Integrated pest management and behavior of stored grain beetles

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INTEGRATED PEST MANAGEMENT AND BEHAVIOR OF STORED GRAIN BEETLES

A Dissertation

Submitted to the Graduate Faculty of the Louisiana State University and Agricultural and Mechanical College in partial fulfillment of the requirements for the degree of Doctor of Entomology in

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by

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In memoriam of Emmaline Rose Doherty
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Abstract

The lesser grain borer, *Rhizopertha dominica*, and the rice weevil, *Sitophilus oryzae*, are two of the most destructive pests of stored rice. Their management has relied heavily upon fumigant insecticides. However, amid growing concerns that fumigant-resistant pests are becoming widespread, there is an urgent need for diversified management techniques and integrated pest management. Here, we explored management techniques and the behavior of these beetles. Host plant resistance toward both beetles was evaluated for 13 varieties of rice. We found large differences among varieties for both beetles in all resistance measures. We found that while nutritional components of a variety did not influence resistance, grain dimensions did influence susceptibility to damage by *R. dominica* adults, larval damage, progeny counts, and progeny mass. Insecticide efficacy was examined by evaluating methoprene, deltamethrin, commercial formulations of methoprene + deltamethrin, β-cyfluthrin, and diatomaceous earth applied to rough rice over six-month periods. We found that methoprene, or a combination of methoprene and deltamethrin were the most effective controls of stored rice pests. *Beauveria bassiana* (strain: GHA) and *Cordyceps fumosorosea* (strain: FE9901) were evaluated as biological control agents of stored grain beetles, alone, together, and in combination with each other. There were higher infection rates of *B. bassiana* in the presence of diatomaceous earth than without it, while combinations of *B. bassiana* and *C. fumosorosea* sometimes resulted in antagonism. Finally, *R. dominica* and *S. oryzae* host-finding abilities were examined through their behavioral responses to different conditions of stored rice using a two-choice olfactometer. We compared responses to clean rice, damaged rice, conspecifics only, only damaged rice, rice infested with conspecifics, and rice infested by another species. While *S. oryzae* did not distinguish between most treatments, *R. dominica* preferred infested rice to only damaged rice or
only beetles. Volatile profiles built through gas chromatography–mass spectrometry identified 8 volatiles unique between the *R. dominica* infested rice, beetles only, and damage only treatments that may have acted as attractants or deterrents. Taken together, these findings build a foundation for effective and sustainable stored grain pest management.
Chapter 1. Literature Review

1.1. Introduction

The US is among the top rice exporters in the world, exporting $1.83 billion of rice on average for the past 3 years (USDA 2023), and Louisiana is the third largest producer in the country (USDA 2019). However, the value of rice, wheat, and other grains sharply declined around 2014, and the market value has yet to recover (Macrotrends 2020; Trading Economics 2020). As a result, grain producers are increasingly storing their harvests in on-site storage bins. These grain bins are often cylindrical metal structures built to store thousands of bushels of grain for extended periods of time while also keeping it cool and dry. Growers may keep a harvest in them for as long as a year, waiting for the best market prices. Consequently, stored product pests are of growing concern.

Postharvest grain loss to insects is a global problem (Neupane 1995; Deshpande and Singh 2001; USDA 2005; Santos 2006; Yigezu et al. 2010; Jiang 2013; Sharon et al. 2014; Zhang et al. 2021). The US loses 5–10% of its total value from corn and wheat postharvest due to insects (USDA 2005; Yigezu et al. 2010). In India, insects are estimated to be responsible for 5% of losses in some grains (Deshpande and Singh 2001; Sharon et al. 2014). Insects also represent the primary method of grain loss in Brazil (Santos 2006). This grain loss can translate to economic loss in several ways. Growers might earn less from damaged kernels due to the weight loss, or grain damaged at high enough rate might be designated sample grade, restricting the product to sale as animal feed (Harein and Meronuck 1990, USDA 2016). Farmers bringing rice that is infested to the mill might be asked to fumigate immediately, or perhaps rejected entirely.

