-regulated molting that are absent in vertebrates, providing a strong margin of safety (Tunaz and Uygun, 2004). Additionally, methoprene is poorly absorbed through human skin and is rapidly metabolized, further reducing risk in food storage and processing environments (U.S. EPA, 2001; Henrick, 2007).

However, it is important to note that, like all insecticides used in food-related settings, methoprene applications are subject to Maximum Residue Levels (MRLs). These are regulatory limits on the amount of pesticide residue permitted to remain in or on food products (Haddi et al., 2018; Athanassiou and Arthur, 2018). MRLs are established to protect human health, as chronic exposure to residues exceeding MRLs could pose risks, particularly for vulnerable populations such as children or pregnant women (Haddi et al., 2018). For wheat alone, the MRL 25 mg/kg (Food and Agriculture Organization, 2025). Although methoprene is generally considered low-risk, MRL exceedances have been noted in certain contexts, especially when applications were too frequent or when storage conditions prolonged residue persistence (Haddi et al., 2018; Oberlander et al., 1997).

1. 5. 2. Pyrethrins and pyrethroids

Pyrethrins are naturally occurring insecticidal compounds derived from flowers of the species within the genus Chrysanthemum (family Asteraceae). While several species within this genus produce pyrethrins in various plant tissues (Zito et al., 1983), *Chrysanthemum cinerariifolium* is the primary commercial source due to its high pyrethrin content and ease of cultivation. Other species known to produce pyrethrins include *C. coccineum* and *C. balsamita* (Zeng et al., 2021; Bestmann et al., 1987). The insecticidal properties of pyrethrins have a long history of use, with records dating back to the 17th century in the Middle East, where powdered flower extracts were employed to control arthropod pests (McLaughlin, 1973).

The term pyrethrum refers to the dried, powdered plant material or the crude extract obtained from such material via organic solvents (Thatheyus and Selvam, 2013). Chemically, the active insecticidal fraction consists of six related ester compounds, each formed by the combination of a specific alcohol with either chrysanthemic acid or pyrethric acid (Khambay, 2002). Based on the acid moiety, the pyrethrins are categorized into two groups: pyrethrins I (esters of chrysanthemic acid) and pyrethrins II (esters of pyrethric acid) (Khambay, 2002). Each group comprises three individual compounds: pyrethrin I, cinerin I, and jasmolin I in the pyrethrin I group; and pyrethrin II, cinerin II, and jasmolin II in the pyrethrin II group (Khambay, 2002; Soderlund, 2012; Thatheyus and Selvam, 2013). This structural diversity contributes to the broad-spectrum insecticidal activity of pyrethrum extracts (Soderlund, 2012).

The symptoms of pyrethrin poisoning in insects typically follow a characteristic progression, beginning with hyperactivity, followed by ataxia (loss of coordination),

convulsions, and ultimately paralysis (Elliott, 1976; Miller and Adams, 1982). These symptoms result from progressive disruption of neuronal function in both the peripheral and central nervous systems, driven by the action of pyrethrins on voltage-gated sodium channels (VGSCs) (Narahashi, 1971; Miller and Adams, 1982). Under normal physiological conditions, insect neurons generate action potentials through tightly regulated opening and closing of voltage-gated ion channels. Upon stimulation, VGSCs open, allowing an influx of sodium ions (Na^+) that depolarizes the membrane. Rapid inactivation of VGSCs, followed by potassium ion (K+) efflux through potassium channels, restores the resting potential and resets the neuron for subsequent signaling (Hodgkin and Huxley, 1952). This precisely timed cycle allows for controlled neural firing and coordinated motor behavior. Pyrethrins disrupt this process by binding to VGSCs and prolonging their open state, thereby delaying inactivation and causing a persistent inward sodium current (Lund and Narahashi, 1982; Miller and Adams, 1982). This results in repetitive neuronal firing and sustained depolarization. The resultant hyperexcitability of neural circuits explains the initial symptoms of hyperactivity and convulsions (Miller and Adams, 1982; Narahashi, 197).

In the motor system, pyrethrins disrupt motor neuron firing patterns, especially at the neuromuscular junction. Pyrethrins induce repetitive bursts of action potentials in motor axons, either in response to a single stimulus or spontaneously (Hallett, 1971). This results in prolonged muscle contraction and uncoordinated twitching, manifesting as ataxia and convulsions (Miller and Adams, 1982). Over time, the sustained depolarization can lead to conduction block, a state in which neurons fail to propagate action potentials, culminating in what is known as flaccid paralysis (Hirota et al., 1976). In the central nervous system (CNS), pyrethrins induce spontaneous burst discharges in ganglia such as the ventral nerve cord (Pitman, 1971). These bursts disrupt the patterned outputs needed for walking and flight (Sattelle and Lane, 1982). With increasing dose or duration of exposure, CNS neurons may enter depolarization block, where excessive depolarization prevents further action potentials, contributing to the knockdown (KD) and paralysis of arthropod pests.

While natural pyrethrins exhibit potent insecticidal activity and have long been valued for their rapid knockdown effects and relative safety to mammals (Thatheyus and Selvam, 2013), they possess significant limitations that restrict their use in modern pest management. Chief among these is their rapid degradation under environmental conditions. Pyrethrins are highly susceptible to photodegradation in sunlight and to oxidation in air, leading to short-lived efficacy

(Khambay, 2002; Thatheyus and Selvam, 2013). In open field applications, their residual activity may persist for only hours to a few days, necessitating frequent reapplication (Khambay, 2002).

The search for more effective and persistent insecticides gained urgency in the mid-20th century, particularly with the rise and subsequent abandonment of organochlorines such as DDT. DDT had been widely used for its remarkable residual activity and broad-spectrum efficacy during and after World War II (Davies et al., 2007, as cited in Field et al., 2017). However, its extreme environmental persistence and bioaccumulation in food chains led to widespread ecological damage and eventual bans in many countries (Davies et al., 2007, as cited in Field et al., 2017; Thatheyus and Selvam, 2013). In response, attention turned to insecticides that combined the rapid knockdown and favorable non-target safety profile of pyrethrins with the residual potency of DDT (Field et al., 2017; Soderlund, 2012).

This led to the development of pyrethroids, beginning in the 1970s (Elliott, 1976). Through modification of the natural pyrethrin molecule, researchers introduced structural elements such as α-cyano groups, and optimized stereochemistry that greatly enhanced photostability, lipophilicity, and VGSC-binding affinity (Elliott, 1976; O'Reilly et al., 2006). The resulting pyrethroids were categorized into two major groups based on their chemical structure and toxicological effects: Type I pyrethroids, which lack an α-cyano group (including compounds such as allethrin, bifenthrin, permethrin, phenothrin, resmethrin, tefluthrin, and tetramethrin), and Type II pyrethroids, which contain an α-cyano group (including cyfluthrin, cyhalothrin, cypermethrin, deltamethrin, fenvalerate, fenpropathrin, flucythrinate, flumethrin, fluvalinate, and tralomethrin) (Thatheyus and Selvam, 2013; Soderlund, 2012).

The distinction between Type I and Type II pyrethroids also underlies significant differences in their potency and neurophysiological effects in arthropods. The key feature of Type II pyrethroids is the presence of an α-cyano group, which greatly enhances their ability to stabilize the open state of VGSCs (O'Reilly et al., 2006). This modification results in prolonged sodium currents, leading to more severe neuronal depolarization and sustained hyperexcitability (Lund and Narahashi, 1982). Consequently, the toxicity of Type II pyrethroids is marked by prolonged tremors, convulsions, and eventual paralysis within insects (Verschoyle and Aldridge, 1980). In contrast, although similar, Type I pyrethroids typically induce tremors, hyperactivity, and ataxia (Verschoyle and Aldridge, 1980). Type II pyrethroids prolong both the open state duration and tail current of sodium channels to a greater extent than Type I compounds (Lund

and Narahashi, 1982). Furthermore, Type II binding exhibits greater state-dependence, becoming especially potent during repetitive neuronal firing (Soderlund, 2012). Structural studies suggest that differences in binding orientation and interactions with key VGSC residues contribute to the enhanced potency of Type II pyrethroids (Vais et al., 2000; Fields et al., 2017; O'Reilly et al., 2006). As a result, compounds such as deltamethrin, cypermethrin, and cyfluthrin are widely used for their high potency and effective knockdown of insect pests (Thatheyus and Selvam, 2013).

1. 5. 3. Spinosad

Spinosads are a group of insecticides derived from the fermentation of the soil actinomycete *Saccharopolyspora spinosa*. These compounds were discovered during an antibiotic screening program and later recognized for their potent insecticidal properties (Kirst et al., 2002). Commercial spinosad formulations primarily consist of two active components: spinosyn A and spinosyn D (Snyder et al., 2007). These structurally related macrocyclic lactones differ in the degree of methylation and hydroxylation, which influences their binding affinity to insect receptors and contributes to synergistic efficacy when combined (Hertlein et al., 2011).

As an active ingredient, spinosad's primary target is the nicotinic acetylcholine receptor (nAChR) in the insect central nervous system. Spinosyns bind to allosteric sites on the α6 subunit of insect nAChRs (Salgado and Sparks, 2005), leading to a conformational change that locks the ion channel in an open state. This results in sustained influx of cations, particularly sodium (Na^+) and calcium (Ca^{2+}), causing prolonged depolarization and overexcitation of motor neurons (Salgado, 1998). The physiological outcome is tremors, paralysis, and eventual death of the insect. Additionally, spinosad exhibits secondary interaction with GABA-gated chloride channels (GABA receptors), reducing inhibitory neurotransmission and further amplifying neurotoxic effects (Snyder et al., 2007; Hertlein et al., 2011; Clark et al., 2013). This dual mechanism contributes to spinosad's broad-spectrum efficacy across diverse insect taxa such as orders Lepidoptera, Diptera, Thysanoptera, Coleoptera, Orthoptera, Hymenoptera, and other taxa (Sparks et al., 1995; Bret et al., 1997) while maintaining a favorable safety profile in mammals, whose homologous receptors exhibit low affinity for spinosyns (Hertlein et al., 2011).

Spinosad can be absorbed by insects via ingestion or cuticular contact. A distinctive feature of commercial formulations is the use of crystalline particles, or solid spinosad embedded in a protective matrix, which improves environmental stability (Hertlein et al., 2011). These

crystals adhere to plant and grain surfaces or directly to insects. Upon ingestion or penetration, spinosyn A and D enter the insect's hemolymph and target the nervous system (Kirst et al., 2002). The crystalline form also protects the active ingredient from abiotic degradation, enabling slow release and extended residual activity (Hertlein et al., 2011; Ujváry, 2001). In stored product protection, crystalline spinosad provides several advantages. It adheres well to grain surfaces, silo walls, and storage structures, offering long-term control even under conditions of intermittent pest activity. Compared to liquid formulations, crystalline spinosad is more resistant to evaporation and wash-off. Its gradual release mitigates the risk of resistance development (Sparks et al., 2012).

Spinosad has been extensively evaluated for its performance in stored grain protection and structural pest management. Its efficacy is influenced by multiple factors, including grain type, surface characteristics, temperature, humidity, UV exposure, and storage conditions. Numerous experimental studies have demonstrated that spinosad is highly effective against primary grain pests such as *R. dominica* and *S. oryzae*, with more variable results against secondary feeders like *T. confusum*. For example, Vayias et al. (2010) reported that spinosad provided complete control of *R. dominica* and *S. oryzae* applied to wheat, barley, and maize for up to six months under typical storage conditions. Similarly, Athanassiou et al. (2011) found that spinosad applied to hard red winter wheat at doses as low as 0.1 ppm resulted in 100% mortality of *R. dominica* and complete suppression of progeny production for at least 65 days, while efficacy against *T. confusum* was more limited, with lower mortality rates even at higher doses.

Grain type is a key factor influencing efficacy. Vayias et al. (2010) demonstrated that spinosad exhibited greater persistence on wheat and barley compared to maize, where residues declined more rapidly. Similarly, Subramanyam et al. (2007) found that spinosad was highly effective on wheat but showed reduced performance on brown rice and milled rice. These differences are largely attributed to grain surface properties, with smoother surfaces favoring crystal adhesion and insect exposure (Arthur, 1999).

Spinosad also performs well as a contact insecticide when applied to storage structure surfaces. Agrafioti et al. (2025) found that spinosad residues on non-porous surfaces such as plastic and ceramic remained effective for up to six months, whereas efficacy declined more rapidly on porous surfaces such as concrete. These findings are consistent with earlier results from Arthur (2004), who showed that spinosad residues persisted longer on metal and painted

wood surfaces compared to unpainted concrete, due to absorption and binding of spinosad into porous matrices.

The efficacy and stability of spinosad are strongly influenced by abiotic factors. In this regard, temperature is critical. Athanassiou et al. (2008) demonstrated that spinosad exhibits a positive temperature coefficient for insecticidal activity: mortality rates of *R. dominica*, *S. oryzae*, and *P. truncatus* increased significantly as temperatures rose from 20°C to 30°C. However, the chemical stability of spinosad follows a negative temperature coefficient. Cleveland et al. (2002) showed that higher temperatures accelerate hydrolysis, oxidation, and photolysis of spinosad in aqueous systems. Similarly, Ujváry (2001) reported that thermal degradation of spinosad is accelerated at temperatures >35°C. Adak and Mukherjee (2016) further confirmed that spinosad remains chemically stable in dark, dry grain storage at 25–30°C but rapidly degrades when exposed to UV light or direct sunlight.

Humidity also impacts spinosad stability. Adak and Mukherjee (2016) reported that spinosad is more stable under dry conditions, whereas high relative humidity promotes hydrolytic breakdown, particularly on porous surfaces. Similarly, Vayias et al. (2010) observed that efficacy declined more rapidly under high humidity storage. pH is another factor: Cleveland et al. (2002) and Adak and Mukherjee (2016) demonstrated that spinosad is most stable under neutral to slightly acidic pH, while highly alkaline conditions accelerate degradation. Fortunately, most cereal grains maintain a near-neutral pH, which supports long-term stability.

In real-world storage conditions, spinosad has demonstrated a capacity for great persistence. Vayias et al. (2010) documented that residues remained active on grain surfaces for 6 to 12 months, depending on grain type and environmental conditions. Athanassiou et al. (2011) also reported long-term efficacy on wheat for up to three to six months, depending on pest species. General reviews of stored product IPM confirm that under cool to moderately warm (25–30°C), dry, and UV-protected storage, spinosad provides consistent protection (Hertlein et al., 2011). Application methods include direct grain treatment, surface sprays, aerosol applications, and emerging techniques such as packaging impregnation and top-dressing (Athanassiou et al., 2009; Clark et al., 2013).

1. 6. Insecticidal Tolerance and Resistance

1. 6. 2. Resistance

As insecticides are continually being utilized, many pest species have developed resistance driven by genetic mutations (Yang et al., 2018). This resistance can emerge through various mechanisms, including metabolic changes (e.g., enhanced enzyme activity that degrades insecticides), alterations to target sites where active ingredients bind, and modifications to the insect cuticle composition sometimes occurring in combination (Ye et al., 2022; Wilson and Ashok, 2018; Balabanidou et al., 2018). These genetic alterations, combined with repeated exposure to sublethal doses of insecticides, enable resistant insect populations to expand despite continued insecticide application, complicating pest management efforts (Yang et al., 2018). By identifying key resistance pathways, researchers and industry professionals can work toward more sustainable, long-term solutions to manage resistant pest populations, such as rotating chemical classes, integrating biological control methods, and improving grain storage practices.

1. 6. 1. 1. Metabolic resistance

Among the most significant detoxifying enzymes, cytochrome P450 monooxygenases (P450s) play a particularly versatile role in insecticide metabolism (Ye et al., 2022). These enzymes primarily function by introducing oxygen atoms into insecticide molecules, increasing their polarity and making them more water-soluble for excretion. P450s have been widely implicated in the resistance to pyrethrins and pyrethroids, as their oxidative modifications significantly reduce the potency of these insecticides (Ibrahim et al., 2016). In resistant insect populations, P450 overexpression has been observed, allowing for the rapid detoxification of insecticides before they can reach their molecular targets within the nervous system (Ye et al., 2022). This increased metabolic capacity not only extends insect survival but also allows populations to tolerate doses of insecticides that would otherwise be fatal (Ibrahim et al., 2016). Furthermore, variations in P450 gene regulation across different insect species contribute to differences in resistance levels, with some pests developing resistance faster than others. The adaptability of P450 enzymes makes them a major concern for pest management, as their broad substrate range enables them to counteract multiple insecticide classes, complicating efforts to control resistant populations (Ye et al., 2022).

In addition to P450s, esterases are another key group of enzymes involved in insecticide detoxification, particularly targeting pyrethroids and other active ingredients such as organophosphates (Bhatt et al., 2020; Wei et al., 2020). These enzymes hydrolyze ester bonds within insecticide molecules, breaking them down into non-toxic metabolites. Some resistant

insect populations exhibit enhanced esterase activity due to gene amplification or upregulation, leading to increased degradation of insecticides before they can accumulate to lethal levels (Bhatt et al., 2020; Wei et al., 2020). This metabolic adaptation is particularly concerning in agricultural and stored product pests, where prolonged insecticide exposure selects for populations with elevated esterase activity, reducing the effectiveness of chemical control measures (Nayak et al., 2015).

Similarly, glutathione *S*-transferases (GSTs) play a critical role in the detoxification of insecticides, particularly organophosphates and organochlorines, by catalyzing their conjugation with glutathione, a tripeptide composed of glutamate, cysteine, and glycine (Pavlidi et al., 2018). This conjugation process makes toxic compounds more water-soluble, facilitating their excretion and preventing them from interfering with essential physiological functions (Pavlidi et al., 2018). Over time, increased GST activity in certain insect populations has been linked to enhanced resistance, as higher levels of these enzymes enable more rapid detoxification (Enayati et al., 2005). Some insect species exhibit GST gene amplification or upregulated expression, leading to greater enzymatic efficiency in neutralizing insecticides (Pavlidi et al., 2018). This heightened metabolic capability allows resistant populations to persist in environments where insecticide application is routine, reducing the efficacy of chemical control measures.

In addition to detoxifying organophosphates and organochlorines, GSTs can also contribute to resistance against pyrethroids, although their role in pyrethroid metabolism is generally secondary to that of P450s and esterases (Oruni et al., 2024). Some studies suggest that GSTs help protect insect nervous systems from oxidative stress induced by insecticides, rather than directly breaking down the compounds themselves (Koirala et al., 2022). By mitigating oxidative damage, GSTs allow insects to endure prolonged exposure to insecticides without experiencing fatal physiological effects (Koirala et al., 2022). Understanding the genetic and biochemical basis of these resistance mechanisms is crucial for designing new control strategies that can counteract metabolic resistance and improve the long-term effectiveness of insecticide treatments.

Spinosad resistance in *R. dominica* has been associated with mutations in the nicotinic acetylcholine receptor (nAChR) α6 subunit, a critical component of neural signal transmission (Wang et al., 2018). Resistant strains exhibit multiple alterations to this receptor, including structural modifications such as a reduced N-terminal region, a frame-shift mutation, and

deletions in the TM2 transmembrane domain (Wang et al., 2018). These changes impair the receptor's ability to bind spinosad, rendering the insecticide ineffective. Furthermore, reduced expression of the nAChR α6 gene has been observed in resistant *R. dominica*, suggesting that resistance is driven by both structural mutations and regulatory changes that limit the production of the target protein (Wang et al., 2018). When target site mutations occur alongside metabolic resistance mechanisms, stored product pests become significantly more difficult to control. The combination of multiple resistance pathways within a single population reduces the efficacy of traditional insecticides, making it essential to explore alternative pest management strategies and continuously monitor resistance trends to maintain effective control measures.

1. 6. 1. 2. Target-site mutations

Stored product pests develop resistance to insecticides not only through metabolic adaptations but also via modifications to their target sites (Wilson and Ashok, 2018). Many insecticides, such as methoprene, pyrethrins, and spinosads, act by interfering with essential neural proteins, disrupting their function and leading to toxicity; however, when genetic mutations alter the structure of these target site proteins, the insecticides lose their ability to bind effectively, reducing their potency (Davies et al., 2008; Wilson and Ashok, 2018; Ureña et al., 2019). As a result, resistant insects can maintain normal nerve function despite exposure, allowing them to survive chemical treatments that would otherwise be lethal (Wilson and Ashok, 2018).

In *Sitophilus* spp., resistance to pyrethrins has been linked to specific genetic changes in the voltage-gated sodium channel, which pyrethroids target to disrupt nerve signaling (Araújo et al., 2011). Mutations such as L1014F and T929I modify the structure of these channels, preventing pyrethroids from binding effectively and allowing insects to continue normal nervous system function even in treated environments. These mutations have been observed in resistant populations, with *S. oryzae* predominantly carrying the L1014F mutation in certain regions, while *S. zeamais* tends to exhibit T929I (Araújo et al., 2011).

1. 6. 1. 3. Penetration rate

The insect cuticle, a multi-layered structure composed primarily of proteins, chitin, and lipids, serves as the first line of defense against external threats, including insecticides. In resistant insect populations, modifications to the cuticle via mutations in the genome can reduce

the rate at which insecticides penetrate the body, thus delaying or preventing their toxic effects (Balabanidou et al., 2018). Some insects develop a thicker cuticle through increased chitin synthesis, creating a denser barrier that slows the penetration of insecticides (Wang et al., 2025). Additionally, alterations in cuticular composition, such as increased hydrophobicity due to elevated lipid content, can further hinder insecticide absorption by repelling water-soluble formulations (Balabanidou et al., 2018). This type of resistance is particularly effective when an individual makes contact with insecticides that rely on rapid penetration before detoxification mechanisms can neutralize them. As a result, higher concentrations or longer exposure times are required for insecticides to achieve their intended effects, reducing the efficacy of conventional chemical treatments. This form of resistance, when combined with metabolic detoxification and target site insensitivity, makes managing resistant insect populations increasingly challenging, as slower penetration allows detoxification enzymes more time to break down toxic compounds before they reach their target sites (Balabanidou et al., 2018).

1. 6. 2. Means of tolerance

Tolerance and resistance to insecticides may seem similar, but their origins and implications are distinct. Tolerance emerges when environmental pressures gradually shape a population, giving certain individuals an advantage against insecticides through behavioral shifts or physiological adaptations (Guo et al., 2018; Li et al., 2024). In contrast, resistance is driven by genetic changes that fundamentally alter an insect's biology, allowing it to withstand chemical treatments (Liang et al., 2025). Because tolerance is shaped by external conditions rather than direct genetic changes, insect populations may slowly develop a heightened ability to endure protectants, even without evolving true resistance (Li et al., 2024). This gradual buildup of tolerance remains a significant challenge in pest management since the environment in which a pest population exists plays a crucial role in shaping its adaptive responses. Failure to account for these environmental influences could mean underestimating a population's ability to tolerate treatments, potentially leading to ineffective control measures.

1. 6. 2. 1. Temperature

As ectotherms, insects rely on external environmental temperatures to regulate their body heat, meaning functions such as their metabolism are directly influenced by the ambient temperature (Mirhosseini et al., 2017). Warmer temperatures typically increase the rate of

metabolic processes, which in turn can enhance the ability of insects to detoxify or degrade toxicants (Kumar et al., 2023). With elevated metabolic activity, insects can more efficiently neutralize or eliminate specific insecticides, leading to a reduced effectiveness of chemical treatments (Johnson, 1990). Additionally, increased temperatures can affect the persistence and degradation rate of some insecticides, potentially shortening their residual effectiveness in stored grain environments (Soliman, 2012).

This accelerated detoxification allows resistant individuals to survive exposure to insecticides that would otherwise be lethal, promoting the development of resistance over time (Liang et al., 2025). This type of tolerance is particularly challenging when using pyrethrin or pyrethroids, which have been shown to have negative temperature coefficients, thus exhibiting lower efficacy at higher temperatures, yet higher efficacy at lower temperatures (Kan et al., 2014). However, depending on the insecticide being used, some active ingredients, such as spinosad, have demonstrated increased efficacy under higher temperatures. In an experiment conducted by Athanassiou et al. (2008) where the effects of spinosad applied directly to grain was tested on *R. dominica*, *S. oryzae*, and *P. truncatus*, higher temperatures of 30°C resulted in significantly increased mortality rates compared to treatments at 20°C and 25°C, particularly for *R. dominica* and *S. oryzae*. However, *P. truncatus* exhibited lower susceptibility across all temperature conditions, suggesting species-specific variations in response to Spinosad (Athanassiou et al., 2008).

In addition to metabolic changes, temperature influences the geographical distribution of insect pests. Warmer winters shorten the overwintering period, enabling insects to survive in areas that were previously too cold for them (Marshall et al., 2020). Because insects are ectothermic, their ability to thrive and reproduce is highly dependent on temperature, and warmer conditions can open up new habitats for species that were previously confined to more temperate zones (Marshall et al., 2020). This expansion of range means that insects can now travel to regions with different pest management practices, where they may encounter new insecticides that have been adapted to local pest populations (Ma et al., 2021). This is of particular concern for species such as *P. truncatus*, which have yet to cross from Latin America into the U.S. due to climatic limitations but could one day become an invasive species if temperatures continue to rise (Quellhorst et al., 2020).

1. 6. 3. Combating resistance and tolerance

Effective pest management in grain storage requires a multifaceted approach to combat resistance and tolerance to insecticides. To address this challenge, several strategies have been explored, including the use of synergists to enhance insecticide potency, multi-active ingredient formulations to target pests through multiple pathways, and strategic timing of treatments to exploit pest vulnerabilities. Additionally, influencing environmental storage parameters such as temperature and moisture control can play a crucial role in both pest prevention and improving treatment efficacy (Mutalov et al., 2025). Emerging biotechnological approaches, such as RNA interference (RNAi), offer promising avenues for disrupting resistance mechanisms at a genetic level, although practical challenges remain. By integrating these methods, storage facilities can optimize pest control strategies, reduce reliance on chemical treatments, and mitigate the risk of further resistance development.

1. 6. 3. 1. Synergists

Synergists, such as piperonyl butoxide (PBO), have been explored to counteract metabolic resistance in stored product pests by enhancing the efficacy of grain protectants (Whickham, 1999). Synergists are used in conjunction with other more lethal grain protectants by inhibiting the activity of detoxifying enzymes, particularly cytochrome P450 enzymes, glutathione *S*-transferases, and esterases (Samadieh et al., 2024). By binding to and inhibiting these detoxification enzymes, the target species is prevented from breaking down toxicants. By blocking the metabolic pathways responsible for detoxifying the chemicals, synergists effectively nullify resistance mechanisms, allowing the insecticides to retain their potency (Samadieh et al., 2024). This allows for lower rates of insecticides and can be used to achieve effective pest control, potentially reducing environmental impact and preventing further resistance development (Samadieh et al., 2024). In the context of stored product pests, the use of PBO as a synergist has proven particularly valuable for combating resistance in species like *R. dominica* which have developed metabolic resistance over time due to prolonged exposure to grain protectants (Ortega et al., 2021).