Management of stored rice pests in the US is heavily reliant on fumigant insecticides
(Hagstrum et al. 2012). These fumigants are an effective way for farmers to clean infested grain before shipping, but there are some problems associated with them. For a period, methyl bromide and phosphine gas were the dominant fumigants on the market. However, methyl bromide usage has been limited due to its harmful environmental effects (US EPA 2020), which in turn has increased reliance on phosphine gas. Moreover, this change comes amid growing concerns that phosphine-resistant pests may become widespread. There are already phosphine-resistant populations of stored grain beetles in the United States, Brazil, and other countries (Lorini et al. 2007; Opit et al. 2012; Nayak et al. 2019). The need for diversified techniques and integrated pest management (IPM), the use of multiple control methods, has become urgent.

Many of these pest insects have ranges that are nearly cosmopolitan, and they are non-native to most of those regions, including the United States. The wide distribution of stored products, and the large transportation networks used to move them, may be partially responsible for the successful establishment of so many species to new regions. Grain pests can be categorized into primary pests that attack sound grain, like the lesser grain borer, *Rhizopertha dominica* (F.) (Bostrichidae: Coleoptera), and rice weevil (*Sitophilus oryzae* (L.) (Curculionidae: Coleoptera), or secondary pests that only attack damaged grain. Secondary pests will struggle to establish without damaged grain, and thus can be managed through grain cleaning operations (USDA 2015). For these reasons, this research focuses on primary pests, their biology, and management practices, with the goals to improve management of pests of stored rice and examine the factors which influence population dynamics.

1.2. Primary Pests

1.2.1 Lesser Grain Borer
*Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae) is the most damaging stored rice pest in the US (USDA 2015). They prefer to infest wheat, corn, and rice, but are capable of surviving on all stored grain (Hagstrum et al. 2012). They can also be found outside grain bins, in wooded areas, where they can feed on seeds and acorns (Jia et al. 2008a; Mahroof et al. 2010a). They are native to the tropical regions of Asia, but like many stored rice pests, they are now established on several continents. *Rhyzopertha dominica* are also called the Australian wheat weevil due to heavy infestation in Australia in the early 1900s (Cotton and Good 1937). Infestations in the US can be costly, thus effective management is essential. Su et al. (2019) estimated that proper management of *R. dominica* could improve rice value by $0.35/kg, possibly more.

Adults are commonly dark brown to black, and among the smallest grain pests, at approximately 2–3 mm (Hagstrum et al. 2012). Development can occur between 18.2–39°C, while optimum grain moisture content is between 12–14% (Birch 1953; Longstaff 1999). Brood development will fail at moisture content less than 8% (Birch 1953). Naik et al. (2016) found that at 28°C, egg eclosion occurred after 4–6 days, pupation occurred after another 28–33 days, and adults eclosed after another 5–7 days, making the egg to adult period approximately 37–46 days.

Mating can occur early, even within the first 24h after eclosing (Thompson 1996). Males produce a sex pheromone that is attractive to both males and females, thus males will attempt to mate with males, but females will not attempt to mate with females (Khorramshahi and Burkholder 1981). Both sexes can mate multiple times, and females cannot fertilize all of their eggs without multiple matings (Thompson 1996). Adult females may oviposit up to 500 eggs in their lifetime, between 33–45 per day. These can be oviposited as single eggs or in clusters
outside the grain (Edde 2012). As larvae, they spend their lives inside the grain. Upon reaching adulthood they will leave an amorphous exit hole.

1.2.2. Rice Weevil

*Sitophilus oryzae* (L.) (Coleoptera: Curculionidae) is another of the most damaging pests (USDA 2015). Though native to India, it now occurs within grain in all regions of the world and is particularly abundant in warmer climates (Koehler 1994). Adults commonly feed upon the endosperm, while larvae will feed on the endosperm as well as the germ in the grain interior (Gvozdenac et al. 2020). Padín et al. (2002) found that 20 adult *S. oryzae* consumed over 200 of 500 grams of wheat across 4 months.

The adult rice weevils have an average lifespan of 4–5 months. They are colored brown or black and are small, usually around 3 mm. As a Curculionidae beetle, it also has a distinctive snout. *Sitophilus oryzae* are sexually dimorphic, and females are typically larger (Halstead 1963; Lum and Baker 1975; Holloway and Smith 1987). Females also typically have a longer, narrower rostrum (Halstead 1963). There are also a number of minor differences in the structure of the abdomen (Lum and Baker 1975).

During adulthood, females can lay as many as 400 eggs (USDA 2016). Unlike *R. dominica*, which lays its eggs outside of the grain, *S. oryzae* adults often bore holes in the grain, and oviposit their eggs inside. *Sitophilus oryzae* are capable of acclimating to temperatures as low as 15°C (Evans 1977), while 14°C is the lower bound for development (Birch 1953). The optimal temperature for development is around 29°C (Birch 1953). Development from egg to adult can take as few as 26 days.
1.3. Pest Management

1.3.1. Insecticides

Insecticides are the most commonly used control method in stored products pest management. Fumigants have been particularly popular as they are broad spectrum, fast acting, leave little to no residue, and can be applied in vehicles in preparation for mills/shipment (Hagstrum et al. 2012). As such, they can be used to quickly respond to an infestation, whereas other insecticides are typically used to prevent infestations. Methyl bromide and phosphine gas are the most prevalent fumigants used in stored products. Methyl bromide is thought to kill insects through enzyme methylation (Price 1985). However, due to methyl bromide’s ozone depleting properties its usage has been limited to critical uses (US EPA 2020). Phosphine may have several modes of action that suppress insect metabolism, increase acetylcholine transmission in the sympathetic nervous system, and create oxidative stress within cells (Ebert et al. 2011). Its usage is also limited due to instances of resistance development in insects, as there is currently no replacement for it (Hagstrum et al. 2012).

Other stored grain insecticides are commonly applied to the grain bin or the grain itself. A number of these insecticides impair insect nervous system functions. Historically, DDT and organophosphates like malathion were among them, but they were phased out due to safety concerns and development of resistance (Boyer et al. 2012; Edde 2012). More recently, pyrethroids have been used to fill their role. The pyrethroids deltamethrin, β-cyfluthrin, permethrin, and fenvalerate have been tested in stored grain, but deltamethrin and β-cyfluthrin have been found to be the most effective (Williams et al. 1982, Athanassiou, et al. 2004a). There are now multiple insecticidal products for stored grain containing deltamethrin and β-cyfluthrin. These insecticides can be effective against the adults of several major pests for over a year, but
are less effective against larvae, which tend to live inside of kernels and therefore have shorter exposure times (Ghimire et al. 2016, Arthur 2018, 2019a,b).

The juvenile hormone analogue methoprene has also seen success as an insecticide in stored grain. It can be an effective control against larval insects that are exposed during development, even those that are only exposed briefly. For example, a bioassay by Arthur (2016) found that even small amounts of methoprene were able to completely stop *R. dominica* progeny production. Moreover, methoprene can remain effective for several years (Arthur 2016) and be used in combination with deltamethrin (Arthur 2019a).

Diatomaceous earth has been shown to be an effective protectant of stored products (Mewis and Ulrichs 2001, Athanassiou, et al. 2004b, Athanassiou et al. 2005, Wakil et al. 2010). It operates by absorbing the insect’s wax layer, resulting in desiccation (Mewis and Ulrichs 2001) and by disrupting the spiracles (Webb 1946). Diatomaceous earth can also be effectively used alongside other insecticides like β-cyfluthrin (Athanassiou 2006).

Phytochemicals have also been shown to be effective tools against grain pests. López et al. (2008) found a variety of effective compounds in the oils from the seeds of *Coriander sativum* L. and *Carum carvii* L. as well as leaf oils from *Ocimum basilicum* L. against *S. oryzae*, *R. dominica* and *Cryptolestes pusillus* (Coleoptera: Laemophloeidae). Other effective phytochemicals have been found in eucalyptus leaves (Santos 2006) and dried leaves of *Ocimum* sp. (Obeng-Ofori et al. 1998). However, there are not known comparisons of these methods to conventional insecticides.

Exclusive use of insecticides may create opportunities for secondary pests, and harm beneficial insects. Moreover, behavioral, metabolic, and target site resistances have developed in some populations of stored product pests (Boyer et al. 2012). For example, some *R. dominica*
strains are repelled by some pyrethroids as a behavioral resistance mechanism (Lorini and Galley 1999), and some *Tribolium confusum* du Val (Coleoptera: Tenebrionidae) are able to metabolize malathion (Wool and Front 2002).