1. 6. 3. 2. Dual-use active ingredients

Using multiple active ingredient protectants is an effective strategy to combat resistance in stored product pests by targeting different physiological processes, making it harder for pests to evolve resistance to all components simultaneously (Helps et al., 2020). By combining

insecticides with different modes of action, these multi-active ingredient formulations decrease the likelihood of resistance development, as pests are less likely to overcome multiple toxic effects at once (Helps et al., 2020). Several commercial protectants, such as Gravista® (Central Life Sciences, Schamburg, IL), are already formulated with multiple active ingredients, along with a synergist compound, providing a more comprehensive pest control approach.

1. 6. 3. 3. Treatment timing

The timing of treatment applications is crucial to the effectiveness of pest treatments because different stages of an insect's lifecycle can exhibit varying levels of resistance to insecticides (Hagstrum and Flinn, 1990). Insects undergo several developmental stages, from eggs to larvae, pupae, and adults, with each stage potentially showing different vulnerabilities to treatment (Flinn and Hagstrum, 1995). For instance, eggs may be more resistant to insecticides due to their protective outer coatings, making it harder for chemicals to penetrate (Authur, 1996). Similarly, pupae, which are in a non-feeding stage, are often less susceptible to treatments that target metabolic or feeding processes (Scheff et al., 2019). Larvae, on the other hand, may be more vulnerable during their active feeding stages, when they are ingesting the protectants directly, while adults, especially mobile ones, can sometimes avoid contact with the insecticides altogether (Authur, 2004).

By targeting the most vulnerable stages of the pest lifecycle, the effectiveness of treatments can be greatly enhanced (Arthur, 1996). For example, if it is known that *S. oryzae*, a primary feeder, has infested a supply of grain, it may be more ideal to periodically apply treatment if necessary to eliminate any wandering or emerging adults, since targeting internal-feeding larvae may be difficult (Arthur, 2004). On the other hand, applying treatments during the adult stage might be less effective in some instances, as some adult insect species other than *S. oryzae* are more mobile, can avoid treated areas, or may not even consume any product at all, as is the case with *P. interpunctella*. Regarding this tactic, it must again be stated that insect pest identification prior to treatment is crucial.

1. 6. 3. 4. Temperature and moisture control

Temperature and moisture control are crucial not only for preserving grain quality but also for managing insect infestations and enhancing the efficacy of chemical treatments in storage facilities (Mutalov et al., 2025). To mitigate infestations and spoilage, storage facilities

will often use ventilation and cooling systems to regulate temperature and prevent hot spots, while aeration systems help control moisture levels and inhibit fungal growth (Jones and Hardin, 2017). These modern storage facilities increasingly integrate automated systems that monitor and adjust temperature and moisture levels in real time, utilizing sensors to control ventilation, cooling, and aeration, ensuring stable conditions that deter pests and reduce the reliance on chemical treatments (Mutalov et al., 2025).

However, when chemical treatments are necessary, maintaining optimal environmental conditions can improve their efficacy (Hagstrum and Athanassiou, 2019). Many insecticides and fumigants perform best within specific temperature and humidity ranges, and controlled environments help ensure even distribution, reducing the likelihood of resistance development. Additionally, stabilizing temperature and moisture can extend the residual effectiveness of treatments, minimizing the need for frequent reapplications (Hagstrum and Athanassiou, 2019). By integrating precise climate control with chemical treatments, storage facilities can enhance pest management strategies, reduce overall pesticide use, and maintain grain quality more sustainably and efficiently.

1. 6. 3. 5. RNAi treatment

RNAi is a promising approach in pest control, including its potential use for combating resistance in stored product pests. RNAi functions by silencing specific genes, effectively inhibiting the production of target proteins (Christiaens et al., 2020). In pests, RNAi can disrupt critical biological processes and has shown to be effective in overcoming metabolic resistance mechanisms that render traditional insecticides less effective (Christiaens et al., 2020). In this context of metabolic resistance, RNAi can specifically target genes involved in the detoxification pathways that pests use to metabolize insecticides (Jiang et al., 2025). For example, RNAi can be used to silence genes encoding cytochrome P450 enzymes, which are commonly involved in the breakdown of insecticides, thus potentially restoring the effectiveness of insecticides by preventing pests from metabolizing and eliminating the toxic compounds (Jiang et al., 2025). Similarly, glutathione *S*-transferases and other esterases could also be targeted with RNAi to inhibit the ability of pests to neutralize toxicants, further improving the efficacy of pest control strategies (Eakteiman et al., 2018).

RNAi has seen successful applications in field crops, and while it shows promise in combating metabolic resistance in stored product pests, its practical use faces several

environmental and technical challenges (Hoang et al., 2022). In stored grain settings, the delivery of RNAi to pests is difficult, as many stored product pests, like those in the *Sitophilus* genus, lay eggs directly inside grains, shielding larvae from treatments. In contrast, pests like *R. dominica*, *P. truncatus*, and *P. interpunctella*, which lay eggs on the outside or in damaged areas, could be exposed to surface treatments.

Finally, there are concerns about the cost and scalability of RNAi treatments (Christiaens et al., 2020). Although the technology is advancing, producing interfering RNA molecules on a large scale for use in agricultural and storage environments remains expensive and labor-intensive (Verdonckt and Vanden Broeck, 2022). For RNAi to become a viable tool in pest management, it would need to be economically feasible for large-scale applications and easily integrated into existing pest control practices, which are typically reliant on more conventional chemical insecticides. Although RNAi technologies hold promises for use in stored product settings, their application at this stage remains largely confined to laboratory research.

1. 7. Closing remarks

As previously discussed, IPM represents a holistic and scienced-based approach to pest control that requires a comprehensive understanding of both the abiotic and biotic factors influencing pest populations within a given environment. This approach is particularly relevant when considering stored grain pest management, as environmental conditions, grain characteristics, and pest biology all contribute to infestation risks and subsequent product loss. Given the economic and food security importance of stored grains, continuous research is necessary to refine and improve pest management techniques. While significant research has been conducted on stored product susceptibility in major cereal grains such as wheat, rice, and corn, sorghum grain remains comparatively underrepresented in scientific literature. However, as sorghum continues to be a staple crop in many regions worldwide, and as production and storage practices evolve to meet growing demand, it is imperative that similar studies be conducted to assess its susceptibility to post-harvest losses due to insect pests. Research on other grains provides a valuable foundation for identifying key risk factors, but tailored investigations specific to sorghum are necessary to develop effective and sustainable pest management strategies.

One critical area of study is the efficacy of grain protectants over extended storage periods and under varying environmental conditions. Research in this domain could elucidate

how and why certain pests persist longer than others and the mechanisms behind their resistance or tolerance to treatment methods. Understanding these factors would be instrumental in designing targeted interventions that minimize infestations while ensuring the long-term viability of stored sorghum grain. Furthermore, sorghum's inherent versatility as a crop underscores the importance of examining the susceptibility of different sorghum varieties to pest infestations. Given that sorghum can be selectively bred for traits such as pericarp hardness, starch composition, and nutritional content (Elkonin, 2018), it is essential to investigate how these variations influence pest susceptibility. This information would be particularly valuable for farmers and commercial growers who cultivate sorghum for specific market purposes, as they would benefit from a deeper understanding of the potential risks associated with different varieties. Since pest management strategies often impose additional costs on growers, knowledge of inherent pest resistance in certain sorghum strains could inform breeding and storage practices that reduce economic burdens associated with infestation control.

In addition to varietal differences, the nutritional composition of sorghum grain may also play a role in determining pest attraction and survival rates. Stored product pests exhibit diverse dietary preferences and metabolic requirements, and their ability to infest and thrive on sorghum grain may be influenced by factors such as protein and starch content (Stathas et al., 2023). Investigating these relationships could help identify sorghum varieties that are inherently less susceptible to pest infestations, offering a natural means of pest mitigation that complements existing control measures. Likewise, differences in pericarp hardness could significantly impact a grain's vulnerability to infestation.

Finally, in the context of climate change and shifting agricultural landscapes, the production of sorghum is likely to increase due to its resilience in high-temperature and low-water environments (Khalifa and Eltahir, 2023). As global temperatures rise and water availability declines, sorghum may emerge as an even more crucial staple in food systems worldwide. However, with increased production comes the challenge of securing stored supplies against pest-related losses. Given the potential changes in pest populations and behaviors due to climate shifts, it is imperative that the measures outlined in this review be carefully considered and implemented to ensure the protection of sorghum post-harvest and throughout storage. Future research efforts must focus on developing adaptable and sustainable strategies to mitigate

infestation risks and maintain the integrity of stored sorghum grain in an evolving agricultural landscape.

Chapter 2 - Influence of Sorghum Kernel Hardness and Protein Content on the Infestation Dynamics of *Rhyzopertha dominica* (F.) and *Sitophilus oryzae* (L.)

2. 1. Abstract

Stored product pests such as *Rhyzopertha dominica* (F.), lesser grain borer, and *Sitophilus oryzae* (L.), rice weevil, are among the most damaging species in the bulk stored grain environment. Sorghum (*Sorghum bicolor*) is a genetically diverse cereal grain with wide variation in physical and nutritional grain traits, such as kernel hardness and protein content, two factors believed to affect insect infestation and developmental success. However, the extent to which these traits mediate infestation resistance remains poorly understood. This study examined how sorghum kernel hardness and protein content impact infestation, development, and feeding behavior of *R. dominica* and *S. oryzae*. Ten different varieties of sorghum were first categorized into low, medium, and high levels of kernel hardness or protein. Fifty grams of sorghum were placed in a vial and 10 adults of either species were added to vials for 7 d. Adults were removed, assessed for mortality, and the sorghum was held for an additional 8 wks. After 8 wks, sorghum was analyzed for progeny production, frass accumulation, and insect damaged kernels (IDKs). Additionally, kernel cross-sections were analyzed to assess endosperm composition (soft vs. hard), the germ, and larval tissue consumption over time. Results indicated that both species had high progeny and more frass and IDKs on low hardness varieties. Protein content had a more variable influence, suggesting that nutritional quality alone does not predict susceptibility. Larval feeding preferences shifted over time, with both species increasingly targeting germ and soft endosperm tissues during later developmental stages. These findings highlight the importance of grain structure on susceptibility to insect infestation. Breeding sorghum lines with reduced soft endosperm and increased kernel hardness may offer a promising avenue for sustainable pest management.

2. 2. Introduction

Stored product pests present a persistent challenge to global food security, particularly in the post-harvest storage of cereal grains. Among these pests, *Rhyzopertha dominica* (F.)

(Coleoptera: Bostrichidae), lesser grain borer, and *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae), rice weevil, are two of the more economically significant pests of stored grains (Hagstrum and Philips, 2012). Both species complete their larval development entirely within grain kernels, emerging as adults after consuming large portions of the seed's internal tissues. In sorghum (*Sorghum bicolor* (L.) Moench), this concealed feeding not only reduces kernel weight and quality but also complicates detection and control efforts, leading to considerable post-harvest losses (Arthur et al. 2020).

An effective strategy for mitigating such losses is the implementation of integrated pest management (IPM) programs within the post-harvest supply chain. IPM programs emphasize a comprehensive understanding of all components of the pest-host system to monitor, prevent, and control for pest and/or signs of infestation and eliminates the reliance on a single method. IPM tools and techniques include, but are not limited to, insect trapping and monitoring, environmental controls, fumigation, grain protectants, contact insecticides, and physical barriers to prevent and control infestations. Within the IPM ideology, the physical and nutritional traits of the commodity itself are also a major component. Grain traits such as kernel hardness and protein content may influence the insect pest's ability to infest the grain, feeding efficiency, and survival, thereby serving as potential targets for breeding resistant crop varieties (Arthur et al, 2020: Abedi et al, 2024; Gourgouta et al, 2021). Understanding these relationships is essential for developing passive resistance strategies that reduce the likelihood of infestations during storage.

Sorghum is a genetically diverse and highly adaptable crop cultivated globally for food, feed, and industrial uses (Aruna and Cheruku, 2019). Its ability to thrive in a variety of environmental conditions makes it a vital food security crop, particularly in arid and semi-arid regions (Abreha et al. 2022). Due to its broad phenotypic variability, sorghum can be bred for a range of desirable characteristics, including those that may contribute to post-harvest insect resistance (Aruna and Cheruku, 2019). Among these, kernel hardness has been linked to physical resistance to insect entry and feeding in other cereal grains, while protein content may influence nutritional suitability and larval development (Arthur et al, 2020: Abedi et al, 2024; Gourgouta et al, 2021). However, the degree to which these traits affect susceptibility to internal-feeding pests like *R. dominica* and *S. oryzae* on sorghum grain remains insufficiently characterized.

The objective of this study was to evaluate how variation in sorghum grain traits, specifically kernel hardness and protein content effects the susceptibility of sorghum to infestation and damage by *R. dominica* and *S. oryzae*. To address this, a series of laboratory bioassays were conducted using multiple sorghum varieties that differed either in kernel hardness or in crude protein content. For each variety, we quantified adult mortality, progeny production, frass accumulation, and the percentage of insect-damaged kernels. Additionally, cross-sections of uninfested and infested kernels were analyzed to assess endosperm composition (soft vs. hard) and to quantify tissue-specific feeding across developmental time. By integrating insect performance data with detailed kernel trait analysis, this study aims to clarify which grain characteristics are most influential in pest development and feeding, ultimately informing breeding programs and IPM strategies aimed at reducing post-harvest losses.

2. 3. Materials and methods

2. 3. 1. Insect rearing

The *R. dominica* and *S. oryzae* individuals used in this study originated from pesticide-susceptible laboratory colonies maintained at the USDA–ARS, Center for Grain and Animal Health Research (CGAHR) in Manhattan, KS for more than 30 years. Both species were reared on commercial whole sorghum grain (Cargill®, Salina, KS) and maintained in an environmental chamber at 27°C and 60% relative humidity (r.h.) in complete darkness (Percival, Perry, IA). To standardize insect age across treatments, *R. dominica* or *S. oryzae* adults were introduced to jars containing ~300 g of sorghum for 1 wk at 27°C and 60% r.h. After 7 d, parental adults were removed, and jars returned to the environmental chamber and held for adult progeny. All *R. dominica* and *S. oryzae* adults used in experiments had been emerged for around two weeks at the time of testing.

2. 3. 2. Analysis of sorghum varieties for protein content, kernel hardness, and grain structure

Nine experimental varieties, grown in Kansas, plus the standard laboratory sorghum diet (N=10), were selected based off the analyzed harness and protein content. Prior to use in all trials, the sorghum varieties were frozen for a minimum of 48 h to eliminate any pre-existing insect infestation. After freezing, the grain was cleaned using a grain dockage sieve (8/64” TRI, Seedburo Equipment Company, Chicago, IL) and a thrasher (Precision Machine Co. INC.,

Lincoln, NE) to remove glumes, dust, and other foreign materials. All sorghum varieties were analyzed by near-infrared spectroscopy (NIR) analysis to measure protein content in triplicate using a LECO FP 828P nitrogen and protein analyzer (LECO Corporation, St. Joseph, MI). Briefly, 1 g subsamples of each variety (N=3) were ground into a fine powder, and samples were combusted within the LECO analyzer. The nitrogen percentage collected post-combustion was calculated into a crude protein percentage. The experimental varieties were categorized into three groups, low, medium, and high, based on protein content of the kernel.

Kernel hardness was determined using the single kernel characterization system (Perten SKCS 4100, Perten Instruments North America, Springfield, IL) using 100 sorghum kernels per rep (N=3) as described by Bean et al. (2006). The experimental varieties were categorized into three groups, low, medium, and high, based on kernel hardness.

The sorghum kernel internal grain structures were measured by selecting 10 kernels of each variety and longitudinally cutting the kernel in half utilizing the germ as a reference point (Arthur et al. 2020). Images of the internal structures were taken using a Keyence digital microscope (Keyence, VHX-6000, Osaka, Japan). The stage of the microscope was positioned such that the cut surface was perpendicular to the microscope, and the entire germ and endosperm were exposed. Images of sectioned kernels were measured using the image analysis tools available on the microscope for the area of the endosperm, floury/soft endosperm, hard endosperm, and the germ (Figure 2.1A, B, C).

To maintain objectivity and protect the identity of commercially sourced sorghum experimental varieties, all varieties were assigned anonymized labels. Each code reflects the insect species, the grain trait being tested, and the relative ranking of that trait. For experiments involving *R. dominica*, the prefix “LGB” (lesser grain borer, the species common name) was used, while “RW” (rice weevil) was used for *S. oryzae*. The second character in each code, “P” for protein or “H” for hardness, indicates the experimental focus. Trait levels were denoted by “L” (low), “M” (medium), or “H” (high), followed by a numeral (1–3) to distinguish between varieties within each group. For example, LGB-PL1 refers to *R. dominica* and the first low-protein variety used, while RW-HH3 indicates *R. dominica* and the third high-hardness variety tested. This naming convention was consistently applied across all experiments to ensure clarity while minimizing potential bias from variety recognition.

2. 3. 3. Insect Infestations

For each experimental treatment, three replicate samples of 50 g per sorghum variety were selected and placed into 0.12 L plastic vials fitted with screened lids to allow for air exchange. Ten two-week-old mixed sex adult *R. dominica* or *S. oryzae* were introduced into each vial and placed in and environmental chamber set at 27°C and 60% r.h in complete darkness. Adults were held on sorghum samples for 7 d, after which adults were removed and assessed for mortality. The sorghum vials were returned to the environmental chamber and held an additional 8 wks to allow for larval development and adult emergence. This experiment was replicated three times (blocks) on different weeks using different insect colonies, which resulted in a total of 9 independent experimental units per species and sorghum variety combination.

After 8 wks, all samples were frozen for a minimum of 48 h to terminate any remaining insect activity. The contents of each vial were sieved using a sieve stack consisting of #12 (1700 µm), #30 (600 µm), and a catch pan (U.S. Standard sieves, Dual Manufacturing Co., Chicago, IL). Intact sorghum kernels were retained on the #12 sieve, adult beetles were retained on the #30 sieve, and frass/fines were collected in the catch pan. The number of adult progenies was counted, and the frass/fines weight (g) was recorded for each sample.

Grain damage was determined by randomly collecting three 5 g sub-samples from each vial and 100 kernels were examined under a stereomicroscope (AM73515MT8A Dino-Lite Edge 3.0, Dunwell Tech, Inc., Torrance, CA). Kernels were scored as insect-damaged kernels (IDKs) if they exhibited signs of insect activity, including visible adult emergence holes (Figure 2.2A) or surface-level adult feeding damage (Figure 2.2B). The percentage of IDKs per vial was calculated using the following formula:

Equation 2.1: $Percent\ IDKs = \left[\frac{Insect\ damaged\ kernels}{Undamaged\ kernels + Insect\ damaged\ kernels}\right] x\ 100$

2. 3. 4. X-ray Image analysis

During the 8 wk holding period, approximately 100 kernels per sample were randomly selected at weekly intervals and examined under an x-ray imaging (Faxitorn X-ray Corp., Wheeling, IL, MX-20) to detect internal larval development based off Brabec et al. (2010) (Figure 2.3). Kernels that were observed to have larvae, pupae, or adults, were selected and frozen for at least 48 hrs to terminate further development. The infested kernels were longitudinally sectioned, and cross-sectional images were captured using the Keyence digital microscope as described previously. The total area consumed by the larvae, including portions of

the germ and the soft and hard endosperm, was measured by cross-sectioning infested kernels and using the same Keyence digital microscope that was used to measure tissue composition of varieties prior to bioassays. This process was repeated weekly for each sorghum variety and species combination replicate until adult emergence was observed.

2. 3. 5. Data analysis

To evaluate the effects of sorghum grain traits on insect infestation outcomes, all data were analyzed separately by insect species and grain traits, and replicate blocks were treated as independent and combined for analysis. For adult mortality, adjusted mortality values were calculated using Abbott's formula (Abbott, 1925) to account for natural mortality observed in control treatments (Equation 2.2). The percent adjust mortality and IDKs were transformed to angular value and progeny and frass/fines weight was transformed to $\log_{10}(x + 1)$ to satisfy assumptions of normality (Zar, 2010).

Equation 2.2: $$Adjusted\ mortality\ = \left[\frac{Mortality\ in\ treatment + Mortality\ in\ Control}{100 - Mortality\ in\ Control}\right] x\ 100$$

Data was then subjected to a one-way analysis of variance (ANOVA), with either protein content or hardness level as the main factor, depending on the experiment, using the General Linear Model (GLM) in the Statistical Analysis System (Version 9.4, SAS Institute, Cary, NC). If the ANOVA was significant, means were compared using least squares means (LS Means) with Tukey's Honestly Significant Difference (HSD) test ($\alpha = 0.05$). Adjusted mortality values were calculated using Abbott's formula (Abbott, 1925) to account for natural mortality observed in control treatments (Equation 2.2). To assess general larval feeding preferences across kernel tissues, tissue consumption data were pooled across all sorghum varieties within each experiment and analyzed separately for *R. dominica* and *S. oryzae*. The percentage of soft endosperm, hard endosperm, and germ consumed was calculated by dividing the consumed area of each tissue type by its total measured area, soft and hard endosperm were expressed relative to total endosperm area, and germ relative to total kernel area. Total kernel consumption was calculated as the proportion of all consumed tissue relative to the full kernel cross-sectional area. This pooled approach allowed for a generalized evaluation of tissue preference, independent of sorghum variety. All consumption percentage values were transformed to angular value prior to

statistical analysis. One-way ANOVAs were conducted using the GLM in SAS to test for significant differences in tissue consumption across weeks of larval development. If the ANOVA was significant, were compared using least squares means (LS Means) with Tukey's Honestly Significant Difference (HSD) test ($\alpha = 0.05$).

2. 4. Results

2. 4. 1. Effect of kernel hardness on *Rhyzopertha dominica* development

The sorghum varieties selected for the kernel hardness experiment varied widely in physical and anatomical characteristics (Table 2.1). Kernel hardness index ranged from 37.03 ± 1.39 (LGB-HL1) to 97.50 ± 0.74 (LGB-HH3) among all samples (Table 2.1). The sorghum varieties were divided into low (37 – 53), medium (80 – 82), and high (86 – 98) hardness categories. Each category, low, medium, and high, were statistically different from each other ($F = 235.84$; df = 9, 20; $P < 0.05$) and the control variety fell within the medium hardness category.

The germ area (23–33%) and total endosperm area (67–77%) were consistent across varieties and did not vary significantly among the varieties (germ area: $F = 1.37$; df = 9, 90; $P = 0.213$; endosperm area: $F = 1.37$; df = 9, 90; $P = 0.2136$). However, the internal distribution between soft and hard endosperm varied significantly (soft endosperm: $F = 19.40$; df = 9, 90; $P < 0.0001$; hard endosperm: $F = 19.40$; df = 9, 90; $P < 0.05$). The low-hardness varieties (LGB-HL1, HL2, HL3) had a higher percentage of soft endosperm, >72% among the varieties, compared to the high high-hardness varieties, with soft endosperm < 44% (Table 2.1). The control variety had a hardness index and endosperm composition categorized between the low and medium categories.

Sorghum varieties varying in hardness had a significant effect on the amount of frass produced by *R. dominica* ($F = 3.53$; df = 9, 80; $P < 0.05$) and the percentage of IDKs (F = 3.71; df = 9, 80; P = 0.0006), but not on the adjusted adult mortality ($F = 1.42$; df = 8, 72; $P = 0.2029$) or the number of progeny ($F = 1.37$; df = 9, 80; $P = 0.2134$). The adjusted adult mortality was <14% among all the varieties. The low-hardness varieties (LGB-HL1, LGB-HL2, LGB-HL3) overall had the highest mean progeny counts among all hardness varieties tested ranging from 63 – 97 adults, though there was not statistical difference among the varieties. The low hardness varieties also had the highest levels of frass and IDKs, which indicates that these sorghum varieties are highly susceptible to *R. dominica* feeding. Compared to the control sorghum,

progeny counts and IDKs were higher in the low hardness varieties but did not differ statistically. In contrast, the medium-hardness varieties, LGB-HM1, LGB-HM2, and LGB-HM3 had similar progeny counts, frass, and IDKs compared to the hard sorghum varieties (Table 2.2). The exception was LGB-HM2, which had the fewest progeny, frass, and IDKs among all the varieties tested, but was not statistically different from the other medium hardness varieties.

2. 4. 2. Effect of protein content on *Rhyzopertha. dominica* development

Sorghum varieties used in the protein content experiment exhibited a wide range of protein content and internal structural characteristics that varied significantly ($F = 480.97$; df = 9, 19; $P < 0.05$). The sorghum variety had a significant effect on the percent area of soft endosperm ($F = 7.24$; df = 9, 90; $P < 0.05$) and hard endosperm ($F = 7.24$; df = 9, 90; $P < 0.05$), but not for the percent germ area ($F = 0.89$; df = 9, 90; $P = 0.5375$) or the percent total endosperm area ($F = 7.24$; df = 9, 90; $P = 0.5375$).

Protein content ranged from 9.09% (LGB-PL1) to 13.16% (LGB-PH3) and thus the sorghum varieties were separated into low (9.0 – 9.9%), medium (10.0 – 11.5%), and high (11.9 – 13.2%) categories (Table 2.3) and the control variety fell into the low protein category. Due to limited varietal availability, there was some unavoidable statistical overlap among the protein contents. Overall, the percentage germ and endosperm areas of the kernel were consistent among the varieties, except for LGB-PH2. The germ consisted of 53% of the kernel and the endosperm was only 47%, both components were statistically different among all the varieties tested. Soft and hard endosperm distribution varied among the varieties, with the low-protein varieties tending to have higher proportion of soft endosperm (e.g., LGB-PL1 at 72.12 ± 4.83%), while several mid- and high-protein lines had more balanced or hard-endosperm-dominated compositions, such as LGB-PM3 at 61.19 ± 3.44% hard endosperm.

The sorghum variety had significant effects on adult mortality ($F = 2.53$; df = 8, 69; $P < 0.05$), progeny ($F = 3.00$; df = 9, 76; $P < 0.05$), frass ($F = 2.24$; df = 9, 76; $P < 0.05$), and IDKs ($F = 4.04$; df = 9, 76; $P < 0.05$). The adjusted adult *R. dominica* mortality ranged significantly among all the sorghum varieties from 3% in the LGB-PH3 and LGB-PL1 varieties to ~19% for the LGB-PM1 and LGB-PH2 varieties (Table 2.4). The highest adult progeny observed after 8 wks was for varieties LGB-PL1, LGB-PH1, and LGB-PH3, with 75, 76, and 103 adults respectively (Table 2.4). These varieties also have the highest IDK values with 15, 16, and 16% IDKs respectively, and the amount of frass produced, 0.71, 0.60, and 0.84 g respectively. In

general, as the number of progenies increased the amount of frass and IDKs also increased. However, there was not a clear trend among the sorghum varieties when comparing the protein content with progeny, frass or IDKs. The control variety yielded intermediate values across all metrics.

2. 4. 3. Sorghum kernel feeding preference by *Rhyzopertha dominica*

The feeding activity of *R. dominica* larvae varied significantly over time as development progressed within infested sorghum kernels (Table 2.5). One-way ANOVA results showed the main effects of total kernel tissue consumed ($F = 76.62$; df = 3, 210; $P < 0.05$), consumed germ ($F = 33.68$; df = 3, 210; $P < 0.05$), consumed soft endosperm ($F = 63.84$; df = 3, 210; $P < 0.05$), and consumed hard endosperm ($F = 22.82$; df = 3, 210; $P < 0.05$) were significant during the 8 wk storage period. These findings indicate that larval feeding in the kernel changed during the length of the study. There were no larvae were observed in the sorghum kernels when observed under x-ray during the first week post parental adult removal. Larvae were first observed 2 weeks after parental adults were removed. After 2 wks, with the total percent kernel consumed was 1.33% (Table 2.5). As the larvae continued to develop inside the grain kernel the percentage of the kernel consumed increased up to 42% at the end of wks 4 and 5. It should be noted that pupae were observed after 4 wks, post parental adult emergence. The pupal stage of *R. dominica* does not feed, thus there was not an increase in kernel consumption. Initial adult emergence was observed after 6 wks post parental adult emergence.

Comparing the individual constituents of the kernel, as the amount of time increased the amount of each constituent also increased. Two wks post parental adult removal, the germ was the most consumed portion of the kernel (Table 2.5), compared to the soft or hard endosperm, 15%, 10% and 0.1% respectively. However, after 3 wks post parental adult removal, consumption of the kernel significantly increased among all components of the kernel as the larval continued to develop and grow (Figure 2.5). Consumption of soft endosperm increased nearly threefold (28.54 ± 2.65%), and germ consumption also rose to 23.12 ± 2.98%. However, hard endosperm was still consumed at relatively low levels (3.26 ± 1.50%), suggesting it was a primary food source during early to mid-stages of development. Soft endosperm remained the most highly consumed tissue type in wks 4 and 5 of development at 62 and 66% of the total soft endosperm area respectively. The germ portion also continued to increase up to 62% of its total

area 4 wks post parental adult removal. The hard endosperm consumption increased significantly up to a maximum of 31% of the total area after 5 wks.

2. 4. 4. Effect of kernel hardness on *Sitophilus oryzae* development

Sorghum varieties used in the *S. oryzae* kernel hardness experiment exhibited a wide range of kernel hardness and endosperm composition. Hardness values ranged from 42.62 ± 0.67 (RW-HL1) to 83.46 ± 0.77 (RW-HH3) (Table 2.6). Varieties were divided into low (43 – 47), medium (56 – 57), and high (78 – 83) hardness categories and the control variety fell into the hard category. Each category, low, medium and high, were statistically different from one another ($F = 402.69$; df = 9, 20; $P < 0.05$).

The percent germ area (25-31%) and the total endosperm area (69-80%) were relatively consistent across all varieties and did not vary significantly (germ area: $F = 0.97$; df = 9, 90; $P = 0.4693$; endosperm area: $F = 0.97$; df = 9, 90; $P = 0.4693$). The internal distribution percentage for soft and hard endosperm, however, was significant and varied across each variety (soft endosperm: $F = 11.19$; df = 9, 90; $P < 0.05$; hard endosperm: $F = 11.19$; df = 9, 90; $P < 0.05$). The low-hardness varieties (RW-HL1, HL2, HL3) had a higher percentage of soft endosperm, exhibiting >76% soft endosperm area, compared to higher-hardness varieties (RW-HH1, RW-HH2, RW-HH3) that had a soft endosperm area <56% (Table 2.6).

Statistical analysis found significant effects of variety on progeny ($F = 3.11$; df = 9, 90; $P < 0.05$), frass accumulation ($F = 3.80$; df = 9, 90; $P < 0.05$), and the percentage of IDKs ($F = 8.08$; df = 9, 90; $P < 0.05$), but not for the adjusted adult mortality ($F = 1.60$; df = 8, 72; $P = 0.1405$). The adjusted adult mortality among all the varieties was <11% (Table 2.7). Among all the varieties, the high-hardness varieties had the lowest mean progeny counts across all hardness varieties tested, ranging from 4 – 32 adults. These varieties also had the lowest levels of frass and IDKs, indicating that harder sorghum kernels are less susceptible. The RW-HM2 statistically the highest number of progeny among all the varieties, except RW-HL1 and the control. All other medium and low varieties did not different statistically in the number of adult progeny after 8 wks. The RW-HM2 variety also had the highest number of IDKs and frass accumulation compared to all other sorghum varieties. 2. 4. 5. Effect of kernel hardness on *Sitophilus oryzae* development

The experimental sorghum varieties used for the *S. oryzae* protein content experiment exhibited a wide range of protein levels and internal characteristics. Protein levels ranged from

9.35 ± 0.05 (RW-PL1) to 13.60 ± 0.09 (RW-PH3) (Table 2.8). Varieties were divided into low (9.35– 10.52%), medium (11.50 – 11.90%), and high (12.57 – 13.60%) protein level categories. The protein percentage among each category, low, medium, and high were statistically different from each other ($F = 281.11$; df = 9, 19; $P < 0.05$).

Paragraph on the endosperm/germ/etc. (like you did for the other results section) Sorghum varieties varying in protein content had a significant effect on the number of progeny produced ($F = 4.52$; df = 9, 77; $P < 0.05$), frass ($F = 11.66$; df = 9, 77; $P < 0.05$), percentage of IDKs ($F = 5.94$; df = 9, 77; $P < 0.05$), but not on the adjusted adult mortality ($F = 1.68$; df = 8, 69; $P = 0.118$). Among all the varieties the adjusted adult mortality was < 12%. The highest number of progeny was observed on the control sorghum with 93 adults. Among all the experimental varieties, the range in adult progeny was 15 – 82 (Table 2.9) There was a significant amount of overlap in adult progeny, but the control and RW-PL1 had significantly higher percent IDKs and frass compared to all other varieties.

2. 4. 6. Sorghum kernel feeding preference by *Sitophilus oryzae*

The feeding activity of *S. oryzae* larvae varied significantly over time as development progressed within infested sorghum kernels (Table 2.10). One-way ANOVA results showed significant effects of week on the percentage of total kernel tissue consumed ($F = 9.04$; df = 3, 109; $P < 0.05$), as well as the consumption of specific tissue types including the germ ($F = 3.48$; df = 3, 109; $P < 0.05$), soft endosperm ($F = 11.87$; df = 3, 109: $P < 0.05$), and hard endosperm ($F = 3.48$; df = 3, 109; $P < 0.05$). These findings indicate the larval feeding tissue preference changed as the larva developed.

Unlike samples infested with *R. dominica*, *S. oryzae* larvae were observed via x-ray 1 week after parental adults were removed. As the experimental study time increased the total amount of kernel consumed significantly increased from 45% at wk 1 to >72% by week 3 and 4. Three weeks after parental adults were removed, some pupae were observed within kernels and adult emergence was noted at wk 4. Like *R. dominica* pupae, *S. oryzae* pupae do not feed, thus there was not an increase in kernel consumption after week 4.

After 1 wk post parental adult removal, the germ was again the most consumed portion of the grain with nearly 15% of the germ area consumed (Table 2.10) compared to the 8% soft or hard endosperm. This trend remained consistent into wk 3, with the germ portion having the highest percentage consumed. However, as the larvae developed inside the kernel the among of

germ, soft and hard endosperm also increased from week 1 to wk 4, the soft and hard endosperm began to be consumed more than the germ portion of the grain, with the hard endosperm making up the majority of consumed tissue at 40%, whereas the germ and soft endosperm percentage that was consumed being at 33% and 34%, respectively. By the end of wk 4, the hard endosperm remained the most consumed potion of sorghum kernels on average, with nearly 50% of the area making up the hard endosperm being consumed by larvae.

2. 5. Discussion

Rhyzopertha dominica and *S. oryzae* are both internal feeders, however the egg laying behavior of the females is one attribute that distinguishes the two species. *Rhyzopertha dominica* females deposit their eggs outside of the kernel and the neonate larvae must penetrate the kernel in order to develop. The *R. dominica* neonate larvae possess long, sharply pointed mandibles with prominent incisor regions that enable the larvae to penetrate tough plant tissues (Sharifi et al., 2008). These strong mouthparts allow the species to initiate feeding on intact, undamaged kernels with minimal dependence on prior damage or kernel softness. In contrast, *S. oryzae* females rely on a relatively short, less-sclerotized rostrum to drill oviposition sites within an intact kernel and deposit eggs within the grain (Abedi et al., 2024). The successful infestation of the kernel is contingent on the female's ability to breach the kernel surface and deposit an egg. The larvae, which remain confined to the oviposition site, also possess less robust mandibles, limiting their ability to expand into denser tissues during development (Sharifi et al., 2008). This reliance on external conditions and softer entry points makes *S. oryzae* more vulnerable to resistance traits such as increased hardness and pericarp thickness (Mwenda et al., 2019).

Rhyzopertha dominica produced the more progeny, frass, and IDKs on the low-hardness varieties LGB-HL1 and LGB-HL2. However, this relationship was not linear or consistent across all observations. *Rhyzopertha dominica* had significantly lower frass and IDKs on the medium hardness varieties, compared LGB-HM1 and LGB-HM2 but was not significantly different compared to LGB-HH1. Toews et al. (2000) similarly reported that while wheat cultivar influenced *R. dominica* performance, susceptibility did not correlate strictly with hardness, such as smaller kernel size sometimes facilitated larger progeny output. Likewise, Arthur et al. (2020) observed that hardness alone did not explain infestation outcomes in sorghum; for instance, red-tannin and red-waxy varieties with intermediate hardness values produced fewer progeny than soft white and red non-tannin varieties. This demonstrates that kernel hardness is one factor that

determines susceptibility but is likely to interact with other kernel traits to determine resistance levels. Similar to *R. dominica,* the number of *S. oryzae* progeny were varied significantly among the different hardness varieties. In general, the varieties that had the highest progeny counts also had the most frass and IDKs. Kordan et al. (2023) found that on wheat (*Triticum aestivum*), higher kernel hardness was generally associated with lower progeny numbers, less frass production, and reduced grain mass loss by *Sitophilus granarius* (L.), granary weevil, in some cultivars. However, the effect was not consistent across all varieties, some hard cultivars still supported high levels of infestation, suggesting that while hardness can impair larval penetration and development, its protective role is influenced by other factors such as starch or protein content.

In our study, varieties with higher proportions of soft endosperm (e.g., RW-PL1 and LGB-HL1) supported more extensive early larval development (Tables 2,1, 2,4, 2.9, 2.12). As the larvae matured within the grain kernel, both species expanded feeding from the germ into the endosperm of the kernel. This was evident in the progressive tissue consumption increased weekly for each species. Our results and previous studies (Belton et al., 2006) suggest that it is the structure and distribution of these proteins that dictate resistance. For example, RW-PH3 exhibited both high protein and hard endosperm, while RW-PM2 had high protein but retained a higher area of soft endosperm (Table 2.12), supporting earlier conclusions that protein content alone is a poor predictor of resistance (Hamaker et al., 1987). Insect protein digestion studies clarify these findings. Although *R. dominica* can digest kafirins using trypsin-like serine proteases, digestibility declines in high-kafirin, cross-linked environments (Oppert et al., 2000). Jood et al. (1990) observed up to a 55% reduction in protein digestibility following *R. dominica* infestation, suggesting that dense kafirin matrices limit digestion efficiency. Thus, while both species can hydrolyze kafirins to some extent, their success depends on matrix structure and accessibility (Duodu et al., 2003).

The soft endosperm contains loosely arranged starch granules embedded in a thin protein matrix, making it more accessible to insect mandibles, especially in early larval stages (Shull et al., 1990). In contrast, the hard endosperm features tightly packed starch granules encased in a dense, cross-linked protein matrix, which provides structural integrity and reduces tissue accessibility (Hoseney et al., 1974). The early instars of both species are particularly vulnerable to such barriers due to limited boring ability. Arthur et al. (2020) similarly reported significantly

greater *R. dominica* progeny on red-tannin sorghum, a variety with lower kernel hardness, starch content, and endosperm vitreousness, indicating that softer grains are more susceptible. Structural durability may also limit infestation risk post-harvest by reducing kernel fracture during handling and storage. Although studied in rice, Kavallieratos et al. (2012) demonstrated that *R. dominica* progeny output increased significantly when more than 10% of kernels were cracked, even in resistant varieties. This suggests that the physical integrity of the grain plays a key role in mitigating pest establishment. By extension, more vitreous and intact sorghum kernels may be less vulnerable to colonization, supporting integrated resistance breeding and grain handling strategies.

The results of our study have demonstrated the complexity of sorghum grain composition on the infestation and reproductive ability of *R. dominica* and *S. oryzae*. These findings have important implications for stored product pest management and sorghum growers across the U.S. We have identified potential varietal traits in sorghum that could reduce pest infestation during storage, such as kernel hardness and protein content. Sorghum breeding programs can incorporate these findings to select future varietal lines that are less susceptible to internal feeders during storage. However, further studies are warranted on the long-term impacts of kernel hardness, protein content, and other constituents that could prevent or minimize infestation by stored product insects. Furthermore, integrating grain-based traits into IPM strategies enhances system resilience and supports economic and ecological sustainability in post-harvest protection.

2. 6. Figures and tables

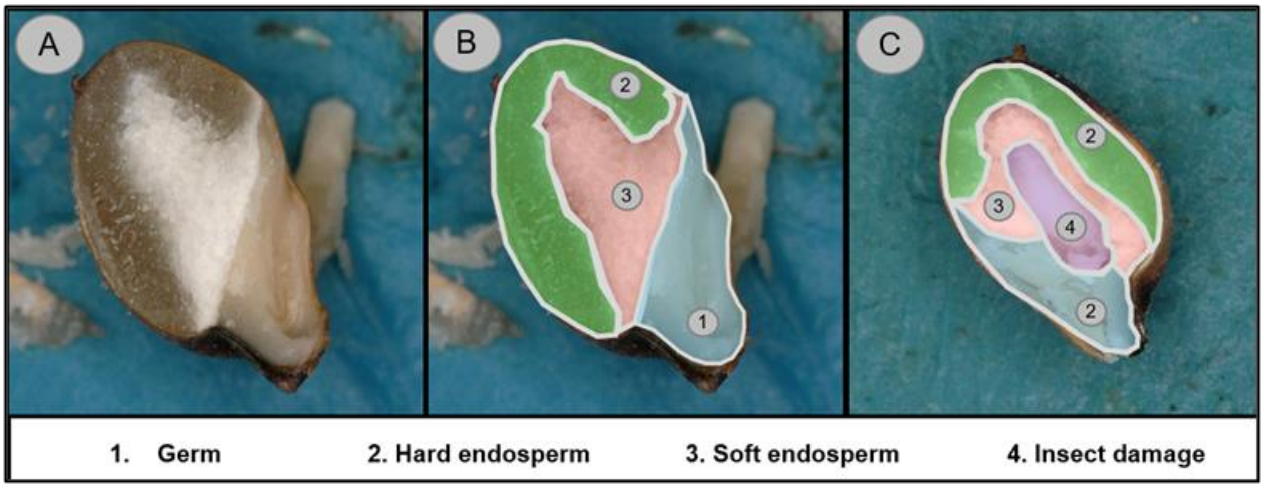

Figure 2.1 Cross-sectioned images of A) sorghum kernel; B) sorghum with the germ in blue (1), hard endosperm in green (2), and the soft endosperm in pink (3) labeled; and C) infested sorghum kernel at 4 wks with insect damage labeled in purple (4).

Figure 2.2 Insect damage kernels (IDKs) resulting from A) adult emergence holes and B) adult insect feeding on the outside of the kernel.

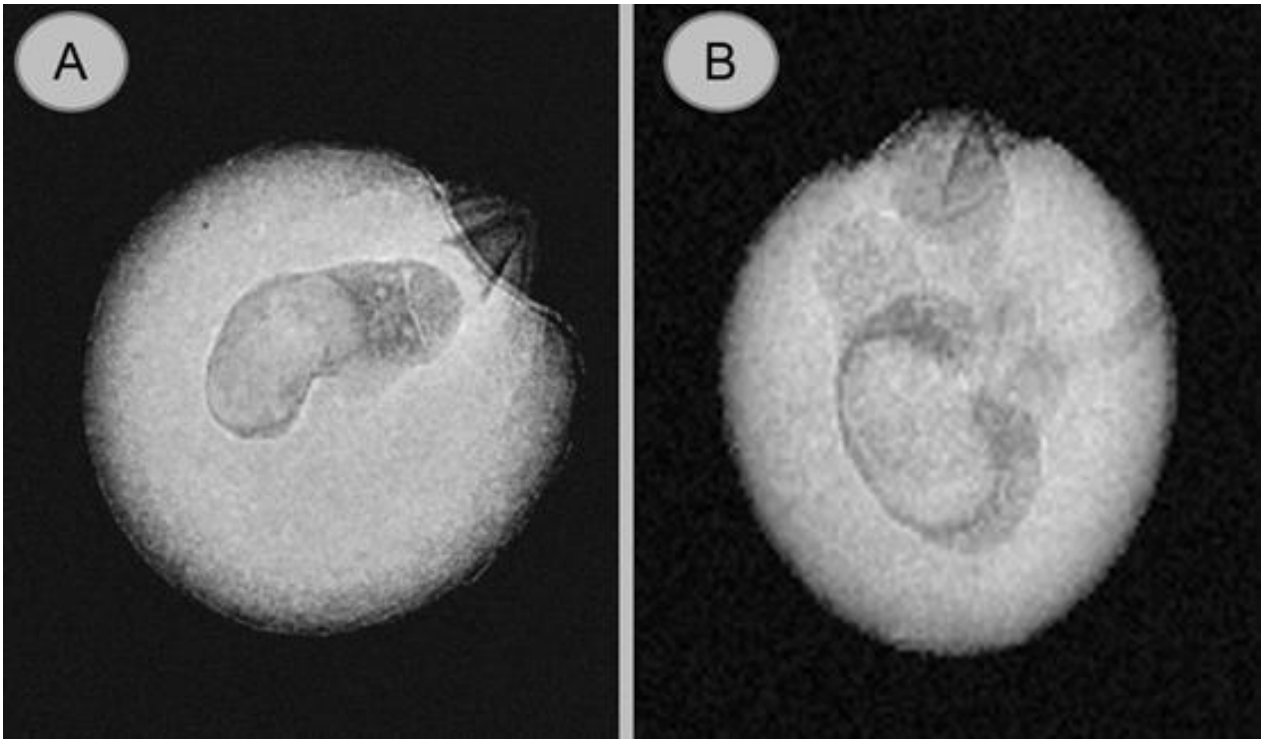

Figure 2.3 X-ray images of sorghum kernels infested with either A) *Rhyzopertha dominica* larvae or B) *Sitophilus oryzae* larvae.

Table 2.1 Mean (± SE) hardness index values, percent germ area, percent total endosperm area, percent soft and percent hard endosperm area for each sorghum variety used in the kernel hardness experiments assessing susceptibility to *Rhyzopertha dominica* infestation. Means within each column followed by a different uppercase letter are significantly different ($P < 0.05$, Proc GLM, LSMeans with Tukey's HSD test).

Variety	Hardnes index	Germ area	Total endosperm	Soft endosperm[a]	Hard endosperm[a]
Control	77.75 ± 0.81C	25.84 ± 1.87	74.16 ± 1.87	54.01 ± 2.45B	45.99 ± 2.45B
LGB-HL1	37.03 ± 1.39F	26.44 ± 0.60	73.56 ± 0.60	82.00 ± 5.01A	18.00 ± 5.01C
LGB-HL2	44.77 ± 2.42E	32.72 ± 4.68	67.28 ± 4.68	78.78 ± 3.94A	21.22 ± 3.94C
LGB-HL3	52.45 ± 0.96D	25.28 ± 2.62	74.72 ± 2.62	72.12 ± 4.83A	27.88 ± 4.83C
LGB-HM1	80.67 ± 1.39C	26.23 ± 0.89	73.77 ± 0.89	45.48 ± 2.42C	54.52 ± 2.42AB
LGB-HM2	80.79 ± 1.00C	25.10 ± 1.40	74.90 ± 1.40	48.82 ± 1.64C	51.18 ±AB 1.64AB
LGB-HM3	81.46 ± 1.88C	23.32 ± 2.60	76.68 ± 2.60	50.03 ± 3.25C	49.97 ±A 3.25
LGB-HH1	86.67 ± 0.93B	25.49 ± 1.34	74.51 ± 1.34	40.78 ± 1.74C	59.22 ± 1AB.74
LGB-HH2	90.88 ± 0.05A	28.57 ± 1.49	71.43 ± 1.49	44.27 ± 3.17C	55.7A3 ± 3.17
LGB-HH3	90.88 ± 0.74A	26.12 ± 0.98	73.88 ± 0.98	42.69 ± 4.91C	57.31 ± 4.91

[a]Soft and hard endosperm percentages are expressed relative to the total endosperm area.

Table 2.2 Mean (± SE) percent adjusted adult mortality, total progeny, frass, and percent IDKs for *Rhyzopertha dominica* on 10 different sorghum varieties varying in kernel hardness levels. Means within each column followed by a different uppercase letter are significantly different ($P < 0.05$, Proc GLM, LSMeans with Tukey's HSD test).

Variety	Adjusted adult mortality[a]	Progeny	Frass (g)	% IDKs
Control	*	55.67 ± 11.62	0.65 ± 0.13AB	18.97 ± 3.42AB
LGB-HL1	9.41 ± 3.85	74.33 ± 20.14	0.87 ± 0.23A	31.65 ± 7.00A
LGB-HL2	3.59 ± 2.33	97.44 ± 30.84	0.98 ± 0.29A	29.63 ± 6.05A
LGB-HL3	9.13 ± 4.65	63.44 ± 19.34	0.68 ± 0.20AB	30.32 ± 4.68A
LGB-HM1	14.02 ± 4.86	23.67 ± 6.07	0.17 ± 0.05C	13.24 ± 2.90BC
LGB-HM2	14.28 ± 6.47	11.89 ± 3.22	0.09 ± 0.02C	6.75 ± 1.09C
LGB-HM3	10.45 ± 5.23	41.00 ± 9.28	0.35 ± 0.09BC	13.76 ± 2.73BC
LGB-HH1	1.11 ± 1.11	55.67 ± 13.59	0.44 ± 0.12ABC	22.57 ± 3.99AB
LGB-HH2	8.18 ± 4.07	48.22 ± 11.53	0.32 ± 0.09BC	14.50 ± 2.67BC
LGB-HH3	9.14 ± 2.53	30.11 ± 11.33	0.22 ± 0.08C	16.44 ± 3.91BC

[a]The adjusted mortality within the control group is zero after implementing Abbott's formula and is noted with a *.

Table 2.3 Mean (± SE) protein content, percent germ area, percent total endosperm area, percent soft, and percent hard endosperm for each sorghum variety used in the kernel protein content experiments assessing susceptibility to *Rhyzopertha dominica* infestation. Means within each column followed by a different uppercase letter are significantly different ($P < 0.05$, Proc GLM, LSMeans with Tukey's HSD test).

Variety	Protein content	Germ area	Total endosperm	Soft endosperm[a]	Hard endosperm[a]
Control	9.25 ± 0.45HI	25.84 ± 1.87	74.16 ± 1.87	54.01 ± 2.45BC	45.99 ± 2.45BC
LGB-PL1	9.09 ± 0.12I	25.28 ± 2.62	74.72 ± 2.62	72.12 ± 4.83A	27.88 ± 4.83D
LGB-PL2	9.35 ± 0.02H	25.10 ± 1.40	74.90 ± 1.40	48.82 ± 1.64D	51.18 ± 1.64AB
LGB-PL3	9.91 ± 0.05G	30.97 ± 7.76	69.03 ± 7.76	53.91 ± 2.28C	46.09 ± 2.28BC
LGB-PM1	10.18 ± 0.06F	20.95 ± 1.32	79.05 ± 1.32	60.11 ± 5.59B	39.89 ± 5.59C
LGB-PM2	10.52 ± 0.05E	26.12 ± 0.98	73.88 ± 0.98	42.69 ± 4.91D	57.31 ± 4.91A
LGB-PM3	11.50 ± 0.07D	28.40 ± 1.39	71.60 ± 1.39	38.81 ± 3.44D	61.19 ± 3.44A
LGB-PH1	11.90 ± 0.08C	25.49 ± 1.34	74.51 ± 1.34	40.78 ± 1.74D	59.22 ± 1.74A
LGB-PH2	12.43 ± 0.04B	52.88 ± 1.11	47.12 ± 5.26	52.88 ± 5.26CD	25.87 ± 1.11AB
LGB-PH3	13.16 ± 0.05A	28.94 ± 1.24	71.06 ± 1.24	44.67 ± 2.22CD	55.33 ± 2.22AB

[a]Soft and hard endosperm percentages are expressed relative to the total endosperm area.

Table 2.4 Mean (± SE) percent adjusted adult mortality, progeny, frass, and percent IDKs for *Rhyzopertha dominica* on 10 different sorghum varieties varying in protein content. Means within each column followed by a different uppercase letter are significantly different ($P < 0.05$, Proc GLM, LSMeans with Tukey's HSD test).

Variety	Adjusted adult mortality[a]	Progeny	Frass (g)	% IDKs
Control	*	71.33 ± 17.27AB	0.65 ± 0.17ABC	12.62 ± 1.85AB
LGB-PL1	2.56 ± 1.46D	74.56 ± 16.49A	0.71 ± 0.18AB	15.16 ± 1.97A
LGB-PL2	17.24 ± 5.57ABC	50.75 ± 22.09D	0.46 ± 0.21ABCD	7.60 ± 2.74C
LGB-PL3	6.01 ± 4.67CD	62.44 ± 13.80AB	0.35 ± 0.07BCD	12.67 ± 1.99AB
LGB-PM1	19.80 ± 7.52AB	29.67 ± 6.38BCD	0.31 ± 0.11CD	9.43 ± 1.79BC
LGB-PM2	6.87 ± 3.72BCD	52.00 ± 11.44ABC	0.38 ± 0.09BCD	14.73 ± 1.91AB
LGB-PM3	14.68 ± 5.31BCD	70.00 ± 22.58ABCD	0.50 ± 0.15ABCD	13.72 ± 2.99AB
LGB-PH1	3.30 ± 2.17D	76.44 ± 6.35A	0.60 ± 0.06ABC	16.14 ± 1.04A
LGB-PH2	18.94 ± 5.70A	22.00 ± 3.89D	0.17 ± 0.03D	5.28 ± 0.88C
LGB-PH3	2.60 ± 1.58CD	103.00 ± 23.69A	0.84 ± 0.27A	15.93 ± 3.07A

[a]The adjusted mortality within the control group is zero after implementing Abbott's formula and is noted with a *.

Table 2.5 Mean (± SE) percent of total kernel, germ, soft endosperm, and hard endosperm area consumed by *Rhyzopertha dominica* larvae after 2, 3, 4, and 5 weeks post parental adult removal. Means followed by different letters within the same column indicate statistically significant difference among the storage weeks (*P* < 0.05, PROC GLM; LSMeans with Tukey's HSD test).