Stored product pest control could extend beyond insecticides to include cultural control, pest resistance in grain, and biological control. As such, integrated pest management (IPM), the use of multiple control methods, may be more effective. The use of economic thresholds would also help growers save on the costs of pest management while protecting their harvests. Unfortunately, research on stored rice IPM is scant.

1.3.2. Grain Resistance

Host plant resistance is the result of a plant’s heritable characteristics which allow it to reduce or tolerate injury inflicted by herbivores. It is a low cost IPM tactic that can be easily implemented alongside other management techniques. Cogburn (1977) demonstrated the value in pursuing varietal resistance to primary pests in stored grain, while Cogburn and Bollich (1990) determined that rice resistances to stored product pests were heritable. More recently, Chanbang et al. (2008b) examined the susceptibility of 28 varieties of rice to *R. dominica* through adult emergence and damage to the grain. Arthur et al. (2013) refined their methodology examining susceptibility to *R. dominica* and *S. cerealella* of 25 rice varieties. They found as large as a 6-fold difference in the amount of damage and as large as a 7-fold difference in the number of progeny. Other papers have seen similar trends with *S. cerealella* (Rizwana et al. 2011; Santos et al. 2015) and with *S. oryzae* (Santos et al. 2015; Pal et al. 2021). However, the varieties studied have largely fallen out of use, and few studies have investigated what qualities result in pest resistance.
A few studies have found that physical defenses can confer resistance in stored grain systems (Breese, 1963; Chanbang et al. 2008a; Kavallieratos et al., 2011; Asiwaju-Bello et al., 2019). In stored rice, Chanbang et al. (2008a) found maximum hull thickness was positively correlated to development time, with thicker hulls adding 2–3 days to development of *R. dominica* and reducing the number of progeny. Length/width ratio has also been positively correlated with *S. oryzae* and *R. dominica* progeny emergence (Asiwaju-Bello et al. 2019). The influence of stored rice nutritional content on susceptibility is less clear. Rizwana et al. (2011) investigated lipid, carbohydrate, and protein content as influencers of susceptibility, but found little evidence to suggest variations in nutrients conferred resistance. Further investigations have found susceptibility to be associated with mineral content (Asiwaju-Bello et al. 2019). Further research into grain nutritional content and morphology could lead breeders towards more resistant strains.

1.3.3. Biological Control

Stored grain systems also have numerous beneficial species. Many of those studied are Hemipterans. These include Reduviidae predators like *Amphibolus venator* (Klug) and *Peregrinator biannulipes* (Montrouzier & Signoret) (Imamura et al. 2008), as well as Anthocorid predators like *Xylocoris sordidus* (Reuter) (Arbogast et al. 1983) and *Cardiastethus pygmaeus* Poppius (Imamura et al. 2008). However, few of these species have followed their hosts/prey outside of Eurasia. Among the predators that occur in US, *Xylocoris flavipes* (Reuter) (Hemiptera: Anthocoridae) is perhaps the most well-studied.

Like many stored grain pests, *X. flavipes* has spread across the world. *Xylocoris flavipes* is a generalist predator, feeding on over 20 species of Coleoptera, including *R. dominica*,
Tribolium castaneum (Herbst) (Tenebrionidae: Coleoptera), and T. confusum (Parajulee and Phillips 1993; Imamura et al. 2008). Like many of Anthocorid insects, it preys on the eggs and small larvae of these beetles. Many grain pests can be effectively controlled by X. flavipes a few weeks following their introduction to grain bins (Brower and Press 1992). In laboratory experiments, Adarkwah et al. (2019) found that X. flavipes was capable of controlling R. dominica in both wheat and rice, reducing F1 progeny by 96.5–98.4% compared to controls. However, X. flavipes is not an effective control of S. oryzae, likely because S. oryzae spends more of its life within the grain.

There are also numerous Hymenoptera that attack grain pests in Eurasia (Ryoo et al. 1991; Eliopoulos et al. 2002; Lucas and Riudavets 2002), including the Pteromalidae Anisopteromalus calandrae (Howard), Lariophagus distinguendus (Forster), Pteromalus cerealellae (Ashmead), Theocolax elegans (Westwood), as well as Holepyris sylvanidis (Bréthes) (Bethylidae), Cephalonomia tarsalis (Ashmead) (Bethylidae), and Venturia canescens (Gravenhorst) (Ichneumonidae). Of these species, A. calandrae and T. elegans occur in the US and parasitize both R. dominica and S. oryzae larvae.