Week	% Kernel total	% Germ	% Soft endosperm	% Hard endosperm
Week 1[a]	-	-	-	-
Week 2	1.33 ± 8.79C	15.20 ± 3.33B	10.31 ± 2.28C	0.11 ± 0.11C
Week 3	13.91 ± 5.18B	23.12 ± 2.98B	28.54 ± 2.65B	3.26 ± 1.50C
Week 4	42.24 ± 6.67A	62.02 ± 4.10A	61.63 ± 3.30A	24.05 ± 4.73B
Week 5	41.99 ± 7.58A	55.99 ± 4.07B	66.17 ± 3.18A	31.31 ± 4.13A

[a]Across all varieties, no images of kernels infested by *R. dominica* larvae were observed at week 1.

Table 2.6 Mean (± SE) hardness index values, percent germ area, percent total endosperm area, percent soft and percent hard endosperm area for each sorghum variety used in the kernel hardness experiments assessing susceptibility to *Sitophilus oryzae* infestation. Means within each column followed by a different uppercase letter are significantly different ($P < 0.05$, Proc GLM, LSMeans with Tukey's HSD test).

Variety	Hardness index	Germ area	Endosperm area	Soft endosperm area[a]	Hard endosperm area[a]
Control	77.75 ± 0.81B	25.84 ± 1.87	74.16 ± 1.87	54.01 ± 2.45CD	45.99 ± 2.45AB
RW-HL1	42.62 ± 0.67F	24.75 ± 0.93	75.25 ± 0.93	76.30 ± 6.41A	23.70 ± 6.41D
RW-HL2	45.67 ± 0.63E	27.82 ± 1.75	72.18 ± 1.75	79.99 ± 5.47A	20.01 ± 5.47D
RW-HL3	47.40 ± 0.68E	25.73 ± 1.35	74.27 ± 1.35	81.91 ± 5.63A	18.09 ± 5.63D
RW-HM1	56.65 ± 0.16D	25.21 ± 2.17	74.79 ± 2.17	61.28 ± 4.35BC	38.72 ± 4.35BC
RW-HM2	56.82 ± 0.66D	25.94 ± 1.55	74.06 ± 1.55	70.78 ± 3.63AB	29.22 ± 3.63DC
RW-HM3	56.94 ± 0.11D	20.54 ± 1.98	79.46 ± 1.98	71.94 ± 2.42AB	28.06 ± 2.42DC
RW-HH1	77.96 ± 0.41C	25.77 ± 0.76	74.23 ± 0.76	47.25 ± 1.78D	52.75 ± 1.78A
RW-HH2	78.44 ± 2.48B	30.97 ± 7.76	69.03 ± 7.76	53.91 ± 2.28DC	46.09 ± 2.28AB
RW-HH3	83.46 ± 0.77A	29.63 ± 1.48	70.37 ± 1.48	44.95 ± 3.57D	55.05 ± 3.57A

[a]Soft and hard endosperm percentages are expressed relative to the total endosperm area.

Table 2.7 Mean (± SE) percent adjusted adult mortality, total progeny, frass, and percent IDKs for *Sitophilus oryzae* on ten different sorghum varieties varying in kernel hardness levels. Means within each column followed by a different uppercase letter are significantly different ($P < 0.05$, Proc GLM, LSMeans with Tukey's HSD test).

Variety	Adjusted adult mortality[a]	Progeny	Frass (g)	% IDKs
Control	*	67.44 ± 15.56AB	0.39 ± 0.11BC	12.98 ± 2.94B
RW-HL1	3.33 ± 1.67	82.78 ± 15.97AB	0.55 ± 0.11B	16.27 ± 1.85B
RW-HL2	3.33 ± 1.67	48.22 ± 8.91BC	0.29 ± 0.06CD	7.61 ± 1.44C
RW-HL3	6.67 ± 3.33	51.22 ± 13.87BC	0.30 ± 0.09CD	7.91 ± 1.68C
RW-HM1	7.78 ± 4.34	45.22 ± 8.12BC	0.28 ± 0.05CD	13.64 ± 2.20B
RW-HM2	0.00 ± 0.00	113.44 ± 11.21A	0.93 ± 0.08A	27.04 ± 1.05A
RW-HM3	2.22 ± 1.47	58.00 ± 10.02B	0.37 ± 0.07BC	14.77 ± 2.25B
RW-HH1	10.00 ± 3.73	31.78 ± 5.26BC	0.14 ± 0.02DE	5.39 ± 0.78C
RW-HH2	11.11 ± 3.89	4.11 ± 1.45D	0.02 ± 0.01E	0.55 ± 0.20D
RW-HH3	5.56 ± 2.42	23.78 ± 3.29C	0.09 ± 0.01E	4.15 ± 0.41C

[a]The adjusted mortality within the control group is zero after implementing Abbott's formula, and is noted with a *.

Table 2.8 Mean (± SE) percent adjusted adult mortality, progeny, frass, and percent IDKs for *Sitophilus oryzae* on 10 different sorghum varieties varying in protein content. Means within each column followed by a different uppercase letter are significantly different ($P < 0.05$, Proc GLM, LSMeans with Tukey's HSD test).

Variety	Protein content	% Germ	% Endosperm	% Soft endosperm[a]	% Hard endosperm[a]
Control	9.25 ± 0.45H	25.84 ± 1.87	74.16 ± 1.87	54.01 ± 2.45C	45.99 ± 2.45C
RW-PL1	9.35 ± 0.05H	26.44 ± 0.60	73.56 ± 0.60	82.00 ± 5.01A	18.00 ± 5.01E
RW-PL2	9.91 ± 0.05G	30.97 ± 7.76	69.03 ± 7.76	53.91 ± 2.28C	46.09 ± 2.28C
RW-PL3	10.52 ± 0.05F	26.12 ± 0.98	73.88 ± 0.98	42.69 ± 4.91DE	57.31 ± 4.91AB
RW-PM1	11.50 ± 0.07E	28.40 ± 1.39	71.60 ± 1.39	38.81 ± 3.44E	61.19 ± 3.44A
RW-PM2	11.77 ± 0.03D	33.21 ± 1.32	66.79 ± 6.60	33.21 ± 6.60B	25.22 ± 1.32D
RW-PM3	11.90 ± 0.08D	25.49 ± 1.34	74.51 ± 1.34	40.78 ± 1.74DE	59.22 ± 1.74B
RW-PH1	12.57 ± 0.21C	23.32 ± 2.60	76.68 ± 2.60	50.03 ± 3.25CD	49.97 ± 3.25BC
RW-PH2	13.16 ± 0.05B	28.94 ± 1.24	71.06 ± 1.24	44.67 ± 2.22CDE	55.33 ± 2.22ABC
RW-PH3	13.60 ± 0.09A	25.77 ± 0.76	74.23 ± 0.76	47.25 ± 1.78CDE	52.75 ± 1.78ABC

[a]Soft and hard endosperm percentages are expressed relative to the total endosperm area.

Table 2.9 Mean (± SE) percent adjusted adult mortality, total progeny, frass, and percent IDKs for *Sitophilus oryzae* on 10 different sorghum varieties varying in protein content. Means within each column followed by a different uppercase letter are significantly different ($P < 0.05$, Proc GLM, LSMeans with Tukey's HSD test).

Variety	Adjusted adult mortality[a]	Progeny	Frass (g)	% IDKs
Control	*	93.00 ± 17.71A	0.43 ± 0.08A	20.51 ± 3.89A
RW-PL1	2.25 ± 1.84	82.33 ± 16.93A	0.59 ± 0.12A	15.28 ± 2.72A
RW-PL2	8.42 ± 4.33	15.22 ± 4.02D	0.06 ± 0.02B	3.46 ± 0.56D
RW-PL3	15.87 ± 7.22	19.11 ± 4.57CD	0.07 ± 0.02B	5.36 ± 0.80CD
RW-PM1	12.30 ± 6.63	22.56 ± 4.72BCD	0.07 ± 0.02B	8.28 ± 2.10C
RW-PM2	0.79 ± 0.52	20.67 ± 6.48CD	0.08 ± 0.02B	8.21 ± 1.43C
RW-PM3	0.79 ± 0.52	38.22 ± 9.66ABC	0.16 ± 0.03B	9.64 ± 1.76BC
RW-PH1	10.72 ± 5.61	47.89 ± 6.31AB	0.17 ± 0.03B	6.74 ± 1.15CD
RW-PH2	0.29 ± 0.29	33.17 ± 2.27ABC	0.09 ± 0.02B	7.44 ± 1.36CD
RW-PH3	7.38 ± 5.09	48.89 ± 13.95ABC	0.16 ± 0.04B	7.35 ± 1.80CD

[a]The adjusted mortality within the control group is zero after implementing Abbott's formula, and is noted with a *.

Table 2.10 Mean (± SE) percent of total kernel, germ, soft endosperm, and hard endosperm area consumed by *Sitophilus oryzae* larvae after 1, 2, 3, and 4 weeks post parental adult removal. Means followed by different letters within the same column indicate statistically significant difference among the storage weeks ($P < 0.05$, PROC GLM; LSMeans with Tukey's HSD test).

Week	% Kernel total	% Germ	% Soft endosperm	% Hard endosperm
Week 1	45.40 ± 5.18B	14.68 ± 6.43B	8.08 ± 5.82B	7.84 ± 3.81B
Week 2	48.39 ± 3.99B	21.11 ± 4.45B	7.87 ± 3.22B	7.39 ± 1.98B
Week 3	76.60 ± 4.40A	32.87 ± 7.89AB	34.14 ± 7.56A	40.11 ± 5.50A
Week 4	72.22 ± 6.22A	41.98 ± 5.93A	42.52 ± 7.58A	49.88 ± 4.86A

Chapter 3 - Comparative Performance of Four Insecticides against Key Stored Grain Pests on Sorghum under Variable Temperatures

3.1 Abstract

Stored-product insect pests pose a major threat to global grain reserves by reducing quality and quantity during post-harvest bulk storage. The application of grain protectants is one control method to prevent infestations during long term storage. This study evaluated the efficacy of four commercially available grain protectants—Diacon® IGR ((S)-methoprene), EverGreen® (natural pyrethrin), Gravista® (deltamethrin + (S)-methoprene + piperonyl butoxide), and Sensat™ (spinosad) applied to sorghum against *Rhyzopertha dominica* (F.), *Sitophilus oryzae* (L.), *Prostephanus truncatus* Horn, and *Plodia interpunctella* (Hübner), at 22, 27, and 32°C. Temperature had a significant effect on insect development, with faster adult emergence observed at 32°C compared 22 or 27°C. The Gravista® and Sensat™ treatments had 70 - 100% adult mortality for both *R. dominica* and *P. truncatus*, resulting in complete suppression of F_1 adult progeny in nearly all trials. These two protectants were also efficient in the suppression of *P. interpunctella* larvae across all temperatures but were not as effective at controlling *S. oryzae* adults and their subsequent progeny. The Diacon® IGR treatment resulted in <1 adult progeny among all the species and temperatures. EverGreen® provided only partial suppression, with variable efficacy across temperature treatments and species. *P. truncatus* did not develop at 22°C. The results of this study highlight the importance of temperature and insect species on the efficacy of four different grain protectants applied to sorghum and how grain protectants can be incorporated into an integrated pest management program for bulk sorghum storage.

3.2 Introduction

Post-harvest losses caused by stored-product insects represent a persistent and global challenge to food security (Stathas et al., 2023), especially in regions where limited storage infrastructure are common (Morrison III et al., 2019). Common stored-product insects such as *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae), lesser grain borer, *Sitophilus oryzae* (L.)

(Coleoptera: Curculionidae), rice weevil, and *Plodia interpunctella* (H.) (Lepidoptera: Pyralidae), Indian meal moth, and *Prostephanus truncatus* Horn (Coleoptera: Bostrichidae), larger grain borer, are capable of infesting a wide range of cereal grains, leading to both quantitative losses through direct consumption and qualitative losses from frass, webbing, and physical damage to kernels (Hagstrum and Phillips, 2012). These pests compromise the marketability and quality of stored products by feeding damage caused to grain and the introduction of allergens and contaminants to stored masses through frass accumulation and cuticle shedding.

Sorghum (*Sorghum bicolor*) (L. Moench) is a drought-tolerant cereal that has gained increasing importance in regions vulnerable to climate variability (Abreha et al., 2022). Its nutritional profile, and storage potential make it a valuable post-harvest commodity. However, sorghum presents a unique set of traits which include higher tannin content, variable kernel hardness, and complex surface chemistry depending on the variety being produced (Aruna and Cheruku, 2019). These factors may alter the kernels susceptibility to infestation. Despite sorghums growing use as a commodity for human consumption (Zheng et al., 2023), to our knowledge relatively few studies have evaluated the susceptibility to stored-product insect infestation or insecticide efficacy on sorghum under different temperature conditions. This gap in our current understanding is especially concerning as post-harvest systems in sorghum-producing regions are often exposed to fluctuating temperatures.

Among the pests evaluated in this study, *P. truncatus* is of particular concern. *P. truncatus* has been noted as a possible pest of concern in the United States (U.S.) as its range is seemingly increasing towards the U.S. border (Quellhorst et al., 2021). While traditionally associated with maize in Central America and as of 1970, sub-Saharan Africa (Gile and Leon, 1975), *P. truncatus* has shown a capacity to spread and adapt to new environments (Quellhorst et al., 2021). Its ability, along with the ability of *R. dominica* and *S. oryzae*, to bore into and feed within hard kernels as primary feeders on stored grain, could make it difficult to control using surface-applied protectants (Hagstrum and Phillips, 2012). Although not yet widespread in the U.S., the presence of *P. truncatus* in parts of Central America and its expanding range make it a potential emerging threat to U.S. grain storage systems, particularly as climate conditions shift, transportation infrastructure is bolstered, and sorghum production and exportation within the U.S. expands (Quellhorst et al., 2021).

Temperature is a key environmental factor influencing the biology, behavior, and chemical susceptibility of stored-product insects. Numerous studies have documented species-specific thermal thresholds and development rates under controlled conditions, allowing researchers to model population dynamics and predict outbreak potential under various storage scenarios (Howe, 1965; Lu et al., 20204; Na and Ryoo, 200; Longstaff, 1999; Bell and Waters, 1982). However, much of this research has centered on staple commodities such as maize, wheat, corn, and rice. The physicochemical differences between these grains and alternative crops, such as sorghum, may influence not only pest development and reproduction but also the performance of insecticidal protectants, especially under different abiotic conditions such as temperature. On the other hand, the efficacy of chemical grain protectants can also be influenced by temperature, as both insect metabolism and insecticide toxicity may shift under different thermal regimes (Mao et al., 2019; Delnat et al., 2021). Compounds such as (S)-methoprene an insect growth regulator and juvenile hormone analog, pyrethrum, deltamethrin, a synthetic pyrethroid, , and spinosad a biologically derived neurotoxin, are widely used in grain protection programs and have all shown to have altered efficacies against pest insects at variable temperatures (Grafius, 1986; Alzogaray and Zerb, 1992; Jensen et al., 2009; Athanassiou et al., 2008; Kalmouni et al., 2025). Their effectiveness has been well documented across traditional grain types, but few studies have evaluated their performance on sorghum, particularly in relation to fluctuating storage temperatures or across multiple pest species.

This study evaluates the efficacy of four commercial insecticide formulations, against four economically important stored product pests of sorghum: *R. dominica*, *S. oryzae*, *P. truncatus*, and *P. interpunctella*. Bioassays were conducted using treated sorghum grain incubated at three different temperatures (22°C, 27°C, and 32°C), with pest responses assessed via multiple endpoints including adult mortality, progeny production, frass accumulation, the percent kernels damage by feeding, larval emergence, and qualitative infestation ratings. By focusing on sorghum as a model commodity and integrating environmental variability into experimental design, this work addresses critical knowledge gaps and provides applied insight for enhancing integrated pest management strategies in post-harvest systems increasingly reliant on this crop.

3.3. Materials and methods

3. 3. 1. Insects and commodity

The insect species utilized in this study were laboratory strains of *R. dominica*, *S. oryzae*, *P. truncatus*, and *P. interpunctella*, maintained in continuous culture at the USDA–ARS, Center for Grain and Animal Health Research (CGAHR), Manhattan, KS. *R. dominica* and *S. oryzae* were reared on commercial whole sorghum grain (Cargill®, Salina, KS) under constant environmental conditions in a Percival environmental chamber (Percival Scientific, Perry, IA), set at 27°C, 60% relative humidity (r.h.), and continuous darkness. To standardize age, adult insects were introduced to fresh sorghum grain and allowed to oviposit for one week, after which parental adults were removed and the colony jars held for adult progeny emergence. One-to-two-week-old mixed-sex adults were used in all experiments.

Prostephanus truncatus was first reared on a white sorghum variety (Nu Life™, Scott City, KS) at 27°C and 65% r.h. in continual darkness. Colonies were maintained on the white sorghum variety for three generations before being transitioned to the commercial whole sorghum grain (Cargill®, Salina, KS) that both *R. dominica* and *S. oryzae* were also reared on. Adult *P. truncatus* were allowed to oviposit on the grain for two weeks prior to removal, ensuring a consistent cohort of newly emerged adults for experiments. For the bioassays, 0ne-to-two-week-old mixed-sex adults were used in all experiments.

Plodia interpunctella was reared on a standardized laboratory diet consisting of 30.4% cracked wheat, 52.2% wheat shorts, 4.3% wheat germ, 2.2% brewers yeast, 4.3% honey, 4.3% glycerin, and 2.3% water. Approximately 0.5 g of eggs were added to each colony container to maintain uniform population densities across rearing batches. *Plodia interpunctella* eggs, < 24 h old, were used in all experiments.

Prior to treatment, the sorghum grain was frozen for a minimum of 48 hrs to eliminate any naturally occurring insect infestation. The grain was then cleaned by passing it through an 8/64” dockage sieve (Seedburo Equipment Company, Chicago, IL) and processed using a grain thrasher (Precision Machine Co. Inc., Lincoln, NE) to remove glumes, dust, and debris, ensuring uniformity and cleanliness of the experimental substrate.

3. 3. 2. Insecticide formulation and commodity treatment

Four commercially available grain protectants were evaluated alongside a water-only control. The first insecticide used was methoprene with the trade name Diacon® IGR (Central Life Sciences, Schaumburg, IL). The formulation contains 33.6% active ingredient (a.i.) (s)-methoprene and will henceforth be referred to as its trade name Diacon® IGR. Diacon® IGR was

applied at the label rate of 210 mL of insecticide per 3.8 L of water to treat 1000 bushels of sorghum. To treat at this rate, 0.6 mL of product was mixed with 50 mL of water. The second treatment was EverGreen® (MGK, Minneapolis, MN), a 5% a.i. pyrethrin concentrate, mixed at the label rate by combining 2.40 mL with 50 mL of water. The third formulation was Gravista® (Central Life Sciences, Schaumburg, IL), a combination product containing 1.20% a.i. deltamethrin, 2.85% a.i. (S)-methoprene, and 33.30% a.i. piperonyl butoxide (PBO), mixed at the label rate by combining 2.50 mL with 50 mL of water. The fourth treatment was Sensat™ (Bayer CropScience, Research Triangle Park, NC), containing 8.66% spinosad as the a.i., and was prepared by mixing 0.80 mL with 50 mL of water according to label specifications. For each protectant, 0.45 mL of solution was applied to treat 600 g of sorghum.

Insecticide applications were made using an artist spray brush (Model 100™, BADGER, Franklin Park, IL), with water only being used as the control. During the application, 600 g of sorghum was gently poured into a 35.9 × 19.7 × 12.4 × cm (L × W × H) plastic bin (Sterilite, Townsend, MA) while simultaneously spraying the insecticide. After spraying, grain was manually agitated for approximately 20 s to ensure even distribution of the treatment. The bins were fitted with a vented lid and allowed to dry at ambient conditions for 24 h. This treatment process was repeated three times for each combination of active ingredient, insect species, and temperature condition evaluated.

3. 3. 3. Efficacy bioassays of grain protectants

For each treatment, 5 subsamples of 100 g were taken from the initial 600 g of treated grain and transferred into individual 0.18-L plastic vials fitted with vented lids. Each set of 5 replicates were used for each temperature treatment. Twenty-one-to-two-week-old mixed-sex adult *R. dominica*, *S. oryzae*, or *P. truncatus*, were added to each vial. The vials containing adults were placed in environmental chambers maintained at 22, 27, or 32°C at 65% r.h. After 7 d, adult insects were removed and assessed for the number of dead adults. The vials were returned to their respective environmental chambers until the first adult progeny emergence in the control vials was observed and recorded (Table 3.1). One wk after the first recorded emergence, the vials were frozen for a minimum of 48 h before final observations were taken.

After freezing, contents from vials infested by *R. dominica*, *S. oryzae*, or *P. truncatus* were sieved through a stacked sieve set utilizing a of #12 (1700 µm), #30 (600 µm), and a catch pan (U.S. Standard Sieve Series; Dual Manufacturing Co., Chicago, IL). The adult beetles were

retained on the #12 sieve, sorghum kernels were retained on the #30 sieve, and frass was collected in the catch pan. For each vial, the total number of adult progeny and frass weight (g) were recorded. In addition, a 5 g subsamples from each vial were randomly selected and examined under a stereomicroscope (Dino-Lite 3.0 Edge Digital Microscope, Dunwell Tech, Inc., Torrance, CA) to quantify the number of insect-damaged kernels (IDKs). IDKs were identified and counted based on observed insect damage to the kernel (Figure 3.1A-C). The percentage of IDKs was determined using the following formula:

Equation 3.1: $$Percent\ IDKs = [\frac{Insect\ Damaged\ Kernels}{Undamaged\ Kernels + Insect\ Damaged\ Kernels}] \times 100$$

For bioassays involving *P. interpunctella*, 100 eggs were added directly to each vial containing treated sorghum and placed in environmental chambers set at 22, 27, or 32°C and 65% r.h. Weekly observations were made and monitored for the first adult moth emergence in the control (Table 3.1). All the vials we held and additional 2 weeks to allow for adult emergence in the treated vials, and then were frozen for 48 hrs before final analysis. Additionally, 3 replicates of 100 eggs were placed on a glass Petri dish and held at 22, 27, or 32°C and 65 % r.h. Daily observations were made for the number of larvae that emerged. The number of larvae that emerged was divided by 100 to determine the *P. interpunctella* hatchability.

After vials containing *P. interpunctella* larvae and adults were frozen for 48, they were examined for the number of larvae, adults, and an infestation score was determined qualitative damage scale ranging from 0 to 3. A score of 0 indicated no larvae, adult presence, webbing or indications of feeding were observed (Figure 3.2A). A score of 1 indicated the presence of young larvae only. A score of 2 indicated 2 indicated the presence of larvae, webbing , and signs of feeding damage (Figure 3.2B). A score of 3 indicated the presence of larvae, adults, webbing, and signs of insect feeding (Figure 3.2C). Each vial was visually scored according to this scale. Additionally, the total number of larvae and adult moths was recorded. This bioassay protocol was repeated 3 times for each insect species, temperature, and insecticide treatment on separate weeks and with different insect colonies. Therefore there was a total of 15 vials per insect species, temperature, and insecticide.

3. 3. 4. Data analysis

Data were analyzed separately for each insect species. Means and standard errors were calculated for the number of progeny frass and were then transformed to $\log_{10}(x + 1)$ scale for further analysis. Data on the percentage of g initial adult mortality and IDKs were transformed to angular values for statistical analysis (Zar, 2010). Data A two-way analysis of variance (ANOVA) was conducted to assess the main effects of insecticide treatment and temperature on initial adult mortality, number of progeny, frass weight, and percent IDKs (SAS Institute, Version 9.4, Cary, NC). If the ANOVA was significant ($P< 0.05$), differences among the treatments combinations were determined by Tukey's Honestly Significant Difference (HSD) test for multiple comparisons at $P < 0.05$. For *P. interpunctella*, the mean infestation score was also analyzed for using a two-way ANOVA with the main effects of temperature and treatment. If the ANOVA was significant ($P< 0.05$), differences among the treatments combinations were determined by Tukey's HSD test for multiple comparisons at $P < 0.05$.

3.4 Results

3. 4. 1. Insect development time

Temperature had a significant effect on the developmental time for of all insect species evaluated, with the fastest emergence observed at 32°C for all species (Table 3.1). For *R. dominica*, the mean emergence time decreased significantly ($F = 123.83$; df = 2, 6; $P < 0.05$) from approximately 106 d at 22°C to ~28 d at 32°C. However, there was no significant difference in development at 27 and 32°C. The development time required for *S. oryzae*, at 32°C was approximately half that required at 22°C, 30 and 62 d respectively, and significant differences were observed among all temperature treatments ($F = 262.48$; df = 2, 6; $P < 0.05$). There was no *P truncatus* progeny observed in the control vials at 22°C. Subsequent analysis of all the vials failed to find immature stages of this species developing inside the grain kernel, thus it was concluded that this temperature likely falls below its developmental threshold for this species. However, significant differences in development time were observed between 27°C and 32°C ($F = 287.07$; df = 2, 6; $P < 0.05$), and 32°C required ~10 fewer days before adult emergence. Development time for *P. interpunctella* adult emergence was also significant among the temperatures ($F = 1755.80$; df = 2, 6; $P < 0.05$) ranging from 90 – 25 d.

3. 4. 2. Efficacy of four grain protectants on *Rhyzopertha dominica*

There was a significant effect of treatment ($F = 272.86$; df = 4, 217; $P < 0.05$) and the treatment × temperature interaction ($F = 84.92$; df = 14, 207; $P < 0.05$), but not temperature ($F = 0.65$; df = 2, 219; $P = 0.5206$), on the initial adult mortality of *R. dominica* on treated sorghum (Table 3.2). Therefore, we analyzed the effect of treatment for each temperature. At 22°C, significant treatment effects were observed for adult mortality ($F = 56.73$; df = 4, 69; $P < 0.05$). Mortality was lowest in the control (5.79 ± 1.64%) and Diacon® IGR (11.39 ± 2.03%) treated groups, while EverGreen® achieved moderate efficacy (35.09 ± 5.78%). Both Sensat™ (99.37 ± 0.63%) and the Gravista® (72.70 ± 9.71%) produced significantly higher mortality. At 27°C, adult mortality remained significantly affected by treatment ($F = 147.35$; df = 4, 69; $P < 0.05$). Mortality increased in both Sensat™ (97.43 ± 1.40%) and Gravista® (89.67 ± 4.24%), while remaining low in the control (5.43 ± 1.77%) and Diacon® IGR (10.61 ± 2.03%). EverGreen® again yielded moderate mortality (41.17 ± 4.02%). At 32°C, treatment effects on mortality were also significant ($F = 140.05$; df = 4, 70; $P < 0.05$). Sensat™ and the Gravista® achieved complete adult mortality (100.00 ± 0.00%), whereas EverGreen® resulted in 38.60 ± 8.12% mortality.

The main effects of temperature ($F = 14.74$; df = 2, 219; $P < 0.05$), treatment ($F = 36.75$; df = 4, 217; $P < 0.05$), and temperature × treatment ($F = 45.29$; df = 14, 207; $P < 0.05$) were all significant for the number of *R. dominica* progeny (Table 3.2). At 22°C, progeny production was negligible across all treatments, with no meaningful differences detected. At 27°C, the control group produced 107.73 ± 12.11 progeny. In contrast, Sensat™ and Gravista® completely suppressed reproduction. EverGreen® allowed limited reproduction, with an mean of 2.86 ± 1.00 progeny. At 32°C, the control group exhibited peak reproductive activity, producing 184.33 ± 30.97 progeny. Sensat™ and Gravista® again nearly eliminated reproduction (0.13 ± 0.13 and 0.13 ± 0.09 progeny, respectively), while EverGreen® permitted substantial reproduction (59.33 ± 15.94 progeny).

Similarly, frass production was significantly influenced by temperature ($F = 14.74$; df = 2, 219; $P < 0.05$), treatment ($F = 36.75$; df = 4, 217; $P < 0.05$), and their interaction ($F = 45.29$; df = 14, 207; $P < 0.05$) (Table 3.2). For percent IDKs, treatment ($F = 8.04$; df = 4, 217; $P < 0.05$) and temperature x treatment interaction ($F = 4.42$; df = 14, 207; $P < 0.05$) were significant, while the main effect of temperature was not ($F = 0.94$; df = 2, 219; $P = 0.394$). Accordingly, IDK data are interpreted within temperatures only. At 22°C, frass production was significantly affected by treatment ($F = 25.76$; df = 4, 69; $P < 0.05$). EverGreen®, Sensat™, and Gravista® all resulted in

0.00 g of frass, compared to 0.04 ± 0.01 g in the control. Differences in IDK values were not statistically significant ($F = 0.99$; df = 4, 69; $P = 0.4181$), although Sensat™ showed a slightly higher mean (3.85 ± 3.50%) due to a few outlier samples with extensive kernel damage. At 27°C, treatment effects on frass ($F = 102.07$; df = 4, 68; $P < 0.05$) and IDKs ($F = 15.38$; df = 4, 68; $P < 0.05$) were significant. The control group produced 1.32 ± 0.16 g of frass and 1.90 ± 0.29% kernel damage. Sensat™ and Gravista® reduced frass output to 0.00 and 0.01 g, respectively, and lowered IDKs to 0.26 ± 0.07% and 0.15 ± 0.10%. EverGreen® showed moderate frass production (0.08 ± 0.02 g). At 32°C, treatment effects on frass ($F = 36.84$; df = 4, 70; $P < 0.05$) and IDKs ($F = 8.73$; df = 4, 70; $P < 0.05$) were significant, although the main effect of temperature on IDKs was not, so no across-temperature comparisons were made. The control group had the highest frass (3.48 ± 0.86 g) and kernel damage (3.15 ± 0.77%). Both Sensat™ and Gravista® treatments nearly eliminated frass production (0.01 ± 0.01 and 0.01 ± 0.00 g, respectively). EverGreen® allowed higher frass accumulation (0.81 ± 0.19 g).

3. 4. 3. Efficacy of four grain protectants on *Sitophilus oryzae*

Adult *S. oryzae* mortality was significantly affected by temperature ($F = 3.07$; df = 2, 220; $P < 0.05$), treatment ($F = 63.38$; df = 4, 218; $P < 0.05$), and their interaction ($F = 26.51$; df = 14, 208; $P < 0.05$) (Table 3.3). At 22°C, treatment effects were significant for adult mortality ($F = 81.13$; df = 4, 69; $P < 0.05$). Mortality was minimal for Diacon® IGR (2.88 ± 1.01%) and EverGreen® (2.49 ± 1.05%), while Sensat™ (38.78 ± 5.91%) and Gravista® (69.32 ± 4.70%) achieved significantly higher mortality. At 27°C, treatment effects on mortality remained significant ($F = 29.42$; df = 4, 70; $P < 0.05$). Sensat™ showed the highest efficacy (54.81 ± 8.92%), followed by Gravista® (13.16 ± 2.44%). The control and Diacon® IGR groups exhibited the lowest mortality rates. At 32°C, mortality differences were again significant ($F = 25.18$; df = 4, 69; $P < 0.05$). Sensat™ achieved the highest adult mortality (70.62 ± 8.99%), followed by Gravista® (24.67 ± 4.68%). Across temperatures, significant variations in mortality were observed for the performance of Gravista® ($F = 52.85$; df = 2, 42; $P < 0.05$), Sensat™ ($F = 3.89$; df = 2, 42; $P = 0,05$), and EverGreen® ($F = 3.26$; df = 2, 42; $P < 0.05$). Mortality increased with temperature for Sensat™ and EverGreen®, whereas Gravista® performed best at 22°C. Mortality under control and Diacon® IGR treatments did not vary significantly across temperatures ($P > 0.05$).

Progeny production of *S. oryzae* adults exposed to treated sorghum grain was significantly affected by temperature ($F = 67.77$; df = 2, 220; $P < 0.05$), treatment ($F = 9.63$; df = 4, 218; $P < 0.05$), and their interaction ($F = 20.46$; df = 14, 208; $P < 0.05$) (Table 3.3). At 22°C, treatment effects were significant for progeny production ($F = 12.84$; df = 4, 69; $P < 0.05$). All insecticide treatments significantly reduced reproduction compared to the control (60.07 ± 9.16). The most effective was Gravista® (7.07 ± 2.19 progeny) and Sensat™ (23.33 ± 4.84). At 27°C, treatment effects were not statistically significant for progeny production ($F = 2.42$; df = 4, 70; $P = 0.0562$). Control (165.40 ± 16.57) and Diacon® IGR (177.13 ± 20.85) allowed the highest reproduction. Sensat™ and Gravista® permitted >130 progeny, indicating lower efficacy at this temperature. At 32°C, treatment effects were marginally significant ($F = 2.50$; df = 4, 69; $P = 0.05$). The control group produced 65.14 ± 12.43 progeny. Gravista® treated groups resulted in the greatest suppression (25.53 ± 5.36), while Sensat™ reduced progeny to 43.07 ± 9.82. Across temperatures, significant variation in progeny production was detected for the control ($F = 20.79$; df = 2, 41; $P < 0.05$), Diacon® IGR ($F = 23.48$; df = 2, 41; $P < 0.05$), EverGreen® ($F = 20.10$; df = 2, 42; $P < 0.05$), Gravista® ($F = 54.71$; df = 2, 42; $P < 0.05$), and Sensat™ ($F = 18.99$; df = 2, 42 $P < 0.05$).

Similarly, frass production was significantly influenced by temperature ($F = 13.84$; df = 2, 220; $P < 0.05$), treatment ($F = 7.65$; df = 4, 218; $P < 0.05$), and their interaction ($F = 5.28$; df = 14, 208; $P < 0.05$) (Table 3.3). Along with frass production, the percentage of IDKs produced by S. oryzae on treated sorghum grain was also significantly influenced by temperature ($F = 71.99$; df = 2, 220; $P < 0.05$), treatment ($F = 7.42$; df = 4, 218; $P < 0.05$), and their interaction ($F = 16.86$; df = 14, 208; $P < 0.05$) (Table 3.3). At 22°C, frass production ($F = 13.33$; df = 4, 69; $P < 0.05$) and IDKs ($F = 13.27$; df = 4, 69; $P < 0.05$) showed significant treatment effects. Gravista® resulted in the lowest frass (0.03 ± 0.01 g) and IDKs (1.59 ± 0.17%). EverGreen® allowed the highest frass accumulation (0.30 ± 0.05 g), likely due to incomplete suppression of larval feeding. At 27°C, frass ($F = 1.37$; df = 4, 70; $P = 0.2531$) was not significantly affected. However, IDK values differed significantly among treatments ($F = 6.07$; df = 4, 70; $P < 0.05$). The control and Diacon® IGR groups showed the highest kernel damage (18.74 ± 1.70% and 15.69 ± 0.94%, respectively). Sensat™ and Gravista® permitted >130 progeny and comparable feeding damage. At 32°C, significant treatment effects were observed for frass ($F = 5.30$; df = 4, 69; $P < 0.05$), but not for IDKs ($F = 1.36$; df = 4, 69; $P = 0.2567$). The control group produced

the most frass (0.38 ± 0.07 g) and kernel damage (8.72 ± 1.07%). Gravista® yielded the lowest frass (0.12 ± 0.03 g) and IDKs (4.79 ± 0.55%). Sensat™ matched this frass suppression (0.12 ± 0.03 g), but kernel damage remained higher (7.01 ± 1.73%).Significant across-temperature differences in frass production were observed for the control ($F = 3.64$; df = 2, 41; $P < 0.05$), Diacon® IGR ($F = 4.67$; df = 2, 41; $P < 0.05$), EverGreen® ($F = 3.26$; df = 2, 42; $P < 0.05$), Gravista® ($F = 17.38$; df = 2, 42; $P < 0.05$), and Sensat™ ($F = 12.37$; df = 2, 42; $P < 0.05$). Similarly, significant differences across temperatures for the percentage of IDKs were observed for each treatment as well: control ($F = 18.60$; df = 2, 41; $P < 0.05$), Diacon® IGR ($F = 25.39$; df = 2, 41; $P < 0.05$), and EverGreen® ($F = 5.54$; df = 2, 42; $P < 0.05$), Gravista® ($F = 104.42$; df = 2, 42; $P < 0.05$), and Sensat™($F = 11.56$; df = 2, 42; $P < 0.05$).

3. 4. 4. Efficacy of four grain protectants on *Prostephanus truncatus*

Adult *P. truncatus* mortality was significantly affected by temperature ($F = 175.58$; df = 4, 218; $P < 0.05$) and temperature × treatment interactions ($F = 59.80$; df = 14, 208; $P < 0.05$) (Table 3.4). The main effect of treatment on mortality, however, was not significant ($F = 0.20$; df = 2, 220; $P = 0.819$), so cross-temperature comparisons for mortality were not interpreted. At 22°C, mortality differences were significant ($F = 81.77$; df = 4, 69; $P < 0.05$), with Sensat™ (77.83 ± 4.82%) and Gravista® (87.79 ± 2.64%) producing significantly higher mortality than other treatments. At 27°C, mortality remained highly significant ($F = 101.05$; df = 4, 69; $P < 0.05$), with Sensat™ achieving the highest mortality (82.61 ± 2.48%) followed by Gravista® (62.69 ± 4.89%). EverGreen® (18.62 ± 1.64%) and Diacon® IGR (5.40 ± 1.34%) performed poorly at this temperature. At 32°C, mortality was again significant ($F = 43.22$; df = 4, 70; $P < 0.05$), and both Sensat™ and Gravista® maintained high efficacy, each exceeding 98% mortality. In contrast, EverGreen® (15.61 ± 1.95%) and Diacon® IGR (15.15 ± 3.18%) again yielded much lower mortality rates.

Treatment effects on progeny production were significant overall ($F = 21.37$; df = 4, 218; $P < 0.05$), along with temperature ($F = 10.09$; df = 2, 220; $P < 0.05$) and the main effect of temperature × treatment interactions ($F = 18.46$; df = 14, 208; $P < 0.05$) (Table 3.4). At 22°C, no progeny emerged from any treatment or control, suggesting that this temperature inhibits complete larval development. At 27°C, differences in progeny production were significant ($F = 23.48$; df = 4, 69; $P < 0.05$). Sensat™ and Gravista® completely suppressed progeny, while EverGreen® and Diacon® IGR permitted low, but nonzero reproduction. The control produced

12.93 ± 6.37 progeny under these conditions. At 32°C, treatment effects remained significant (F = 9.82; df = 4, 70; $P < 0.05$). Sensat™ and Gravista® again eliminated reproduction entirely, whereas EverGreen® and Diacon® IGR allowed higher progeny numbers relative to these two treatments. Across temperatures, significant variation in progeny production occurred for the control (F = 205.38; df = 2, 43; $P < 0.05$), but not for EverGreen® (F = 26.02; df = 2, 43; $P <$ 0.05) or Diacon® IGR (F = 0.53; df = 2, 42; P = 0.5924). Both Gravista® and Sensat™ treat sorghum yielded no progeny for P. truncatus, so there was no significant variation across temperature treatments.

Frass production was shown to be significantly affected by treatment (F = 24.08; df = 4, 217; $P < 0.05$), temperature (F = 9.65; df = 2, 219; $P < 0.05$), and temperature × treatment interactions (F = 15.45; df = 14, 207; $P < 0.05$) (Table 3.4). Furthermore, the percentage of IDKs generated by P. truncatus was also shown to have been significantly affected by treatment (F = 38.77; df = 4, 218; $P < 0.05$), temperature (F = 15.37; df = 2, 220; $P < 0.05$), and temperature × treatment interactions (F = 32.19; df = 14, 208; $P < 0.05$) (Table 3.4). At 22°C, frass differences were significant (F = 3.09; df = 4, 68; $P < 0.05$), with the highest levels in the control (0.13 ± 0.08 g). Gravista® and Sensat™-treated grain had no measurable frass (0.00 g). IDK values were lowest in Gravista® (0.78 ± 0.10%) and highest in the control (2.83 ± 0.55%) and Diacon® IGR (2.79 ± 0.56%). At 27°C, frass differences were significant (F = 17.09; df = 4, 69; $P < 0.05$). The control produced 1.53 ± 0.63 g frass and 26.38 ± 4.47% IDKs, whereas Sensat™ and Gravista® reduced frass to ≤0.01 g and IDKs to ~1.1%. EverGreen® and Diacon® IGR allowed moderate feeding damage despite lower reproduction. At 32°C, frass differences were significant (F = 14.08, $P < 0.0001$). The control produced 0.64 ± 0.20 g frass and 13.90 ± 2.05% IDKs, while Sensat™ and Gravista® kept frass levels at 0.01 g and IDKs below 1.3%. EverGreen® and Diacon® IGR permitted more feeding damage under these conditions. Across temperatures, frass varied significantly for the control (F = 32.52; df = 4, 70; $P < 0.05$), Diacon® IGR (F = 7.95; df = 4, 69; $P < 0.05$), and EverGreen® (F = 20.76; df = 4, 68; $P < 0.05$), but not for Gravista® (F = 1.45; df = 4, 78 P = 0.2472) or Sensat (F = 1.89; df = 4, 68; P = 0.1631). Regarding IDKs, the control group (F = 25.13; df = 2, 42; $P < 0.005$), Diacon® IGR (F = 5.02; df = 2, 42; $P < 0.05$), and EverGreen® (F = 18.66; df = 2, 42; $P < 0.05$) were found to be statistically significant across temperature treatments, whereas Gravista® (F = 0.63; df = 2, 42; P = 0.5371) and Sensat™ (F = 0.45; df = 2, 42; P = 0.6423) were not.

3. 4. 5. Efficacy of four grain protectants on *Plodia interpunctella*

Treatment efficacy against *P. interpunctella* varied significantly across temperature conditions, with particularly strong suppression observed for Sensat™ and Gravista® in all temperature treatments (Table 3.5). Because adult moths were not directly introduced to samples, hatch rate analysis was conducted to assess initial infestation potential. Statistical analyses revealed a significant effect of temperature on hatch rate ($F = 185.53$; df = 3, 6; $P < 0.05$). Mean hatch rate was lowest at 22°C (32.78 ± 1.73%), increased sharply at 27°C (55.78 ± 2.37%), and remained high at 32°C (49.33 ± 3.03%). These results demonstrate that early egg-to-larva development in *P. interpunctella* is highly temperature-dependent, with peak viability occurring at moderate to warm conditions.

Following hatch, larval and adult emergence were both significantly affected by chemical treatment and its interaction with temperature. For larval count, treatment ($F = 400.71$; df = 4, 107; $P < 0.05$) and temperature × treatment interaction ($F = 202.92$; df = 14, 97; $P < 0.05$) were highly significant, while temperature alone had no effect ($F = 0.45$; df = 2, 109; $P = 0.6404$). In contrast, adult emergence was significantly influenced by all three factors: temperature ($F = 8.44$; df = 2, 109; $P < 0.05$), treatment ($F = 170.53$; df = 4, 107; $P < 0.05$), and their interaction ($F = 36.40$; df = 14, 97; $P < 0.05$). At 22°C, treatment effects were significant for both larvae ($F = 603.20$; df = 4, 32; $P < 0.05$) and adults ($F = 101.58$; df = 4, 32; $P < 0.05$). The control group showed a high larval count (27.07 ± 1.45), adult presence (10.73 ± 2.04), webbing presence, and the highest damage ranking (3). Although Diacon® IGR resulted in a similar number of larvae (30.07 ± 2.02), it completely suppressed adult emergence, lowering the damage score to 2. EverGreen® strongly reduced larval numbers (0.87 ± 0.26) and adult emergence (0.27 ± 0.15) but retained a damage score of 3 due to visible webbing. Both Sensat™ and Gravista® completely suppressed larval and adult development, eliminated webbing, and received a damage score of 0.

At 27°C, treatment effects again remained significant for larvae ($F = 121.56$; df = 4, 32; $P < 0.05$) and adults ($F = 439.10$; df = 4, 32; $P < 0.05$). The control produced fewer larvae (6.67 ± 1.34) than at 22°C but substantially more adults (37.67 ± 3.00), along with webbing and maximum damage. Diacon® IGR performed poorly in larval suppression (35.13 ± 3.47) but successfully prevented adult emergence, maintaining a damage score of 2. EverGreen® allowed moderate larval (2.60 ± 0.61) and high adult emergence (26.93 ± 3.85), leading to continued webbing and a damage score of 3. Sensat™ and Gravista® again achieved full suppression across

all infestation metrics. At 32°C, larval ($F = 249.54$; df = 4, 32; $P < 0.05$) and adult ($F = 1449.01$; df = 4, 32; $P = 0.05$) emergence remained significantly different among treatments. The control group showed elevated infestation metrics: 11.27 ± 0.93 larvae, 29.80 ± 1.43 adults, visible webbing, and full damage ranking. Diacon® IGR failed to limit larvae (32.67 ± 3.11) but completely inhibited adult emergence, with a consistent damage score of 2. EverGreen® allowed both larval (4.60 ± 0.95) and adult development (22.40 ± 1.96), maintaining a damage score of 3. As in earlier temperatures, Sensat™ and Gravista® fully suppressed infestation and prevented webbing, each receiving a score of 0.

3. 5. Discussion

Seasonal temperature fluctuations are often associated with changes in the storage and transportation conditions of grain commodities. These temperature variations can significantly influence the development of stored product insects and the efficacy of different stored product protectants, as temperature affects both the detoxification capabilities of insects and the stability or performance of various insecticide formulations (Johnson, 1990). In this study, we examined the efficacy of four commercially available grain protectants, Diacon® IGR, EverGreen®, Gravista®, and Sensat™, against four key stored product insect species under laboratory-simulated temperature conditions. Our study showed that temperature influenced insect development, with faster emergence times observed for all species at higher temperatures (Table 3.1). *Rhyzopertha dominica* required over 100 days to emerge at 22°C but emerged in under 33 days at 27°C and 32°C. *Sitophilus oryzae* showed similar acceleration, while *P. interpunctella* emerged in less than 30 days at 27°C and 32°C. *Prostephanus truncatus* failed to emerge at 22°C, confirming a higher thermal threshold. These results confirm known species-specific thermal sensitivities and underscore the importance of environmental conditions in shaping pest pressure.

Temperature influenced each grain protectant studies. Diacon® IGR exhibited consistent efficacy across all three temperatures, showing no clear decline or enhancement at higher thermal conditions. This stability was particularly evident in *P. interpunctella* and *S. oryzae*, where Diacon® IGR consistently suppressed adult emergence despite varying larval presence. However, its impact on *R. dominica* and *P. truncatus* was limited, as both species showed moderate to low susceptibility regardless of temperature (Table 3.2, Table 3.4). The lack of temperature dependence likely reflects Diacon® IGR's hormonal mode of action. As a juvenile

hormone analog, it disrupts development by binding to the methoprene-tolerant receptor and interfering with molting processes regulated by ecdysteroids (Hiruma and Kaneko, 2013).

Supporting this, Jenson et al. (2009) found that methoprene-treated surfaces suppressed adult emergence of *P. interpunctella* regardless of whether eggs or larvae were exposed, and that temperature had no significant impact on efficacy. Similarly, Li et al. (2013) reported that methoprene consistently suppressed *R. dominica* progeny across temperatures, though efficacy slightly declined at 36°C. While metabolic resistance to methoprene, such as increased cytochrome P450 and esterase activity, has been shown to develop in other insect species (Sparks and Hammock, 1983), these detoxification pathways did not appear to significantly affect field-level efficacy under the thermal conditions tested in this study.

In contrast, the pyrethrum formulation, EverGreen®, showed generally poor efficacy across all temperatures, with little evidence of thermal enhancement. Mortality remained low, and both progeny development and feeding damage persisted in *R. dominica* and *S. oryzae* (Table 3.2, 3.3). *P. interpunctella* responded somewhat more to EverGreen®, but significant larval development, webbing, and feeding persisted (Table 3.5). EverGreen®'s failure to improve with temperature is notable, as pyrethroids typically exhibit a negative temperature coefficient due to their action on voltage-gated sodium channels, which are less disrupted at higher neural firing rates (Alzogaray and Zerba, 1993; Soderland, 2009). Grafius (1986) found that pyrethrums such as cypermethrin and permethrin efficacy decreased by as much as 8-fold between 14°C and 30°C in *Leptinotarsa decemlineata* Say, Colorado potato beetle, supporting the temperature-linked decline in neural sensitivity to pyrethroid action. Similarly, Alzogaray and Zerba (1993) observed that *Triatoma infestans* exposed to pyrethroids recovered from knockdown more quickly at 28°C than at 16°C. Detoxification enzymes further contribute to this loss of efficacy. Therefore, EverGreen®'s inability to suppress pest development at any temperature likely stems from both rapid environmental degradation and thermally enhanced detoxification.

Gravista®, the deltamethrin + (S)-methoprene + PBO mixture, demonstrated strong efficacy across all temperatures, but with some variation in species-specific response. Mortality and progeny suppression were consistently high in *R. dominica* and *P. interpunctella* (Tables 3.2, 3.5), while *S. oryzae* responded best at 22°C, with performance dipping at 27°C before partially recovering at 32°C (Table 3.3). Deltamethrin, like pyrethrum, acts by keeping sodium channels open, leading to repetitive neuronal firing and paralysis. However, its performance often declines

at elevated temperatures due to faster nerve repolarization and increased detoxification (Grafius, 1986; Alzogaray and Zerba, 1993). Li et al. (2013) confirmed that deltamethrin residues degraded more rapidly at 36°C, resulting in reduced suppression of *R. dominica* progeny over time. Nonetheless, the synergist PBO in the mixture likely inhibited P450 enzymes, prolonging deltamethrin's activity under thermal stress (Pimprikar and Georghiou, 1979). Methoprene's role in blocking emergence further buffered against performance dips, especially in species like *P. interpunctella* and *R. dominica*. The combination of knockdown, growth regulation, and metabolic inhibition likely explains the mixture's consistent performance across thermal gradients, including complete suppression of *P. truncatus* in this study.

Sensat™, containing the active ingredient spinosad delivered the most consistent and temperature-enhanced performance of all treatments. Efficacy improved notably with increasing temperature, especially in *S. oryzae*, where adult mortality rose from 38.8% at 22°C to 70.6% at 32°C (Table 3.3). Complete suppression of *P. truncatus* and *P. interpunctella* at all temperatures was achieved (Tables 4, 5). Spinosad targets nicotinic acetylcholine receptors and GABA-gated chloride channels, causing prolonged neuronal excitation and feeding cessation (Sparks et al., 1998). As metabolic rate and neural activity rise with temperature, insects experience faster intoxication and mortality.

Wijayaratne and Rajapakse (2018) found that exposure to spinosad followed by heat stress significantly increased mortality in both *R. dominica* and *S. oryzae*, indicating a strong synergistic interaction between temperature and mode of action. Pozidi-Metaxa and Athanassiou (2013) observed that spinosad killed 100% of *P. truncatus* within 3 days at 30°C, but required up to 7 days at 20°C. Amarasekare and Edelson (2004) similarly reported increasing toxicity of spinosad against *Melanoplus differentialis* as temperatures rose from 15°C to 35°C.

In addition to temperature and chemical treatment, the intrinsic properties of sorghum grain likely contributed to suppressed insect development and reproduction. Sorghum is chemically distinct from other cereals due to its naturally high levels of polyphenolic compounds, including proanthocyanidins, 3-deoxyanthocyanidins, and tannins, which are known to impair insect digestion, reduce nutrient bioavailability, and inhibit larval development by interfering with midgut physiology and digestive enzyme activity (Duodu et al., 2003; Rhodes et al., 2014). In this context, the poor overall performance of pests, especially *P. truncatus,* may be driven not only by kernel size limitations but also by biochemical constraints. *P. truncatus*

performed poorly overall, failing to reproduce at 22°C and showing reduced infestation potential even at higher temperatures (Table 3.4). This performance likely reflects both physical and biochemical limitations imposed by the host grain. As a species better adapted to larger-seeded hosts such as maize, *P. truncatus* appears constrained by sorghum's smaller kernel size, which likely impairs oviposition success and larval development. The limited emergence observed even under favorable conditions suggests that kernel structure plays a key role in host suitability. In addition to physical barriers, sorghum's defensive chemistry may compound developmental stress, further reducing colonization success. These dual constraints, grain morphology and secondary metabolites, create a suboptimal environment, particularly under cooler temperatures. The complete lack of reproduction at 22°C aligns with previous research showing *P. truncatus* exhibits poor cold tolerance, with larvae especially sensitive to low temperatures (Mutamiswa et al., 2021). Together, these findings indicate that *P. truncatus* poses minimal threat to stored sorghum under cooler conditions, although monitoring remains important in warmer storage scenarios where thermal thresholds may be exceeded.

Taken together, these findings suggest that both grain composition and insect feeding ecology play critical roles in shaping protectant performance and overall infestation outcomes. Sorghum's high polyphenol content likely contributes to intrinsic resistance by impairing feeding efficiency, disrupting digestion, and reducing nutrient bioavailability. These biochemical defenses appear to suppress insect development even in untreated controls, and their effects may be amplified when combined with sublethal chemical exposure. Polyphenol concentrations vary widely among sorghum accessions and are highly heritable (Rhodes et al., 2017), offering a promising avenue for selective breeding aimed at enhancing post-harvest resistance. At the same time, the feeding lifestyle of target pests must be considered when evaluating protectant efficacy. The primary feeders in this study, *R. dominica*, *S. oryzae*, and *P. truncatus,* develop within the kernel, limiting their exposure to surface residues. This contrasts with *P. interpunctella*, a secondary feeder whose external feeding behavior increases contact with chemical treatments and likely explains its higher susceptibility across compounds. These ecological differences reinforce that protectant performance cannot be generalized across pest taxa and must be matched to species biology and behavior.