Additionally, both parasitoids work well with Xylocoris flavipes. Berger et al. (2017) found that the effects of A. calandrae and X. flavipes were additive in their measures of beetle control. Xylocoris flavipes has also been tested alongside T. elegans (Adarkwah et al. 2019). Alone, X. flavipes reduced R. dominica progeny 96.5–98.4%, while T. elegans resulted in 94–98.4% reduction. Together R. dominica progeny were reduced by 98–99.7%. Similar effects were seen for control of S. oryzae. Unfortunately, X. flavipes and these parasitoids are not currently commercially available as biological control agents in the US.

In addition to predators and parasitoids, entomopathogens have recently been explored as
biological control agents for stored grain systems. Several entomopathogenic fungi (EPF) including *Beauveria bassiana* (Bals.) Vuill., *Metarhizium anisopliae* (Metsch.) Sorok and *Cordyceps fumosorosea* (Wize) have been explored as control agents of *S. oryzae* and *R. dominica* (Sheeba et al. 2001; Vassilakos et al. 2006; Riasat et al. 2011; Kavallieratos et al. 2014; Wakil et al. 2015, 2022; Saeed et al. 2020). For example, Sheeba et al. (2001) found that *B. bassiana* (strain: MTCC 2028) could provide up to 75% mortality of adult *S. oryzae*. Additionally, Wakil et al. (2022) found that applications of *B. bassiana* not only provided control of *R. dominica*, but also that control increased as temperature increased. Some research has also found that EPFs have variable interactions with insecticides, like diatomaceous earth, often acting synergistically or additively (Riasat et al. 2011; Shafighi et al. 2014; Wakil et al. 2015, 2021; Ashraf et al. 2017; Rizwan et al. 2019; Saeed et al. 2020). The mechanisms behind this synergy are unclear, but it may be due to diatomaceous earth increasing fungal attachment rates, or perhaps the drier conditions improving fungal viability, which typically degrades once applied to stored grain (Lord 2001, 2005). Despite the potential of EPFs displayed in these studies, there are currently no commercially available fungi labeled for use in stored grain system.

1.3.4. Cultural Control

Stored-product insects can also be managed by cultural control, environmental manipulations. This can be done outside the grain bin by limiting nearby sources of food, water, warmth, and shelter (IAOM 2016). For instance, ditches where water accumulates can attract insects. Proper drainage can eliminate this risk. Similarly, nearby plants that are attractive to insects can pose a significant risk (Beach 2012). Thus, landscaping nearby can also be an important part of stored product IPM. Even the type of lighting used at night could be
detrimental if not considered carefully. Sodium vapor lights are less attractive to insects than mercury vapor lights (IAOM 2016).

In contrast to controls that reduce attractive properties, some research has sought to use the attractive properties of semiochemicals to lead stored product pests away from grain and toward traps. Semiochemicals are chemicals that communicate information between organisms, and consequently alter their behavior, host-seeking or mate-seeking behavior (Law and Regnier 1971). Cox (2004) proposed that grain beetles might be manipulated by mass-trapping with the proper chemical composition and dose. Some research into the aggregation pheromones of *S. oryzae* and *R. dominica* have shown their potential as a trapping tool (Likhayo and Hodges 2000; Edde 2012). However, thus far they have been used for monitoring, not mass-trapping.

Residual grain in the bin and old grain spills can act as sources of pests, so cleaning can also act as pre-binning treatment (IAOM 2016). Moreover, proper cleaning directly after emptying a bin is also important. Sanitation is also important for the efficacy of insecticides, as poor sanitation reduces pesticide efficacy by 8-fold on average (Morrison et al. 2019). Poor sanitation can also reduce the efficacy of other cultural control methods like heat treatment and grain chilling (Morrison et al. 2019).