Future research should continue to explore the intersection of grain chemistry, pest feeding ecology, and environmental context. In particular, temperature emerged as a key

modulator of protectant efficacy in this study, with certain compounds, such as spinosad, performing better at elevated temperatures, while others (e.g., pyrethrum and pyrethroids) showed reduced persistence. These interactions warrant further investigation, especially under real-world storage conditions where temperature fluctuations are common. Expanding this work to include a broader range of sorghum varieties, differing in kernel hardness, polyphenol content, and nutritional composition, would help clarify how grain traits interact with thermal conditions to shape pest suppression. By combining chemical, biological, and environmental insights, future studies can move toward more integrated and climate-resilient strategies for post-harvest grain protection.

3. 6. Figures and tables

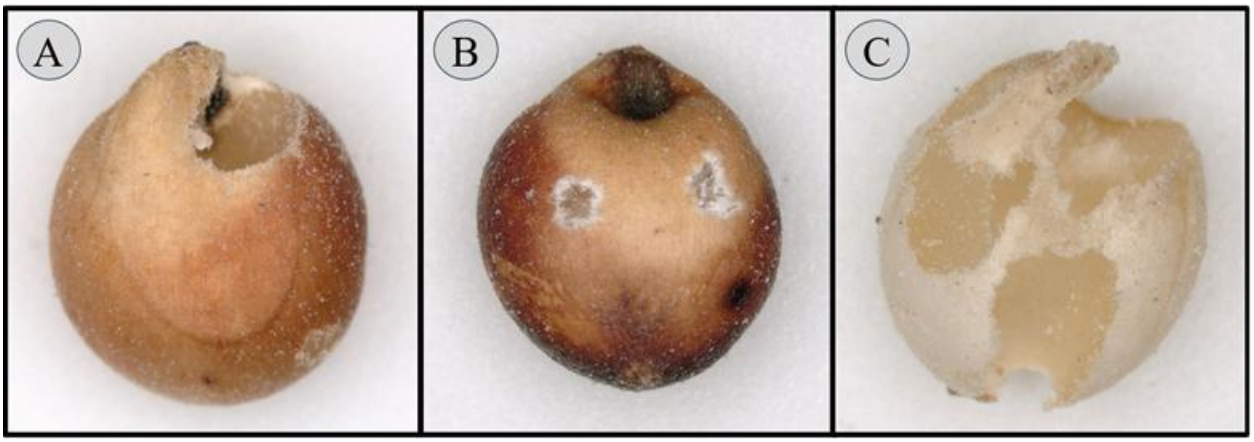

Figure 3.1 Photos of sorghum grain exhibiting A) adult insect emergence damage, B) adult feeding, and C) extensive damage caused by insects.

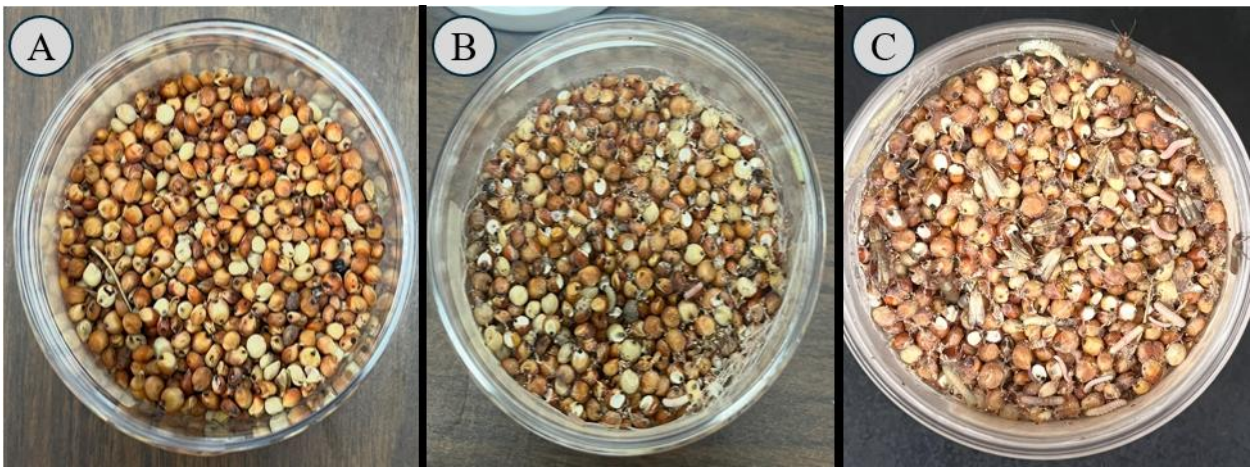

Figure 3.2 Damage scaling for *Plodia interpunctella* where A) there was no larvae or damage indicated by a score of 0, B) larvae and webbing present indicated by a score of 2 and C) larvae, adults, webbing, and signs of feeding indicated by a score of 3.

Table 3.1 Mean (± SE) number of days to for the first adult emergence on control sorghum for *Rhyzopertha dominica*, *Sitophilus oryzae, Prostephanus truncatus, and Plodia interpunctella* at held at 22, 27, or 32°C and 65% r.h. Means followed by different uppercase letters within the same column (species) are statistically different $P < 0.05$, Tukey's HSD test).

Temperature	*R. dominica*	*S. oryzae*	*P. truncatus*[a]	*P. interpunctella*
22º C	106.33 ± 6.57A	62.00 ± 1.73A	-	89.67 ± 1.20A
27º C	32.67 ± 1.45B	34.33 ± 0.33B	32.67 ± 0.22A	29.67 ± 0.67B
32º C	27.67 ± 1.33B	30.00 ± 0.58C	27.67 ± 0.20B	25.00 ± 0.58C

[a]Emergence data for *P. truncatus* at 22°C are not reported due to complete developmental failure at this temperature.

Table 3.2 Efficacy of four different grain protectants against *Rhyzopertha dominica* at three temperature conditions (22, 27, and 32°C) on the mean (± SE) percent adult mortality, number of adult progeny, amount of frass and percent IDKs. Significant difference between treatments among each temperature ($P < 0.05$, Tukey's Honestly Significant Difference Test (HSD)) are denoted by different lowercase letters in each column. Significant differences between temperatures among treatment ($P < 0.05$, Tukey's HSD test) are denoted by different uppercase letters in each column.

Temp.	Treatment	Adult mortality	Progeny	Frass (g)	% IDKs
22°C	Control	5.79 ± 1.64d	0.73 ± 0.25B	0.04 ± 0.01aC	0.72 ± 0.11C
	Diacon® IGR	11.39 ± 2.03d	0.79 ± 0.24	0.02 ± 0.00bB	0.44 ± 0.09
	Evegreen®	35.09 ± 5.78c	0.60 ± 0.19B	0.00 ± 0.00cB	0.37 ± 0.08
	Gravista®	72.70 ± 9.71bB	0.87 ± 0.59	0.00 ± 0.00c	0.32 ± 0.09
	Sensat™	99.37 ± 0.63a	0.00 ± 0.00	0.00 ± 0.00c	3.85 ± 3.50
27°C	Control	5.43 ± 1.77c	107.73 ± 12.11aA	1.32 ± 0.16aB	1.90 ± 0.29aB
	Diacon® IGR	10.61 ± 2.03c	1.00 ± 0.28b	0.03 ± 0.01bA	0.67 ± 0.28b
	Evegreen®	41.17 ± 4.02b	2.86 ± 1.00cB	0.08 ± 0.02bB	0.37 ± 0.14b
	Gravista®	89.67 ± 4.24aAB	0.00 ± 0.00d	0.01 ± 0.00b	0.15 ± 0.10c
	Sensat™	97.43 ± 1.40a	0.20 ± 0.11d	0.00 ± 0.00b	0.26 ± 0.07c
32°C	Control	7.02 ± 1.27c	184.33 ± 30.97aA	3.48 ± 0.86aA	3.15 ± 0.77aA
	Diacon® IGR	13.97 ± 3.26c	0.73 ± 0.33c	0.04 ± 0.00cA	0.39 ± 0.08b
	Evegreen®	38.60 ± 8.12b	59.33 ± 15.94bB	0.81 ± 0.19bA	3.50 ± 2.42a
	Gravista®	100.00 ± 0.00aA	0.13 ± 0.09c	0.01 ± 0.00c	0.17 ± 0.06b
	Sensat™	100.00 ± 0.00a	0.13 ± 0.13c	0.01 ± 0.01c	0.24 ± 0.07b

Table 3.3 Efficacy of four different grain protectants against *Sitophilus oryzae* at three temperature conditions (22, 27, and 32°C) on the mean (± SE) percent adult mortality, number of adult progeny, amount of frass and percent IDKs. Significant difference between treatments among each temperature ($P < 0.05$, Tukey's Honestly Significant Difference (HSD) test) are denoted by different lowercase letters in each column. Significant differences between temperatures among treatment ($P < 0.05$, Tukey's HSD test) are denoted by different uppercase letters in each column.

Temp.	Treatment	Adult mortality	Progeny	Frass (g)	% IDKs
22°C	Control	2.64 ± 0.80c	60.07 ± 9.16aB	0.20 ± 0.03bB	8.84 ± 1.12abB
	Diacon® IGR	2.88 ± 1.01c	46.14 ± 7.89aB	0.17 ± 0.03bB	6.31 ± 1.04bB
	Evegreen®	2.49 ± 1.05cB	53.00 ± 4.52aB	0.30 ± 0.05aA	9.52 ± 1.49abAB
	Gravista®	69.32 ± 4.70aA	7.07 ± 2.19bB	0.03 ± 0.01cB	1.59 ± 0.17cC
	Sensat™	38.78 ± 5.91bB	23.33 ± 4.84bB	0.08 ± 0.02cB	2.84 ± 0.43cC
27°C	Control	1.44 ± 0.64bc	165.40 ± 16.57A	0.37 ± 0.06A	18.74 ± 1.70abA
	Diacon® IGR	1.20 ± 0.91c	177.13 ± 20.85A	0.41 ± 0.06A	15.69 ± 0.94abA
	Evegreen®	3.53 ± 1.46bcB	154.93 ± 17.32A	0.26 ± 0.04A	12.29 ± 0.83cA
	Gravista®	13.16 ± 2.44bB	131.40 ± 14.60A	0.29 ± 0.05A	13.85 ± 0.91bcA
	Sensat™	54.81 ± 8.92bAB	111.67 ± 14.82	0.31 ± 0.05A	11.48 ± 1.29cA
32°C	Control	4.38 ± 1.58c	65.14 ± 12.43aB	0.38 ± 0.07A	8.72 ± 1.07B
	Diacon® IGR	4.40 ± 1.37c	59.87 ± 12.17aB	0.32 ± 0.07aAB	7.10 ± 1.13B
	Evegreen®	14.85 ± 6.33bcA	63.40 ± 12.21aB	0.37 ± 0.07aA	6.69 ± 1.15B
	Gravista®	24.67 ± 4.68bB	25.53 ± 5.36bB	0.12 ± 0.03bB	4.79 ± 0.55B
	Sensat™	70.62 ± 8.99aA	43.07 ± 9.82abA	0.12 ± 0.03bB	7.01 ± 1.73B

Table 3.4 Efficacy of four different grain protectants against *Prostephanus truncatus* at three temperature conditions (22, 27, and 32°C) on the mean (± SE) percent adult mortality, number of adult progeny, amount of frass and percent IDKs. Significant difference between treatments among each temperature ($P < 0.05$, Tukey's Honestly Significant Difference (HSD) test) are denoted by different lowercase letters in each column. Significant differences between temperatures among treatment ($P < 0.05$, Tukey's HSD test) are denoted by different uppercase letters in each column.

Temp.	Treatment	Adult mortality	Progeny	Frass (g)	% IDKs
22°C	Control	5.71 ± 1.61c	0.00 ± 0.00C	0.13 ± 0.08aB	2.83 ± 0.55aC
	Diacon® IGR	6.59 ± 2.12cB	0.00 ± 0.00	0.07 ± 0.02abB	2.79 ± 0.56aB
	Evegreen®	25.18 ± 5.36b	0.00 ± 0.00	0.02 ± 0.00bB	1.35 ± 0.18bB
	Gravista®	87.79 ± 2.64aA	0.00 ± 0.00	0.00 ± 0.00b	0.78 ± 0.10b
	Sensat™	77.83 ± 4.82a	0.00 ± 0.00	0.00 ± 0.00b	0.96 ± 0.18b
27°C	Control	12.51 ± 1.74c	12.93 ± 6.37aA	1.53 ± 0.63aA	26.38 ± 4.47aA
	Diacon® IGR	5.40 ± 1.34dB	0.20 ± 0.11b	0.22 ± 0.04bA	6.74 ± 1.10bA
	Evegreen®	18.62 ± 1.64c	0.87 ± 0.59b	0.22 ± 0.09bA	5.89 ± 1.63bA
	Gravista®	62.69 ± 4.89bB	0.00 ± 0.00b	0.01 ± 0.00b	1.16 ± 0.17c
	Sensat™	82.61 ± 2.48a	0.00 ± 0.00b	0.01 ± 0.00b	1.13 ± 0.17c
32°C	Control	10.72 ± 2.36c	3.07 ± 1.34aB	0.64 ± 0.20aA	13.90 ± 2.05aB
	Diacon® IGR	15.15 ± 3.18cA	0.20 ± 0.14b	0.22 ± 0.05bA	4.84 ± 0.87cAB
	Evegreen®	15.61 ± 1.95c	0.67 ± 0.36b	0.31 ± 0.06bA	8.03 ± 0.93bA
	Gravista®	60.43 ± 6.66bB	0.00 ± 0.00b	0.01 ± 0.00c	1.03 ± 0.25d
	Sensat™	78.54 ± 4.41a	0.00 ± 0.00b	0.01 ± 0.00c	1.22 ± 0.25d

Table 3.5 Efficacy of four different grain protectants against *Plodia interpunctella* at three temperature conditions (22, 27, and 32°C), with water serving as the control. For each temperature condition, means followed by different lowercase letters denote statistically significant differences among insecticide treatments for a given response variable ($P < 0.05$, Tukey's Honestly Significant Difference (HSD) test). Means followed by different uppercase letters indicate significant differences among temperature conditions for a given variable ($P < 0.05$, Tukey's HSD test).

Temp.	Treatment	Larva	Webbing (Y/N)	Adults	IDK ranking[a]
22°C	Control	27.07 ± 1.45aA	Yes	10.73 ± 2.04aB	3
	Diacon® IGR	30.07 ± 2.02a	Yes	0.00 ± 0.00b	2
	Evegreen®	0.87 ± 0.26bC	Yes	0.27 ± 0.15bB	3
	Gravista®	0.00 ± 0.00c	No	0.00 ± 0.00b	0
	Sensat™	0.00 ± 0.00c	No	0.00 ± 0.00b	0
27°C	Control	6.67 ± 1.34bC	Yes	37.67 ± 3.00aA	3
	Diacon® IGR	35.13 ± 3.47a	Yes	0.00 ± 0.00c	2
	Evegreen®	2.60 ± 0.61cB	Yes	26.93 ± 3.85bA	3
	Gravista®	0.00 ± 0.00d	No	0.00 ± 0.00c	0
	Sensat™	0.00 ± 0.00d	No	0.00 ± 0.00c	0
32°C	Control	11.27 ± 0.93bB	Yes	29.80 ± 1.43aA	3
	Diacon® IGR	32.67 ± 3.11a	Yes	0.00 ± 0.00c	2
	Evegreen®	4.60 ± 0.95cA	Yes	22.40 ± 1.96bA	3
	Gravista®	0.00 ± 0.00d	No	0.00 ± 0.00c	0
	Sensat™	0.00 ± 0.00d	No	0.00 ± 0.00c	0

[a]Damage and infestation were assessed using four metrics: number of larvae per bottle, presence or absence of webbing, number of adult moths, and a composite damage rating scale (0–3). The damage rating was defined as follows: 0 = no larvae, webbing, or adults present; 1 = larvae only; 2 = larvae and webbing present; 3 = larvae, webbing, and adults present.

Chapter 4 - Evaluating the Long-Term Efficacy of Four Active Ingredients against *Rhyzopertha dominica* and *Sitophilus oryzae* on Stored Sorghum

4.1. Abstract

Stored-product pests such as *Rhyzopertha dominica* (F.) and *Sitophilus oryzae* (L.) are two major contributors to post-harvest grain losses globally. Grain protectants can be applied to stored grains to provide long term protection of the commodity; however, their long-term efficacy can depend on pest species, environmental conditions, and the type of grain treated. The objective of this study was to determine the residual efficacy of four commercially available grain protectants 1) Gravista® (deltamethrin + (S)-methoprene + PBO), 2) Diacon® IGR (S)-methoprene), 3) Sensat™ (spinosad), and 4) EverGreen® (pyrethrin), applied to lots of sorghum and stored for 28 weeks under simulated storage conditions. Subsamples were collected every 4 weeks, and 10 adults of either *R. dominica* or *S. oryzae* were placed into vials that contained the treated or untreated grain. Initial adult mortality was assessed after 7 d, adults were removed, and vials were held for 8 wks and then assessed for the number of progeny, frass weight, and the percentage of insect-damaged kernels. There was 100% adult mortality for *R. dominica* on spinosad treated grain across all sampling periods compared to 30 – 39% *S. oryzae* adult mortality over 28 wks. *Rhyzopertha dominica* progeny was lower on all treated grain over the 28 wk storage periods. In contrast, only treated grain stored for > 20 wks had fewer *S. oryzae* progeny compared to the untreated grain. Overall, *R. dominica* was more susceptible to all 4 insecticide treatments compared to *S. oryzae.* These findings highlight the importance of species-specific evaluations when selecting grain protectants for long-term grain storage. The results demonstrate that while protectants can be effective on sorghum against stored-product pests, their success is influenced by pest biology, environmental conditions, and active ingredients.

4. 2. Introduction

Stored-product pests are a specialized group of arthropods that infest and consume dried stored products, posing a significant threat to global food security. These pests, which can be found in storage facilities, shipping containers, and commercial markets, target both intact and

broken grain kernels within storage facilities and across supply chains (Hagstrum et al., 2012). In the United States (U.S.), damage caused by these insets due to infestations contribute to an estimated $2.5 billion in annual economic losses due to product degradation, damage, and contamination (Dorsett, 2021; Hubert et al., 2018). Integrated pest management (IPM) is a comprehensive approach to prevent, control, and manage product loss due by combining preventative and corrective measures to safeguard stored grain supplies (Environmental Protection Agency, 2024).

One key method of pest control for stored bulk grain is the application of grain protectants (Arthur and Subramanyam, 2012). These are formulated insecticides that are applied directly to freshly harvested, non-infested grains as they are loaded into storage bins or silos (Arthur and Subramanyam, 2012). For optimal efficacy, insects must come into direct contact with the insecticide either through ingestion of treated grain or through penetration of the exoskeleton when the insect is traversing treated grain (Authur, 1996). A wide range of commercially available protectants exists in the U.S., each with distinct modes of action determined by the active ingredients (a.i.) binding to specific target-site receptors in insects. Some act as broad-spectrum insecticides effective against all life stages, while others function as insect growth regulators (IGRs) that disrupt normal juvenile development and prevent pests from reaching adulthood (Tunaz and Uygun, 2004). The selection of the appropriate grain protectant is influenced by the target pest, the commodity being treated, and the duration of storage, with certain situations benefiting from the use of multiple agents to ensure comprehensive, long-term protection (Arthur, 2004; Arthur, 2015).The type of commodity being treated can significantly influence the adhesion and residual efficacy of the grain protectant during storage (Scheff et al., 2024). Factors such as grain surface texture and size, moisture content, and chemical composition impact how the protectant coats the kernel and how quickly it degrades over time (Authur, 1996). Extensive research on several different grains has revealed notable differences in protectant persistence and effectiveness based on different commodities (Scheff et al., 2024; Athanassiou et al., 2009). For example, Scheff et al. (2024) tested the efficacy of two commercially available grain protectants, Diacon® IGR *Plus* (a.i. methoprene and deltamethrin) and Gravista® (a.i. methoprene, deltamethrin, and the synergist piperonyl butoxide), against *Trogoderma granarium* Everts, khapra beetle, larvae when applied to wheat, maize, and brown rice. Both protectants demonstrated high efficacy on maize, with significant suppression of larval

development and adult emergence for up to 12 months. Wheat exhibited moderate control, with a delayed increase in adult emergence after 6 months. In contrast, brown rice showed poor treatment efficacy compared to the other two grains tested, with adult emergence approaching control levels by month six, indicating limited residual activity long-term (Scheff et al., 2024).

Insecticidal active ingredients degrade at varying rates depending on abiotic factors such as temperature (Ding et al., 2023), relative humidity (Ding et al., 2023), and exposure to ultraviolet (UV) light (Soliman, 2012). Soliman (2012) investigated the impact of UV light exposure and elevated temperature on the stability and efficacy of five insecticides (methomyl, buprofenzin, pirimicarb, imidacloprid, and ES-fenvalerate) applied to *Bemisia tabaci* (Hemiptera: Aleyrodidae) immature stages. The study found that methomyl and buprofenzin degraded rapidly, with > 90% loss of active ingredient and a corresponding increase in LC_{50} values, particularly after extended UV exposure up to 6 hours and high-temperature storage up to 45°C. In contrast, ES-fenvalerate and pirimicarb demonstrated greater stability, retaining higher residue concentrations and insecticidal potency under the same conditions. Daylight also accelerated degradation relative to dark storage, confirming that light, heat, and storage duration play critical roles in insecticide persistence and effectiveness. Moreover, Ding et al. (2023) evaluated how relative humidity influences the degradation of five insecticide active ingredients (triazophos, chlorpyrifos, malathion, fenitrothion, and cypermethrin) when applied to wheat and wheat flour under controlled storage conditions. The study found that higher relative humidity up to 80%, particularly at elevated temperatures around 35°C, significantly reduced the half-lives of all tested compounds. For instance, malathion and chlorpyrifos exhibited rapid degradation with half-lives of 6.6 and 8.1 days, respectively, under these conditions, while their half-lives were more than 12 days at lower humidity (60%). Cypermethrin, though more stable, also showed reduced persistence, with its half-life dropping from over 30 days at low humidity to about 18.5 days at high humidity. Degradation occurred faster in wheat flour than in whole wheat, possibly due to a larger surface area and moisture absorption.

Sorghum bicolor (L. Moench), widely referred to as sorghum, great millet, or milo, ranks among the five most significant cereal crops worldwide (Grain: World Markets and Trade (n.d.)). While primarily valued in Western countries such as the U.S. and Australia for biofuel production and livestock feed, sorghum serves as a staple food for an estimated 300–500 million people in many parts of Africa and Asia (Zheng et al., 2023). In 2023, global sorghum

production reached 118.9 million metric tons, with the U.S. ranking among the top producers at 20.46 million metric tons (United States Department of Agriculture, 2024). Yet despite its widespread cultivation and economic significance worldwide, research on the long-term effectiveness of grain protectants on sorghum is lacking compared to other staple cereal grains like wheat, rice, and maize. While studies on these grains provide a useful framework for understanding how protectants might behave on sorghum, key differences in grain structure, chemical composition, and storage conditions mean that direct studies on sorghum are necessary to develop optimized treatment strategies. Without targeted research, assumptions based on other grains may not fully capture the unique challenges associated with protecting stored sorghum from infestation and contamination. Given the growing demand for sorghum as both a food and industrial crop (Visarada, 2018), further investigation into its susceptibility to stored-product pests and the long-term efficacy of available insecticidal treatments is essential to ensure stored product security. Therefore, the objective of this study was to evaluate the residual efficacy of commercially available grain protectants, Gravista®, Diacon® IGR, EverGreen®, and Sensat™, applied to sorghum against *Rhyzopertha dominica* (L.) (Coleoptera: Bostrichidae) and *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae) infestations during simulated storage.

4. 3. Materials and methods

4. 3. 1. Insects and commodity

The insect species used in this study were lab strains of *R. dominica* and *S. oryzae* from colonies maintained at the USDA–ARS, Center for Grain and Animal Health Research (CGAHR) in Manhattan, KS for more than 30 years *R. dominica* and *S. oryzae* were reared on a commercial whole sorghum (Cargill®, Salina, KS) in an environmental chamber set at 27°C and 60% relative humidity (r.h.), in continual darkness (Percival, Perry, IA). Adult *R. dominica* and *S. oryzae* were added to sorghum and removed after one week, thus ensuring emerging adults used in all studies would be of similar age. Two-week-old mixed sex adult *R. dominica* and *S. oryzae* were used for all experiments.

The sorghum used in all studies was first frozen for a minimum of 48 h to kill any insects that may be present in the grain. The sorghum was sieved using a grain dockage sieve (Seedburo Equipment Company, Chicago, IL), with grain first being processed through as #6 (5/64”, 1981 µm) sieve and a #10 (2.5/64”, 990 µm) sieve. The sorghum was then processed through a

thresher machine (Precision Machine Co. INC., Lincoln, NE) to remove any glumes, dust, and any other foreign material.

4. 3. 2. Insecticide formulations and commodity treatments

Four different commercially available grain protectant formulations were used in this study, with water serving as the control. The first contained the active ingredient (*S*)-methoprene, with the tradename Diacon® IGR (Central Life Sciences, Schamburg, IL). The formulation consists of 33.6% a.i. (*S*)-methoprene. Diacon® IGR was applied at the label rate of 210 ml insecticide per 18.9 L water for sorghum. To treat at this rate, 0.6 mL of Diacon® IGR was mixed with 50 mL water and 1.9 ml of insecticide was applied to treat 2.5 kg of sorghum.

The second insecticide used in this study was a 5% a.i. pyrethrin concentrate with the tradename EverGreen® (MGK, Minneapolis, MN) and was applied at the label rate of 6 fl. Oz per 3.7 L of water for sorghum. To treat at this rate, 2.4 ml of EverGreen® was mixed with 50 ml of water and applied at the rate of 1.9 ml to treat 2.5 kg of sorghum.

The third grain protectant used in this study consisted of a combination of deltamethrin + methoprene + piperonyl butoxide (PBO), with the tradename of Gravista® (Central Life Sciences, Schamburg, IL), and henceforth referred to by the trade name Gravista®. The formulation consists of 1.20% active ingredient (a.i.) deltamethrin, 2.85% a.i. (*S)*-methoprene, and 33.30% a.i. (PBO). Gravista® was applied at the label rate of 937 ml insecticide per 18.9 L water for sorghum. To treat at this rate, 2.5 mL of Gravista® was mixed with 50 mL of water and 1.9 ml of insecticide was applied to treat 2.5 kg of sorghum.

The fourth grain protectant to be used contained the active ingredient spinosad, with the tradename Sensat™ (Bayer CropScience, Research Triangle Park, NC), and will henceforth be referred to the tradename Sensat™. The formulation consists of 8.66% a.i. spinosad and 91.34% a.i. other ingredients. Sensat was applied at the label rate of 290 ml insecticide per 18.9 L water for sorghum. To treat at this rate 0.8 ml of Sensat was mixed with 50 ml water and 1.9 ml of insecticide was applied to treat 2.5 kg of sorghum.

Insecticide applications were made using an artist spray brush (Model 100™, Badger, Franklin Park, IL), with only water being applied as the control on the sorghum. During the application, sorghum was gently poured into a 0.29 × 0.29 m, 15 L plastic bin (Sterlite®, Townsend, TN) while simultaneously being sprayed with the insecticide to mimic applications of a grain protectant being applied during grain bin loading process. After the insecticide

application, the grain was manually shaken for 20 s to ensure an even coating of the insecticide. The bins were fitted with a vented lid and allowed to dry for 24 h in ambient conditions. This application process was replicated three times for each insecticide treatment and treatment applications were conducted on separate weeks for each species.

4. 3. 3. Efficacy bioassays of grain protectants

Approximately 24 h after insecticide applications, the treated sorghum was transferred to an 18.9 L plastic bucket fitted with two screened holes on the lid to allow for air movement. Prior to closing the lids, five 50 g subsamples were taken from each bucket and placed in a 0.18 L plastic vials with a vented lid for a total of 15 vials per insecticide (wk 0). The buckets with the remaining sorghum were placed in an empty 112 metric ton grain bin located at the USDA-ARS-CGAHR campus along with 3 separate HOBO temperature/humidity data loggers (Onset, Bourne, MA) placed inside each bucket containing the untreated sorghum.

The vials containing the 50 g subsamples were taken into the lab and 10 one-to-two-week-old mixed-sex adults of *R. dominica* or *S. oryzae* were added to each vial and held for 7 d in an environmental chamber set at 27°C and 65% r.h. After 7 d, adult *R. dominica* or *S. oryzae,* were removed and assessed for mortality. The vials were returned to the chamber and held for an additional 8 wks to allow for progeny emergence. After 8 wks, vials were frozen for 48 h and then the sorghum was sifted through a #12 (1700 µm), #30 (600 µm), and a catch pan sieve stack (U.S. Standard Sieve Series, Dual Manufacturing Co., Chicago, IL). The adult beetles were retained on the top of the #30 sieve, sorghum kernels were retained on the #12 sieve, and frass was collected in the catch pan. The number of adult progeny and weight (g) of the frass was recorded. After sorghum samples were sieved, three subsamples of approximately 5 g were randomly taken from each vial and analyzed under a stereomicroscope (Dino-Lite 3.0 Edge Digital Microscope, Dunwell Tech, Inc. Torrance, CA) to observe any insect damaged kernels (IDKs). IDKs consisted of kernels that exhibited adult emergence holes (Figure 4.1A), signs of adult feeding (Figure 4.1B) and extensive feeding damage (Figure 4.1C). The total percentage of IDKs from each vial due to infestation was calculated using equation 4.1.

Equation 4.1: $$Percent\ IDKs = \left[\frac{Insect\ Damaged\ Kernels}{Undamaged\ Kernels + Insect\ Damaged\ Kernels}\right] \times 100$$

Grain moisture content was measured every sample week by using a grain moisture analyzer (Mini Gac® 2500, DICKEY-john®, Auburn, IL). Three different random samples of ~100 g of sorghum were taken from the control groups and were averaged for each sample week. The entire procedure described previously was repeated every 4 wks for a total of 28 wks for each species and treatment from May 2023 through November 2023.

4. 3. 4. Data analysis

The average temperature and relative humidity recorded by the 3 HOBO units were averaged per wk and the mean and standard error (± SE) were calculated. Changes in grain moisture over time were averaged for each sample wk, and the mean and standard error (± SE) were calculated similarly.

Replicate blocks (buckets) were considered to be independent and thus the subsamples from each block (bucket) were combined for analysis. Data were first analyzed by insect species. The mean and standard errors for progeny and frass were transformed via $\log_{10}(x + 1)$ for statistical analysis (Zar, 2010). The percentage of initial adult mortality, IDKs were transformed into angular values prior to statistical analysis (Zar, 2010). Data on the temperature and relative humidity within grain bins was subjected to a one-way analysis of variance (ANOVA), with the main factor being time (weeks) using Statistical Analysis Software (SAS Version 9.4, SAS Institute, Cary, NC). Data on the percentage of adult mortality, progeny, frass, and percentage IDKs were subjected to a two-way ANOVA, with the main factors of insecticide treatment and weeks of storage as the main factors. If the ANOVA was significant ($P < 0.05$), differences among the treatments were determined by separating means using LS Means with Tukey's Honestly Significant Difference (HSD) test ($P < 0.05$).

4.4. Results

4. 4. 1.Seasonal effects of temperature, relative humidity, and sorghum moisture content

The main effect of storage time (weeks) was significant for the mean weekly temperature ($F = 141.86$; df = 27, 560; $P < 0.0001$) and relative humidity ($F = 7.32$; df = 27, 560; $P < 0.0001$). The mean weekly temperature increased steadily from ~25°C in wk 1 to a peak of ~35°C after 16 wks of storage. Following this peak, temperatures generally declined, with a particularly sharp drop to ~ 4°C by wk 26 (Figure 4.2). The mean weekly relative humidity

peaked at 63% after 2 wks of storage (Figure 4.2). In the subsequent weeks, the relative humidity fluctuated between 38 and 60%.

The mean grain moisture content significantly declined steadily over the 28-week storage period ($F = 40.39$; df = 7, 16; $P < 0.0001$) (Table 4.1). At the start of the trial (wk 0), mean moisture content was 13.30%, but by wk 8 it had significantly dropped to 12.03%. This downward trend continued through the remainder of the study, with moisture falling to 9.9% after 28 wks of storage.

4. 4. 2. Effect of Grain Protectants on Adult Mortality

4. 4. 2. 1. Rhyzopertha dominica

The main effects of insecticide treatment ($F = 78.6$; df = 4, 595; $P < 0.05$) and the interaction of treatment × storage week ($F = 20.31$; df = 39, 560; $P < 0.05$) were significant for the initial *R. dominica* adult mortality. However, storage time was not significant ($F = 0.87$; df = 7, 592; $P = 0.5337$), which indicates that the active ingredients in all the formulations had a consistent effect on *R. dominica* adults throughout the 28 wk storage period. This was most evident in the Sensat ™ treatment where 100% mortality was achieved at every time interval (Table 4.2). Furthermore, the initial adult mortality for *R. dominica* on sorghum treated with Gravista® ranged between 67 – 95%, with only wks 24 and 28 having a significant reduction in mortality. In contrast, EverGreen® and Diacon® IGR were less effective overall. Mortality with EverGreen® increased to a peak of 25.27% at wk 16 but remained significantly lower than Gravista® and Sensat™ at all time points. Diacon® IGR expectedly showed low mortality values throughout the course of this experiment, ranging between 0–11.82%.

4. 4. 2. 2. Sitophilus oryzae

All main effects were statistically significant for *S. oryzae* adult mortality (Week: $F = 15.11$; df = 7, 592; $P < 0.05$; Treatment: $F = 78.60$; df = 4, 595; $P < 0.05$; Week × Treatment: $F = 20.31$; df = 7, 560; $P < 0.05$), indicating that both the type of treatment and storage duration significantly influenced adult mortality, with the response varying notably over time depending on the treatment applied. Similar to the results observed for *R. dominica* initial adult mortality, the insecticide Sensat™ had the highest mortality among all the treatments. However, mortality ranged from 30 – 39% (Table 4.3), contrasting the high mortality percentage for this insecticide against *R. dominica*. This trend was similar for Gravista®, although it had a much larger range in

mortality that spanned 0 – 41%. Adult mortality of Diacon® IGR and EverGreen® treated sorghum remained low throughout the storage period, except for the 20 wk storage period (Table 4.3). This was the only period where these insecticides had significant increase in mortality compared to the control.

Over the 28-wk storage period, Sensat™ maintained relatively consistent adult mortality levels for S. oryzae, with percentages ranging from 30% at wk 8 to a peak of 39% at week 4. Gravista® showed a more variable trend, with negligible mortality early in storage (0% at wk 8) but a pronounced increase beginning at wk 12 (12%), peaking sharply at wk 20 (41%), suggesting varied efficacy of this insecticide against this species. EverGreen® and Diacon® IGR exhibited low mortality across most wks, generally remaining below 8%, with one marked exception: both treatments showed statistically significant increases at wk 20, with EverGreen® reaching 42%, and Diacon® IGR reaching 22%. This isolated spike suggests a potential interaction between storage conditions and insecticide potency during that period. Outside of this anomaly, mortality levels in EverGreen® and Diacon® IGR treatments were not significantly different from the control.

4. 4. 3. Effect on progeny production

4. 4. 3. 1. Rhyzopertha dominica

All main effects were statistically significant for *R. dominica* progeny production (Week: $F = 7.78$ df = 7, 592, $P < 0.05$; Treatment: $F = 178.02$ df = 4, 595, $P < 0.05$; Week × Treatment: $F = 82.84$, df = 39, 560, $P < 0.05$), indicating that both the type of insecticidal treatment and storage duration played critical roles in progeny suppression. With the exception of EverGreen® at 12 wks, all insecticidal treatments had significantly fewer progeny compared to the control up to 20 wks of storage. Sorghum treated with Gravista®, and Sensat™ had the fewest progeny over the 28 wk storage period ranging from 0 – 10 and 0 – 6 individuals respectively (Table 4.4). Despite minimal effect on *R. dominica* adult mortality, both Diacon® IGR and EverGreen® significantly reduced adult progeny. However, it should be pointed out that control progeny product decreased over the same storage period.

During the course of this experiment, there was a notable decline in progeny production from 93 individuals at wk 0 to 1 individual by wk 28 for the control group, indicating that there may have been changes in the commodity itself over time which could have been affecting

progeny output. Furthermore, there was a sharp dip in progeny development for the control group at wk 12, with only 3 individuals emerging. This trend is also somewhat observed for sorghum treated with EverGreen®, as trial wk 0 yielded 13 individuals and was lowered to less than 1 individual on average by wk 28. However, there was an uptick in progeny output at wk 20 with 10 individuals on average emerging from treated samples. Regarding Diacon® IGR, Gravista®, and Sensat™, there was steady rate of suppression of progeny emergence for each of these treatments throughout the span of 28 wks. Similar to EverGreen®, however, spikes in progeny production was observed at wk 16 for Gravista® with 6 individuals on average and 10 individuals emerging on average at wk 20 for samples treated with Sensat™. These results so far indicate that there may have been environmental factors at play that affected the sorghum grain in storage, which may also explain spikes in progeny output in some experimental wks for some of the chemical treatments tested.

4. 4. 3. 2. Sitophilus. oryzae

All main effects were statistically significant for *R. dominica* progeny production (Week: $F = 58.63$ df = 7, 587, $P < 0.05$; Treatment: $F = 3.18$ df = 4, 590, $P < 0.05$; Week × Treatment: $F = 14.4$, df = 39, 555, $P < 0.05$), indicating that both the type of insecticidal treatment and storage duration played critical roles in progeny suppression. Similar to results observed from *R. dominica* trials, these results indicate that both the type of insecticidal treatment and storage duration were significant in the suppression of *S. oryzae* progeny emergence. In stark contrast to *R. dominica* trials, however, none of the chemical treatments suppressed progeny emergence of *S. oryzae* during the course of this experiment, with all of them somewhat matching progeny trends seen in the control group. While the rate of progeny suppression for Sensat™ against *S. oryzae* was not as effective when compared to the suppression seen in *R. dominica* trials (Table 4.4), Sensat™ did still perform the best overall compared to the other treatments, with it limiting progeny emergence to only 64-1 individuals during the course of this experiment, whereas the rest of the treatments had progeny numbers that ranged from 84-6 individuals in the case of Diacon® IGR, to 104-8 and 114-6 individuals for both EverGreen® and Gravista®, respectively. These results indicate possible resistance or tolerance mechanisms for S. oryzae when compared to results observed for *R. dominica*.

During the course of this experiment, there was, again, a general decrease in progeny emergence across all treatment groups and control (Table 4.5). This trend is more evident with

trials regarding *S. oryzae* since progeny numbers across all treatments were much larger than those seen in *R. dominica* trials, which also experienced the same outcome in the control group (Table 4.4). Regarding each treatment group, progeny numbers were largest at the beginning of the experiment (wk 0) and gradually declined to fewer than 10 individuals for all treatments and the control by wk 28. Because this general decrease in progeny over time has been noted for both species tested, it may again be concluded that there are abiotic effects within the storage environment that are affecting sorghum grain characteristics over time which may be partially inhibiting larval development and emergence.

4. 4. 4. Effect on grain quality attributes

4. 4. 4. 1. Rhyzopertha. dominica

The extent of grain damage caused by *R. dominica* differed significantly by treatment, week, and their interactions as measured by both frass (Week: $F = 4.26$, df = 7, 592, $P < 0.05$: Treatment: $F = 140.94$, df = 4, 595, $P < 0.05$; Week × Treatment: $F = 26.79$, df = 39, 560, $P < 0.05$) and IDKs (Week: $F = 16.82$, df = 7, 592, $P < 0.05$: Treatment: $F = 190.64$, df = 4, 595, $P < 0.05$; Week ×Treatment: $F = 103.83$, df = 39, 560, $P < 0.05$). these results indicate a significance for both the type of insecticidal treatment and storage time on the suppression of damage caused by *R. dominica*.

Among all treatments, Sensat™ and Gravista® consistently provided the highest level of grain protection from insect feeding damage (Table 4.6, 4.7). Both resulted in minimal frass accumulation across the study period. Sorghum treated with Gravista® had < 0.04 g of frass compared to < 0.06 g on sorghum treated with Sensat™ across the 28 wk testing period. The IDK percentage values mirrored these results as Gravista® maintained kernel damage below 1.1% throughout, ranging from 0.10% to 1.02%, and Sensat™ achieved similarly low levels, with 0.11% at wk 20 and 0.02% at wk 28. Diacon® IGR showed moderate efficacy, with frass levels peaking at 0.14 g at wk 0 and 0.07 g at wk 24. IDK values under Diacon® IGR ranged from 0.42% to 2.69%, indicating intermediate but relatively consistent suppression when compared to the control. In contrast, EverGreen® was the least effective treatment. Frass production remained elevated, notably at wks 0 (0.31 g), 12 (0.51 g), and 20 (0.25 g), and IDK values ranged widely from 0.68% to 8.54%, indicating substantial grain damage despite treatment.

Over time, the effectiveness of each protectant varied to a degree. Gravista® and Sensat™ maintained consistently low levels of frass and kernel damage across all time points (Table 4.6, 4.7), showing no meaningful degradation in efficacy over the 28 wks. Their protection profiles suggest a stable, long-lasting impact. In contrast, Diacon® IGR exhibited more variation over time, with higher frass accumulation at the beginning (wk 0) and modest rebounds later (wk 24), suggesting a gradual decline in effectiveness or periodic surges in insect activity. EverGreen® was especially affected by time: although some fluctuations were observed at specific points such as the small drop in frass accumulation and IDK percentage wk 16. EverGreen®'s overall pattern showed persistent and sometimes increasing damage, indicating that its protective effects were both weak and inconsistent over the duration of the study.

Once again, a general decline in both frass accumulation and IDK percentages were observed for the control group of this species similar to the decline seen in the progeny production (Table 4.4, 4.6, 4.7). This decline in destruction coupled with the decline in progeny production over time indicates that there may be a hindrance on larval development and emergence possibly due to changes in sorghum grain characteristics during storage.

4. 4. 4. 2. Sitophilus oryzae

Grain damage caused by *S. oryzae* differed significantly by treatment, week, and their interactions as measured by both frass (Week: $F = 25.63$, df = 7, 587, $P < 0.05$: Treatment: $F = 3.75$, df = 4, 590, $P < 0.05$; Week × Treatment: $F = 9.38$, df = 39, 555, $P < 0.05$) and IDKs (Week: $F = 51.98$, df = 7, 585, $P < 0.05$: Treatment: $F = 10.19$, df = 4, 588, $P < 0.05$; Week × Treatment: $F = 16.45$, df = 39, 553, $P < 0.05$), indicating a significance for both the type of insecticidal treatment and storage time on the suppression of damage. Compared to *R. dominica*, the ability of the treatments to suppress damage caused by *S. oryzae* was rather inconsistent, with some treatments experiencing higher levels of both frass levels and percentages of IDKs expanding above that found in the control group during certain wks (Table 8, 9). Furthermore, damage caused by *S. oryzae* was generally greater across all treatments when compared to damage caused by *R. dominica* (Table 4.6, 4.7).

Frass accumulation varied across treatments over time, with no treatment achieving full suppression throughout the 28-wk trial (Table 4.8). Although Gravista® and Sensat™ showed the most effective overall control, all treatments exhibited fluctuations, with periodic spikes in frass levels despite initial strong performance. For example, Diacon® IGR began with the highest

initial frass at 0.95 g at wk 0, followed by a gradual but uneven decline, including a resurgence at wk 20 before falling to 0.09 g by wk 28. EverGreen® was among the most inconsistent treatments as it exhibited elevated frass levels at wk 8 with 0.39 g and wk 20 with 0.26 g, with only a modest reduction to 0.14 g by wk 28. Gravista® and Sensat™, while superior overall, were not immune to transient lapses. Gravista® produced a spike at wk 4 with 0.41 g of accumulated frass, nearly matching levels seen in the previous treatments and the control group, before quickly declining and stabilizing at low values like 0.04 g at wk 28. Sensat™, which achieved high levels of suppression at later part in the experiment, such as wk 20 (0.05 g) and wk 28 (0.04 g), also exhibited early and mid-study increases, including 0.39 g at week 8 and 0.18 g at week 12.

The IDK percentages also varied across treatments over time, with no insecticide maintaining complete suppression of kernel damage throughout the 28-wk trial (Table 4.9). Although Gravista® and Sensat™ consistently achieved the strongest overall reductions in insect-damaged kernels, all treatments exhibited fluctuations in efficacy, with intermittent increases in IDK values despite periods of strong performance. Diacon® IGR began with elevated kernel damage at 13.29% in wk 0 and, although levels generally declined, it experienced a resurgence at wk 20 (8.30%) before reaching rates of 1.92% by wk 28. EverGreen® showed an erratic performance again, with high IDK values early and mid-study,9.95% at wk 0 and a peak of 10.41% at wk 20, followed by a modest decrease to 1.37% by the end of the trial.

Gravista® and Sensat™, while outperforming all other treatments in overall protection, were not without temporary lapses. Gravista® maintained IDK values below 6% for much of the trial but reached 9.80% at wk 8 before dropping sharply to 0.72% at wk 28. Sensat™ recorded the lowest IDK value at 1.64% by wk 28, but also showed a notable rise mid-study, including 7.21% at wk 8 and 5.85% at wk 12. These results highlight the long-term potential of Gravista® and Sensat™, while also emphasizing the challenge of maintaining uninterrupted suppression under continuous insect pressure.

4. 5. Discussion

One notable aspect of this study that could have a major impact on the effectiveness of the grain protectants was the changes in the physical condition of the grain, particularly the reduction in moisture content. The decrease in moisture content during the 28 wk storage period

could be due to the increase in the ambient temperature and decrease in the relative humidity in the grain bin where the sorghum was stored. The effect of the reduced grain moisture content can be observed in the control data, whereby there was a general reduction in progeny, frass, and IDKs over the 28 wk storage period. This is likely due to the fact both species are dependent on adequate grain moisture, with *R. dominica* performing optimally at 12 – 14% (Birch, 1953) and *S. oryzae* at 14 – 16% (Abedi et al., 2024). As grain dried during storage, it is likely that reduced moisture impaired egg viability, slowed development, and increased immature-stage mortality (Manu et al., 2019).

Diacon® IGR is a commercially available grain protectant that contains the active ingredient (S)-methoprene, an insect growth regulator (IGR). The active ingredient's mode of action acts by disrupting the development of juvenile insects, preventing them from successfully maturing into adults after encountering the treated grain either through surface exposure or ingestion (Wijayaratne et al., 2018). Because methoprene primarily targets immature developmental stages, the low adult mortality observed for both species in this study aligns with its expected mode of action. However, research by Chanbang et al. (2008) demonstrated that even in the absence of significant adult mortality, methoprene can induce notable sublethal effects on adult insects. In their study, *R. dominica* adults exposed to rough rice treated with 1 ppm methoprene showed a dramatic reduction in fecundity, laying only 12.5 eggs per female, compared to 52.1 eggs per female in untreated controls, equating to a 76% decline, underscoring that while methoprene may not directly kill adults, it can severely impair their reproductive output (Chanbang et al., 2008).

Despite the minimal adult mortality, Diacon® IGR was significantly more effective at suppressing larval development and emergence in *R dominica* than in *S. oryzae*. This is evident in the relatively high progeny counts of *S. oryzae* on Diacon® IGR treated sorghum. In contrast, there was little to no progeny produced by *R. dominica* on similarly treated grain. However, despite the low progeny numbers for *R. dominica*, a notable accumulation of frass in some treated samples suggests that limited larval development had still occurred. The lack of suppressive effect against *S. oryzae* was further highlighted by the greater extent of damage (frass accumulation, IDKs) observed in sorghum samples infested with this species, across all experimental weeks, compared to those infested by *R. dominica*.

EverGreen®, which contains natural botanical pyrethrins as its active ingredient, is relatively underperformed in comparison to the other protectants applied to sorghum grain. Despite adult mortality for *R. dominica* on samples treated with this protectant being higher when compared to Diacon® IGR and the control group, with peak adult mortality being only at 25%, this insecticide's ability to affect adult mortality did not compare to the high rates seen in samples treated with Gravista® and Sensat™. Although it was not consistent in its complete suppression, there were still levels of progeny suppression for adult *R. dominica* exposed to EverGreen®-treated sorghum grain. Moreover, it had very low rates of adult mortality for *S. oryzae,* and the progeny produced by this species on sorghum treated with this protectant were high, mirroring the progeny numbers seen in the control group. The low adult mortality and inability to fully suppress progeny production for both species resulted in rates of damage that were higher than what was experienced in samples treated with other protectants in this study. This aligns with findings from Bomzan et al. (2018), who observed that natural pyrethrum applied to wheat and barley alone yielded only moderate adult mortality in *S. oryzae* and *R. dominica* even at high doses whereas much higher efficacy was achieved when pyrethrum was synergized with synthetic pyrethroids like deltamethrin or cypermethrin. This underscores the limited standalone potency of botanical pyrethrins and suggests that the relatively poor performance of EverGreen® may reflect this limitation of natural pyrethrum-based formulations.

Gravista®, a formulation containing the active ingredients (*S*)-methoprene, deltamethrin, and piperonyl butoxide (PBO), demonstrated strong efficacy in suppressing *R. dominica* infestations. High adult mortality rates were recorded for *R. dominica*, reaching approximately 90% within the initial wks of the experiment. However, starting at wk 20, mortality began to decline, dropping to around 80% and to 66% by the conclusion of the study, possibly indicating levels of residual efficacy loss. Notably, this decline followed the warmest recorded storage temperatures during the trial period, suggesting that elevated heat may have contributed to the degradation of the active ingredient and the reduced adult mortality observed in *R. dominica* trials. Similarly, in Arthur's (2018) study, deltamethrin applied to concrete surfaces showed substantial degradation under high summer temperatures, with *Tribolium castaneum* knockdown dropping to as low as 15.3 – 34% after 3 hrs of exposure by 42 –70 d in grain bin conditions, compared to 84.7 – 100% in cooler, controlled environments. However, the decline in adult

mortality we observed for *R. dominica* occurred around 140 days after treatment, indicating that differences in surfaces that are treated also have an effect on degradation rate.

Yet despite this marginal drop in adult mortality for *R. dominica*, the high adult mortality trend likely contributed to the significant suppression of progeny, with few to no larvae observed throughout the study. In contrast, *S. oryzae* exhibited greater tolerance to Gravista® under identical treatment conditions. Although Gravista® remained more effective than EverGreen®, it was less effective overall against *S. oryzae* as adult mortality for this species was minimal, and the treated grain supported considerable progeny production, leading to substantial grain damage. Nevertheless, the amount of frass and the IDK percentage observed for *S. oryzae* exposed to Gravista® was still lower than in both the control group and the group treated with EverGreen™, suggesting partial protective efficacy despite higher overall tolerance.

A possible reason for Gravista®'s performance in suppressing infestations compared to protectants such as EverGreen™, could be held in its multi-active ingredient formulation and inclusion of a synergist. With both methoprene, an insect growth regulator, and deltamethrin, a potent contact insecticide, acting simultaneously on exposed insects, multiple physiological pathways are targeted which enhances the overall efficacy. Furthermore, the inclusion of a synergist like piperonyl butoxide (PBO) may inhibit the insect's ability to detoxify the active compounds, particularly deltamethrin, by blocking key metabolic enzymes that detoxify compounds such as pyrethrums and pyrethroids such as cytochrome P450 (Hodgson et al., 1999). Support for this dual-use insecticide mechanism is found in a study by Arthur (2019), which tested combinations of methoprene + deltamethrin on stored commodities including wheat, corn, and brown rice. On wheat, adult *R. dominica* exhibited nearly complete suppression, with progeny counts reduced to near zero and less than 0.01% weight loss observed (Arthur 2019). For *S. oryzae*, although some progeny were produced, the combination of 1.0 ppm deltamethrin and 2.5 ppm methoprene resulted in a sharp reduction in adult numbers and grain damage compared to untreated controls (Arthur 2019).

Sensat™, which contains the active ingredient spinosad, consistently yielded the highest adult mortality among all treatments for both insect species, with particularly strong efficacy against *R. dominica*. In fact, 100% adult mortality was maintained across all sampling wks, demonstrating sustained residual activity on sorghum grain with no apparent degradation over the span of 28 wks. In contrast, while *S. oryzae* also showed the highest adult mortality under

Sensat™ treatment compared to other protectants, mortality reached only about 35%, indicating that Sensat™ was substantially less effective against this species. Progeny production of *R. dominica* on Sensat™-treated sorghum remained low throughout the trial, with only a single late-week progeny spike of 10 individuals emerged, potentially resulting from delayed mortality of the initially exposed adults. This strong suppression of adult survival and reproduction in *R. dominica* corresponded with a markedly reduced level of grain damage, which was consistently lower than that observed under other treatments and significantly lower than in the untreated control. As with previous trials, *S. oryzae* responded differently. Despite moderately elevated adult mortality under Sensat™, progeny production remained relatively high across all weeks, which also could indicate a delay in mortality. Nevertheless, the number of progeny and the resulting grain damage were both considerably lower than those observed with other protectants, suggesting partial effectiveness of Sensat™ in suppressing *S. oryzae* populations in storage environments. These findings are consistent with the results of Vayias et al. (2010), who evaluated spinosad applied at 0.1, 0.5, and 1.0 ppm to wheat, maize, and barley. They reported that *R. dominica* exhibited near-complete mortality and almost no progeny emergence across a 6-month storage period, particularly at 1.0 ppm, highlighting the insecticide's strong residual efficacy. In contrast, *S. oryzae* showed significant tolerance, with viable progeny still emerging even 30 days after application, especially at lower concentrations. Their results, in combination with out, emphasize the interspecies differences in spinosad susceptibility and reinforce its strong but selective effectiveness as a grain protectant.