Within the grain bin it is important to manage temperature, moisture, and sanitation. Stored product insects typically perform best at 25–32°C (Fields 1992). One form of treatment is to briefly raise the grain’s temperature to a suboptimal or lethal temperature for the insects. For instance, temporarily raising the temperature to 45°C for 48–72h can completely stop adult emergence of *T. castaneum* (Saxena et al. 1992). The reverse is also true; lowering the temperature to 20°C will stop the development of most insects (Howe 1965). Grain bins are often kept at lower temperatures for this reason. However, manipulating temperature can be
energy intensive and expensive depending on regional climate and season (Yang et al. 2017).

Managing humidity, and therefore moisture content, is another large part of stored grain cultural control. Insects may struggle to complete development on grain with particularly low moisture content. For example, *R. dominica* is unable to develop at less than 30% humidity or 9% moisture content (Edde 2012). Moreover, an insect’s tolerance for moisture content is linked to their temperature tolerance (Beckett et al. 1998). At higher moisture contents, *R. dominica* and *S. oryzae* are able to withstand high temperatures for longer, which can then affect heat treatment schedules.

1.4. Research Goals

There are a wide variety of pest management tools available to growers, and several more that could be made available after additional research and development. Through integrated pest management, growers can use combinations of these tools; however, current store rice pest management relies heavily on fumigants. The primary goal of this research is to improve stored rice pest management through integrated pest management, thereby reducing the impact of pests, improving the environmental sustainability of pest management, and extending the longevity of their available tools. Chapters 2 and 3 explores host plant resistance, Chapter 4 evaluates stored grain insecticides, Chapter 5 investigates entomopathogens as biological control agents, and Chapter 6 studies pest host-finding behaviors. Through an improved understanding of the problems and available tools in this system, we hope to make integrated controls a more accessible and achievable goal for rice growers.
Chapter 2. Stored rice varietal resistance towards *Sitophilus oryzae*

2.1. Introduction

Grain loss to pests is a global problem, with 5–30% of postharvest grain destroyed by insects (Neupane 1995; Deshpande and Singh 2001; USDA 2005; Santos 2006; Yigezu et al. 2010; Jiang 2013; Sharon et al. 2014; Zhang et al. 2021). Rice (*Oryza sativa* (L.)) value can be reduced through weight loss, designation as animal feed, or requirements for additional insect control. The rice weevil, *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae), is among the most damaging pests of stored grains, feeding on rice, corn, wheat, and many other cereals (Cogburn, 1977; Padín et al., 2002; USDA, 2016; Vijay & Bhuvaneswari, 2018; Charles Kasozi et al., 2018). It occurs within grain in all regions of the world and is particularly abundant in warmer climates. *Sitophilus oryzae* is a primary pest of grains, meaning that it attacks sound, undamaged grain. Once damaged, the grain then becomes more susceptible to secondary pests, exacerbating problems further.

Both *S. oryzae* adults and larvae feed on grain. Adults commonly feed upon the endosperm under the hull, while larvae will also eat the germ in the grain interior (Gvozdenac et al. 2020). Adult females bore holes into kernels to oviposit an egg within, laying as many as 400 eggs in their lifetime (USDA 2016). Once hatched, *S. oryzae* larvae remain feeding within the grain, until they emerge as adults. Their average lifespan is 4–5 months over which a single weevil can consume 10–25 g of grain (Padín et al. 2002).

Presently, phosphine fumigation is the primary form of stored grain pest management in many regions. While fumigation can quickly and reactively rid stored grain of pests, concerns over phosphine resistance are growing, particularly because there is not a suitable substitute fumigant (Hagstrum et al. 2012; Nayak et al. 2020). Thus, the development of other pest...
management tools, such as host resistance, is of great importance to sustainable grain storage.

Varietal host resistance to *S. oryzae* has been identified in numerous stored grains. In wheat (*Triticum aestivum* L.), Chaudhary & Regmi (2021) found large differences in damage and weight loss due to *S. oryzae* between varieties. Sorghum (*Sorghum* spp.) has also been studied for varietal resistance, and physical characteristics like grain size have been shown to affect *S. oryzae* choice and development (Russell 1962). In rice, differential varietal resistance and susceptibility to stored grain pests has also been previously studied. Cogburn (1977) demonstrated the value of stored rice varietal resistance, finding that varieties had large differences in damage due to *S. oryzae* as well as the Angoumois grain moth, *Sitotroga cerealella* (Olivier) (Lepidoptera: Gelechiidae), and the lesser grain borer, *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae). Further studies have made similar findings about the effects of rice variety upon damage and pest biology (Cogburn and Bollich 1990b; Chanbang et al. 2008a); however, none of the varieties tested in these studies are commonly grown any longer.

More recently, research efforts have been made to examine susceptibility of rice on modern varieties. In Nepal, Pal et al. (2021) found that susceptible rice varieties lost twice as much weight due to injury by pests as resistant rice varieties. In Benin, Santos et al. (2015) found rice variety was responsible for over 7-fold differences in the number of damaged kernels. In the USA, *S. cerealella* have been found to produce over 7-fold differences in progeny between rice varieties, while *R. dominica* produce over 28-fold differences in progeny between varieties (Arthur et al. 2013b). However, no published studies have examined *S. oryzae* varietal susceptibility among the modern inbred and hybrid lines commercially produced in the USA. Recently developed specialty rice such as the high protein, low-glycemic rice, Frontière (Boyd, 2021), likely also differ in pest susceptibility from conventional rice. Further, modern producers
frequently store rice of multiple varieties together in the same grain silos, but the influence of these mixtures on pest susceptibility is unknown.

In some cropping and urban landscape systems, intraspecific diversity has been shown to produce effects which reduce herbivore fitness beyond what it would be in a monoculture (Tooker and Frank 2012; Grettenberger and Tooker 2016; Doherty et al. 2019). For example, in St. Augustinegrass, *Stenotaphrum secundatum* (Walt.) Kuntz, genotypic diversity has been shown to affect the development time, larval weight, and herbivory (Doherty et al. 2019). Similarly, in wheat (*Triticum aestivum* L.), genotypic diversity affected *Rhopalosiphum padi* L. (Hemiptera: Aphididae) resulting in smaller reproductive individuals and fewer progeny (Grettenberger and Tooker 2016). These effects may be a result of associational resistance, whereby a neighboring plant confers resistance to a target plant (Tahvanainen and Root 1972). However, intraspecific diversity may also result in associational susceptibility, whereby a neighboring plant confers susceptibility to a target. Mixes of stored rice varieties principles may also influence pests in stored grain systems, but it has yet to be investigated.

Here, we aim to reexamine influences of rice variety on *S. oryzae* biology. Our specific objectives are to (1) compare *S. oryzae* development and reproduction on modern USA rice varieties, and to (2) examine *S. oryzae* development and reproduction on varietal mixes.

### 2.2. Methods

#### 2.2.1 Varietal Resistance

We acquired freshly harvested rough rice of 13 varieties from the LSU Rice Research Station, including: three imidazolinone herbicide-resistant inbred varieties (CL111, CL151, and
CL153), two imidazolinone herbicide-resistant hybrids (CLXL745 and Gemini 214CL), a conventional hybrid (XP753), two conventional long grain inbred varieties (Mermentau and Cheniere), two conventional medium grain inbred varieties (Jupiter and Caffey), a quizalofop-\(p\)-ethyl (QPE)-resistant long grain (PVL02), an aromatic Jasmine-type variety (Jazzman 2), and the high protein, low-glycemic variety, Frontière (Harrell et al., 2021). These rice varieties represent a diverse genetic background of commercial varieties that are widely grown in the USA Mid-South. Rice varieties were refrigerated at 4°C until needed. Before use, rice was sieved and tempered to 12% moisture content (MC) according to the following equation (AACC 2009):

\[
\text{mL water} = \left( \frac{100 - \text{original moisture (\%)} \cdot \left(100 - \text{desired moisture (\%)}\right) - 1}{\text{sample mass}} \right) \]

We then added 24 g of rice to 10-dram plastic vials. Ten mixed sex \textit{S. oryzae} adults from a laboratory colony reared on wheat were then added to each vial, and vials were kept in a growth chamber (27 ± 1°C, continual darkness). The sex ratio of introduced beetles was assumed to be 1:1 based on prior samples of our colony and published literature (Holloway 1985). Adults were given 2 weeks to feed and reproduce, then were removed from the vials to allow for quantification of larval feeding. After removal, adults were counted as alive or dead, and sexed. The rice of each vial was sieved (#12 mesh) and adult feeding damage was assessed by weighing rice fragments and frass. The intact rice was then returned to the vials and placed into the growth chamber for another 8 weeks.