Across all protectants evaluated, *R. dominica* was more effectively controlled than *S oryzae*, though each product exhibited varying degrees of success depending on its formulation and mode of action. Sensat™ emerged as the most effective protectant overall for *R. dominica*, achieving 100% adult mortality throughout the 28-wk trial and maintaining consistently low progeny levels and minimal grain damage. Gravista® also performed strongly against *R. dominica*, with adult mortality initially reaching 90% and remaining above 80% until late in the experiment; this led to substantial progeny suppression and low grain damage. Diacon® IGR, while not causing significant adult mortality, effectively inhibited progeny development in *R. dominica*, aligning with its insect growth regulator mechanism. EverGreen®, however, had only moderate success with this species, showing low adult mortality and inconsistent progeny suppression.

In contrast, *S. oryzae* proved more resistant across all treatments. Sensat™ resulted in the highest adult mortality among treatments (~35%), yet progeny production remained relatively high, indicating levels of limited suppression. Gravista® showed moderate efficacy, with lower grain damage and progeny counts than the control or EverGreen® but still lacked the capacity to significantly reduce adult survival or fully prevent reproduction and grain damage. Despite its reduced adult mortality Diacon® IGR was still relatively as effective against S. oryzae as the other protectants in this study. EverGreen® performed the poorest overall, with negligible adult mortality and high progeny output for *S. oryzae*, resulting in high levels of grain damage. Overall, protectant performance was species-dependent, with Sensat™ and Gravista® demonstrating the most consistent efficacy, particularly against *R. dominica*.

R. dominica and *S. oryzae* are two key primary pests of bulk-stored grains. As internal feeders, both species complete their development within individual kernels, consuming the germ and endosperm before emerging as adults. However, a critical behavioral difference that may explain why *R. dominica* showed more susceptibility to insecticide treatments compared to *S. oryzae* could lie in how each species initiates infestation. Adult *R. dominica* deposit eggs on or near the grain surface, after which the mobile larvae actively seek out and bore into kernels. In contrast, *S. oryzae* females use their rostrum to bore directly into a kernel and oviposit internally, effectively isolating both the egg and the developing larva from external grain surfaces. This difference in oviposition behavior may partially explain why *S. oryzae* exhibited greater success in terms of progeny production and general damage to sorghum samples (frass and IDKs) across treatments compared to *R. dominica*. Because *S. oryzae* females deposit eggs directly into individual kernels, thus bypassing the grain surface entirely, developing stages are less likely to encounter surface-applied insecticides. In contrast, *R. dominica* eggs and larvae would have an increased chance of being exposed to insecticide residues on the surface of grains. This increased exposure risk is supported by findings from Chanbang et al. (2008), who also reported that *R. dominica* egg hatch was significantly reduced by surface-applied methoprene. At the labeled rate, hatch dropped to 52% and fell as low as 26.7% at higher concentrations. These results highlight the vulnerability of surface-deposited eggs to insecticide residues and help explain *R. dominica*'s reduced success in treated environments.

Furthermore, a study by Wijerathna et al. showed that differences in the cuticular composition of each species had a significant effect on their susceptibility to botanical

insecticides. In toxicity trials using *Acorus calamus* essential oil, *R. dominica* exhibited a much lower LC_{50} value (4.78 µL/g) than *S. oryzae* (9.61 µL/g), indicating that *S. oryzae* was substantially more tolerant to the compound. Scanning electron microscopy revealed more extensive cuticular damage in *R. dominica*, suggesting that its cuticle may be thinner or more permeable, allowing for greater absorption and internal toxicity (Balabanidou et al., 2018). In contrast, *S. oryzae* likely possesses a thicker or more hydrophobic epicuticle, limiting the penetration of lipophilic compounds and reducing the efficacy of contact insecticides (Balabanidou et al., 2018). These physiological differences offer a plausible explanation for the patterns observed in the present study, where *R. dominica* consistently exhibited higher adult mortality and lower progeny production across all chemical treatments compared to *S. oryzae*. The lower absorptive capacity of *S. oryzae*'s cuticle likely reduced the uptake of active ingredients, contributing to its greater resilience under chemical stress.

Beyond differences in behavior and structural differences in the insect cuticle, metabolic detoxification mechanisms also play a critical role in mediating insecticide susceptibility. A comparative study by López et al. (2010) found that *S. oryzae* developed tolerance to multiple monoterpenoids through enhanced esterase activity, while *R. dominica* failed to exhibit similar adaptive capacity despite possessing cytochrome P450 monooxygenases. This study suggests that *S. oryzae* has a more flexible and robust enzymatic detoxification system, which may contribute to its ability to withstand chemical treatments. Supporting this, Getchell and Subramanyam (2008) reported that *S. oryzae* experienced delayed or reduced mortality compared to *R. dominica* when exposed to spinosad, implying either slower uptake, faster detoxification, or both. These physiological and biochemical traits likely explain why *S. oryzae* demonstrated greater resilience across all protectants tested in this study.

The varied responses of *R. dominica* and *S. oryzae* across treatments reinforce the fact that no single protectant is universally effective, and that pest-specific biology and behavior must be considered in post-harvest control strategies (Hagstrum and Athanassiou, 2019). While some products demonstrated excellent efficacy against one species, they were notably less effective against the other highlighting the danger of extrapolating efficacy data from other grains or pest species to sorghum without direct evaluation (Hagstrum and Athanassiou, 2019). Importantly, this study reinforces the necessity of commodity- and species-specific testing under realistic storage conditions. Such research is vital not only to identify effective insecticide formulations

for sorghum, but also to understand their limitations in practical use scenarios. Moreover, future work should extend beyond stand-alone treatments to consider how grain protectants integrate with other IPM components, such as the efficacy of these protectants under different storage temperatures on sorghum grain, to build more comprehensive, resilient pest management programs. Further investigation is essential, particularly involving other key pest species and environmental variables that affect protectant efficacy such as storage temperature, to advance the protection of this globally important crop and ensure the long-term preservation of stored sorghum in diverse agricultural contexts.

4. 6. Figures and tables

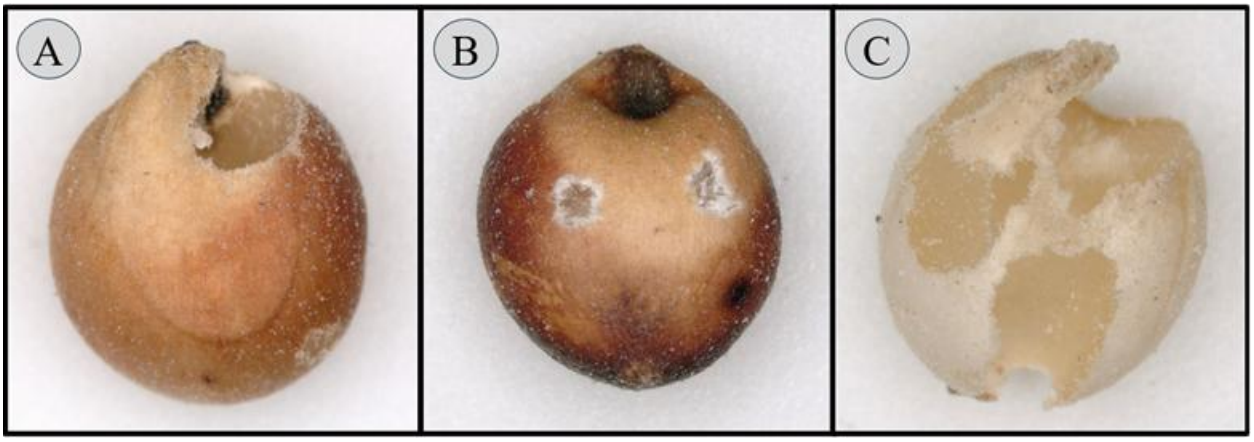

Figure 4.1 Photos of sorghum grain exhibiting A) adult insect emergence damage, B) adult feeding, and C) extensive damage caused by insects.

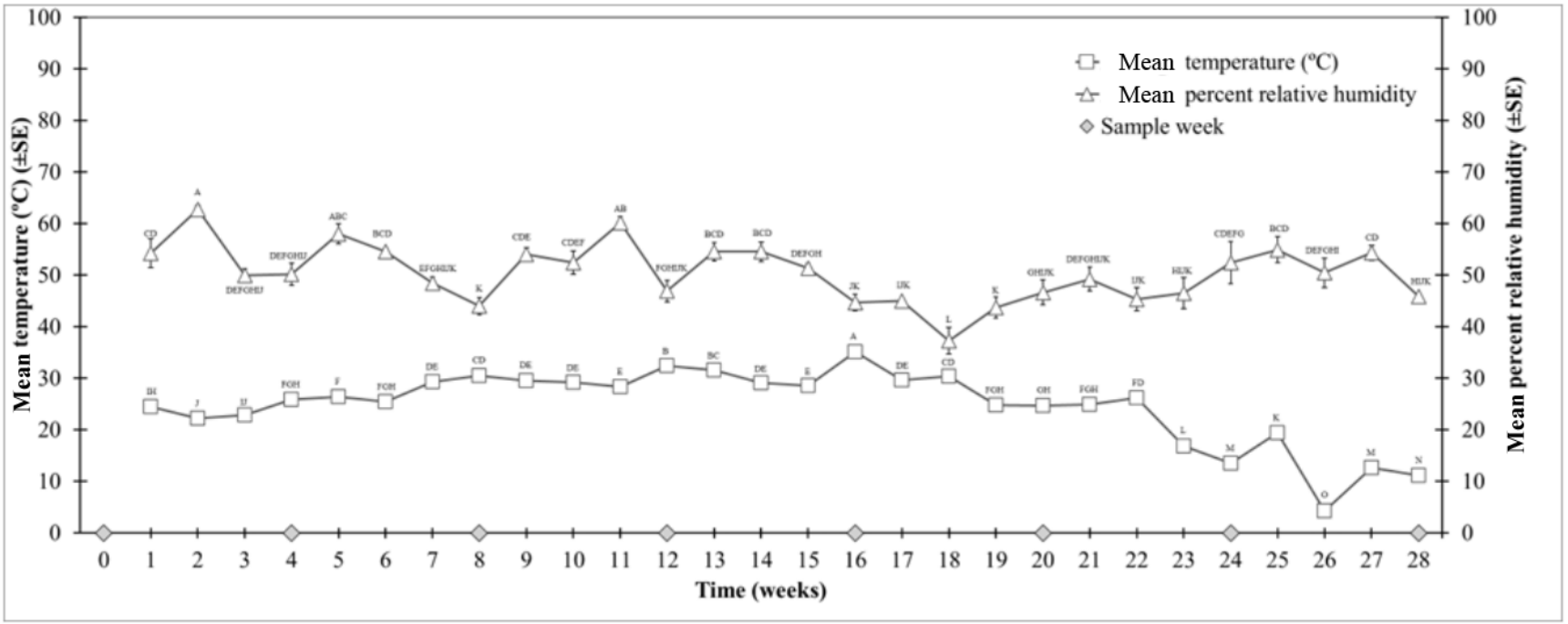

Figure 4.2 Trends in the mean weekly temperature (°C) and percent relative humidity (r.h.) in experimental storage bins over the course of 28 weeks. The black lines and triangles represent the mean temperature each week, black lines and squares represent the mean relative humidity each week, and the gray diamonds are the weeks sorghum sub-samples were pulled from the buckets in storage. Different uppercase letters indicate significant differences ($P < 0.05$, Proc GLM, LSMeans with Tukey's HSD test)

Table 4.1 Mean (± SE) percent moisture content of sorghum held in 30 L plastic bucket in an empty grain bin over a 28-week period. Means followed by different letters within the same column indicate statistically significant differences among the trial weeks ($P < 0.05$, Proc GLM, LSMeans with Tukey's HSD test).

Trial week	Moisture content
0	13.30 ± 0.26A
4	12.83 ± 0.15A
8	12.03 ± 0.18B
12	11.40 ± 0.29CD
16	11.57 ± 0.17BC
20	10.87 ± 0.12DE
24	10.50 ± 0.06E
28	9.93 ± 0.09F

Table 4.2 Mean (± SE) percent adult mortality of adult *Rhyzopertha dominica* exposed on Sorghum grain treated with either water (control), Diacon® IGR, EverGreen®, Gravista®, Sensat™. Means for rows within weeks for each treatment are noted with different lowercase letters indicating significant differences among treatments and means within columns for each treatment are noted with different uppercase letters denoting significant differences among weeks within a specific treatment ($P < 0.05$, Proc GLM, LSMeans with Tukey's HSD test).

Trial week	Control	Diacon® IGR	EverGreen®	Gravista®	Sensat™
0	8.67 ± 2.15cAB	4.67 ± 1.92cdABC	2.00 ± 1.17dD	89.33 ± 3.58bA	100.0 ± 0.0a
4	5.33 ± 1.65cBC	5.33 ± 1.65cAB	3.41 ± 1.29cD	90.00 ± 6.62bA	100.00 ± 0.00a
8	3.83 ± 1.59cCD	7.33 ± 2.28cAB	24.07 ± 5.04bAB	92.67 ± 5.39aA	100.00 ± 0.00a
12	8.83 ± 2.75cABC	4.00 ± 2.35dBC	12.81 ± 1.87cBC	94.67 ± 2.15bA	100.00 ± 0.00a
16	4.15 ± 1.36cBCD	6.00 ± 1.63cAB	25.27 ± 6.10bA	94.67 ± 2.56aA	100.00 ± 0.00a
20	8.16± 3.42cBC	11.82 ± 3.66cA	12.00 ± 2.99cC	80.00 ± 10.69bAB	100.00 ± 0.00a
24	0.67 ± 0.67cD	0.00 ± 0.00cC	2.00 ± 1.07cD	72.76 ± 4.90bBC	100.00 ± 0.00a
28	14.09 ± 2.67cA	5.65 ± 2.46dABC	23.26 ± 4.61cAB	66.67 ± 6.45bC	100.00 ± 0.00a

Table 4.3 Mean (± SE) percent adult mortality of adult Sitophilus oryzae exposed on Sorghum grain treated with either water (control), Diacon® IGR, EverGreen®, Gravista®, Sensat™. Means for rows within weeks for each treatment are noted with different lowercase letters indicating significant differences among treatments and means within columns for each treatment are noted with different uppercase letters denoting significant differences among weeks within a specific treatment (*P*< 0.05, Proc GLM, LSMeans with Tukey's HSD test).

Trial week	Control	Diacon® IGR	EverGreen®	Gravista®	Sensat™
0	1.33 ± 0.91cBC	1.33 ± 0.91cC	0.00 ± 0.00cD	14.00 ± 3.49bC	36.67 ± 5.58aA
4	3.33 ± 1.26cBC	3.33 ± 1.59cBC	4.48 ± 1.56bcBC	13.10 ± 4.04bC	38.82 ± 5.65aA
8	0.67 ± 0.67bC	1.33 ± 0.91bC	0.67 ± 0.67bCD	0.00 ± 0.00bE	30.00 ± 4.58aA
12	4.67 ± 1.65cB	7.94 ± 2.22bcB	6.00 ± 2.35bcB	12.00 ± 2.79bC	34.00 ± 6.31aA
16	3.33 ± 1.26bBC	2.67 ± 1.18bBC	4.00 ± 1.63bBCD	3.33 ± 1.59bDE	37.33 ± 6.05aA
20	4.15 ± 1.36cB	21.63 ± 5.93bA	41.78 ± 6.11aA	40.67 ± 6.86aA	38.00 ± 5.45aA
24	0.61 ± 0.61cC	4.00 ± 1.31bcBC	3.33 ± 1.25bcBCD	6.67 ± 2.11bCD	30.00 ± 4.8aA
28	12.83 ± 2.66bA	14.00 ± 2.14bA	8.00 ± 2.00bB	27.33 ± 5.30aB	34.93 ± 3.49aA

Table 4.4 Mean (± SE) progeny of adult *Rhyzopertha dominica* exposed on Sorghum grain treated with either water (control), Diacon® IGR, EverGreen®, Gravista®, Sensat™. Means for rows within weeks for each treatment are noted with different lowercase letters indicating significant differences among treatments and means within columns for each treatment are noted with different uppercase letters denoting significant differences among weeks within a specific treatment (*P*< 0.05, Proc GLM, LSMeans with Tukey's HSD test).

Trial week	Control	Diacon® IGR	EverGreen®	Gravista®	Sensat™
0	93.33 ± 11.23aA	0.07 ± 0.07cC	12.80 ± 2.04bA	0.00 ± 0.00cC	0.00 ± 0.00cC
4	81.87 ± 9.53aA	0.87 ± 0.27cAB	5.67 ± 1.39bB	0.60 ± 0.24cB	0.27 ± 0.12cBC
8	72.67 ± 8.63aA	2.73 ± 0.99cA	5.60 ± 1.27bB	0.53 ± 0.24cdB	0.20 ± 0.14dC
12	3.53 ± 0.67aD	0.47 ± 0.17bBC	4.27 ± 1.27aB	0.20 ± 0.11bBC	0.20 ± 0.14bC
16	49.67 ± 7.01aB	0.47 ± 0.19cBC	0.33 ± 0.16cD	5.93 ± 1.28bA	0.07 ± 0.07cC
20	29.53 ± 4.86aBC	0.67 ± 0.23cBC	9.67 ± 1.64bA	0.27 ± 0.15cBC	10.40 ± 0.79bA
24	20.47 ± 3.50aC	0.33 ± 0.13cBC	2.53 ± 0.57bBC	0.13 ± 0.09cBC	1.00 ± 0.65cB
28	1.27 ± 0.32aD	0.53 ± 0.17bcBC	0.87 ± 0.17abCD	0.20 ± 0.20cBC	0.13 ± 0.13cC

Table 4.5 Mean (± SE) progeny of adult *Sitophilus oryzae* exposed on Sorghum grain treated with either water (control), Diacon® IGR, EverGreen®, Gravista®, Sensat™. Means for rows within weeks for each treatment are noted with different lowercase letters indicating significant differences among treatments and means within columns for each treatment are noted with different uppercase letters denoting significant differences among weeks within a specific treatment ($P < 0.05$, Proc GLM, LSMeans with Tukey's HSD test).

Trial week	Control	Diacon® IGR	EverGreen®	Gravista®	Sensat™
0	107.07 ± 10.49A	83.87 ± 15.28A	104.13 ± 10.20A	114.00 ± 15.57A	64.33 ± 8.63A
4	41.00 ± 6.73BCD	64.20 ± 9.67A	48.07 ± 8.85BC	49.73 ± 9.46B	42.87 ± 6.45AB
8	47.60 ± 5.35BC	48.60 ± 6.81A	45.73 ± 8.15BC	60.73 ± 8.83AB	43.13 ± 8.37ABC
12	28.67 ± 3.81D	23.13 ± 3.10B	29.53 ± 5.38C	25.13 ± 4.76CD	20.13 ± 2.50C
16	40.73 ± 3.63BC	35.20 ± 4.56AB	31.47 ± 5.02BC	37.47 ± 5.01BC	42.67 ± 3.54AB
20	53.53 ± 6.17aB	45.80 ± 4.99aA	58.60 ± 6.88aB	22.53 ± 3.80bD	13.67 ± 3.29cD
24	34.33 ± 4.54aCD	18.40 ± 2.53bB	26.87 ± 3.96abC	19.20 ± 4.23bD	26.00 ± 3.70abBC
28	8.47 ± 1.52aE	6.13 ± 1.93abC	8.33 ± 1.27aD	3.80 ± 0.88bE	8.80 ± 1.73aD

Table 4.6 Mean (± SE) frass produced (g) from adult *Rhyzopertha dominica* exposed on sorghum grain treated with either water (control), Diacon® IGR, EverGreen®, Gravista®, Sensat™. Means for rows within weeks for each treatment are noted with different lowercase letters indicating significant differences among treatments and means within columns for each treatment are noted with different uppercase letters denoting significant differences among weeks within a specific treatment (*P* < 0.05, Proc GLM, LSMeans with Tukey's HSD test).

Trial week	Control	Diacon® IGR	EverGreen®	Gravista®	Sensat™
0	0.54 ± 0.08aB	0.14 ± 0.05cA	0.31 ± 0.07bB	0.01 ± 0.00dBC	0.00 ± 0.00dB
4	0.99 ± 0.11aA	0.07 ± 0.04cAB	0.17 ± 0.03bB	0.02 ± 0.01cABC	0.03 ± 0.03cAB
8	0.25 ± 0.08aCD	0.02 ± 0.01bB	0.17 ± 0.03aB	0.00 ± 0.00bC	0.00 ± 0.00bB
12	0.87 ± 0.12aA	0.03 ± 0.00cB	0.51 ± 0.09bA	0.04 ± 0.01cA	0.00 ± 0.00cB
16	0.44 ± 0.07aB	0.03 ± 0.01cB	0.23 ± 0.03bB	0.03 ± 0.01cAB	0.06 ± 0.03cA
20	0.48 ± 0.06aB	0.01 ± 0.00cB	0.25 ± 0.03bB	0.00 ± 0.00cC	0.00 ± 0.00cB
24	0.39 ± 0.05aBC	0.07 ± 0.04cAB	0.18 ± 0.02bB	0.01 ± 0.01cBC	0.01 ± 0.01cB
28	0.15 ± 0.06aD	0.10 ± 0.08abAB	0.22 ± 0.11aB	0.00 ± 0.00bC	0.00 ± 0.00bB

Table 4.7 Mean (± SE) percent insect-damaged kernels (IDKs) of adult *Rhyzopertha dominica* exposed on sorghum grain treated with either water (control), Diacon® IGR, EverGreen®, Gravista®, Sensat™. Means for rows within weeks for each treatment are noted with different lowercase letters indicating significant differences among treatments and means within columns for each treatment are noted with different uppercase letters denoting significant differences among weeks within a specific treatment ($P < 0.05$, Proc GLM, LSMeans with Tukey's HSD test).

Trial week	Control	Diacon® IGR	EverGreen®	Gravista®	Sensat™
0	26.68 ± 2.35aA	2.52 ± 0.29cAB	7.21 ± 0.84bA	0.65 ± 0.09dAB	0.94 ± 0.24dB
4	28.21 ± 1.76aA	2.69 ± 0.20cA	6.36 ± 0.58bA	1.01 ± 0.08dA	0.99 ± 0.15dB
8	18.84 ± 1.88aB	2.53 ± 0.29cAB	7.89 ± 0.65bA	0.97 ± 0.13dA	0.79 ± 0.14dB
12	14.11 ± 1.39aC	1.91 ± 0.24cB	8.54 ± 1.08bA	1.02 ± 0.17cdA	0.80 ± 0.20dB
16	10.14 ± 0.88aC	2.17 ± 0.23cAB	0.68 ± 0.11dC	0.73 ± 0.12dA	4.11 ± 0.54bA
20	5.85 ± 0.70aD	1.07 ± 0.16cC	3.60 ± 0.28bB	0.52 ± 0.21dBC	0.11 ± 0.08eC
24	5.04 ± 0.36aD	1.87 ± 0.17cB	3.26 ± 0.40bB	0.22 ± 0.06dCD	0.22 ± 0.08dC
28	2.17 ± 0.35aE	0.42 ± 0.08bD	0.77 ± 0.16bC	0.10 ± 0.06cD	0.02 ± 0.02cC

Table 4.8 Mean(± SE) frass produced (g) of adult *Sitophilus oryzae* exposed on sorghum grain treated with either water (control), Diacon® IGR, EverGreen®, Gravista®, Sensat™. Means for rows within weeks for each treatment are noted with different lowercase letters indicating significant differences among treatments and means within columns for each treatment are noted with different uppercase letters denoting significant differences among weeks within a specific treatment ($P < 0.05$, Proc GLM, LSMeans with Tukey's HSD test).

Trial week	Control	Diacon® IGR	EverGreen®	Gravista®	Sensat™
0	0.42 ± 0.07bAB	0.95 ± 0.25aA	0.26 ± 0.02bcA	0.14 ± 0.02cBCD	0.26 ± 0.04bcAB
4	0.29 ± 0.09BA	0.27 ± 0.04B	0.37 ± 0.08A	0.41 ± 0.15A	0.36 ± 0.09A
8	0.45 ± 0.07aA	0.24 ± 0.04bcBC	0.39 ± 0.09abA	0.16 ± 0.02cB	0.39 ± 0.06abA
12	0.10 ± 0.02abD	0.07 ± 0.01bC	0.09 ± 0.02bB	0.05 ± 0.01bCD	0.18 ± 0.06aBC
16	0.14 ± 0.01CD	0.10 ± 0.01C	0.10 ± 0.02B	0.15 ± 0.01BC	0.12 ± 0.02CD
20	0.27 ± 0.03aBC	0.23 ± 0.03aBC	0.26 ± 0.04aA	0.12 ± 0.02bBCD	0.05 ± 0.01cD
24	0.30 ± 0.12aBC	0.09 ± 0.03bC	0.10 ± 0.02bB	0.11 ± 0.04bBCD	0.08 ± 0.01bCD
28	0.05 ± 0.01D	0.09 ± 0.02C	0.14 ± 0.06B	0.04 ± 0.01D	0.04 ± 0.01D

Table 4.9 Mean (± SE) percent insect-damaged kernels (IDKs) of adult *Sitophilus oryzae* exposed on sorghum grain treated with either water (control), Diacon® IGR, EverGreen®, Gravista®, Sensat™. Means for rows within weeks for each treatment are noted with different lowercase letters indicating significant differences among treatments and means within columns for each treatment are noted with different uppercase letters denoting significant differences among weeks within a specific treatment ($P < 0.05$, Proc GLM, LSMeans with Tukey's HSD test).

Trial week	Control	Diacon® IGR	EverGreen®	Gravista®	Sensat™
0	18.95 ± 1.96aA	13.29 ± 1.97bA	9.95 ± 0.97bcA	8.06 ± 0.89cAB	7.88 ± 1.14cA
4	9.05 ± 1.32aBC	8.59 ± 1.04aB	6.49 ± 1.10abB	5.36 ± 0.76bCD	7.28 ± 1.01abAB
8	10.71 ± 1.21aB	8.52 ± 1.18aB	9.25 ± 1.19aA	9.80 ± 1.20aA	7.21 ± 1.03aAB
12	5.50 ± 0.60D	4.60 ± 0.52DC	4.72 ± 0.83B	3.96 ± 0.40D	5.85 ± 0.93AB
16	9.97 ± 1.15aBC	7.60 ± 1.73abBC	6.41 ± 0.94bB	7.10 ± 0.69abBC	5.26 ± 0.67bB
20	9.09 ± 0.87aBC	8.30 ± 0.84aB	10.41 ± 1.21aA	5.82 ± 0.80bCD	2.67 ± 0.44cC
24	7.09 ± 0.80aDC	2.63 ± 0.34dDE	4.57 ± 0.51bcB	4.12 ± 0.48cD	5.99 ± 0.48abAB
28	2.34 ± 0.28aE	1.92 ± 0.20abE	1.37 ± 0.20bC	0.72 ± 0.14cE	1.64 ± 0.23bC

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