During the 8-week period, vials were checked every two days for emerging progeny. Any adult progeny that emerged were removed from the vials. We recorded time until emergence, weight, and sex. Sex was determined using morphological characteristics, predominantly the width and length of the rostrum and abdomen (Halstead 1963; Lum and Baker 1975). At the end of 8 weeks, we sieved the rice, weighing the broken grains and frass to assess larval damage.
There were 6 replicates of each rice variety in an experimental trial in a completely randomized design, and 4 experimental trials which were done at different times (n = 24 vials/variety).

Unfortunately, some data was lost, including: adult damage data from the third trial, larval damage data from the second trial, and larval damage data for some samples in the third and fourth trials. Additionally, Frontière was not included in the first experimental trial and no larval damage for this rice variety are reported. However, there was still sufficient replication for all measures, except for larval damage in Frontière.

All statistics were performed in JMP Pro 16, with \( \alpha \) set at 0.05. We performed chi-squared analyses to examine the effect of rice variety on progeny sex ratio. Additionally, we ran an analysis of variance examining factors that influenced days to eclosion and adult progeny mass, with sex, variety, and variety × sex as fixed effects, while random effects included experimental trial and trial × variety × replicate. Analyses of variance were also run for adult mortality, introduced sex ratio, total progeny, larval damage, and adult damage using variety as a fixed effect and experimental trial as a random effect. Tukey’s HSD were used for post-hoc analysis between treatments.

**2.2.2. Associational Resistance**

Two susceptible (XP753 and PVL02) and two resistant rice varieties (Mermentau and Caffey) from the varietal resistance study were chosen for this experiment. Rice varieties were tempered to 12% MC before use. There were ten total treatments. Six treatments were created from every unique mixture of the four rice varieties, and an additional four treatments were made from each individual rice variety. Mixtures of two rice varieties were created by stirring 12 g of each variety together. Mixes of rice varieties were referred to by the initials of their component
varieties (ex. Caffey and Mermentau is labeled as C+M). Single variety treatments were created with 24 g of a single variety. These treatments then underwent the same methods as the rice of the varietal resistance experiment. There were 6 replicates of each treatment in a single experimental trial, and 3 experimental trials (n = 18 vials/treatment). Statistical analyses were identical to those of the previous experiment, but replacing variety with treatment.

2.3. Results

2.3.1. Varietal Resistance

Over the course of this experiment over 2,650 beetles were produced, sexed, and weighed. We saw no differences in adult mortality among varieties ($F_{12,226} = 1.29, P = 0.220$). Sex ratio of introduced adults was 1.00:1.12 (male:female), and it did not differ among rice varieties ($F_{12,154} = 0.50, P = 0.914$). We also did not see differences in progeny sex ratio due to rice variety, which was 1.00:0.76 (male:female) ($\chi^2 = 16.65, P = 0.163$). There was an effect of sex on adult mass ($F_{1,2606} = 44.23, P < 0.001$), where males weighed 1.66 ± 0.14 mg (LSM ± MSE), and females weighed 1.81 ± 0.14 mg. However, there was no interaction effect of variety × sex on beetle mass ($F_{1,2604} = 1.33, P = 0.195$). There was also no effect of sex on developmental time ($F_{1,2599} = 0.48, P = 0.489$), which averaged 41.25 ± 1.70 days across sexes and rice varieties. Additionally, there was no interaction effect of variety × sex ($F_{12,2578} = 0.91, P = 0.537$).

Adult damage, larval damage, total progeny, development time, and progeny adult mass were influenced by rice variety (Table 1). Adult damage (weight of frass and broken grains) in PVL02 was approximately 2-fold greater than in the rice variety with the second highest damage, Frontière, and 11-fold greater than in the least susceptible rice variety, Jupiter. Larval damage
was greatest in XP753, followed by CL111, which were 10.2- and 6.5-fold greater, respectively, than the least susceptible rice variety, Mermentau.

XP753 produced the most progeny, nearly 2-fold more than the rice variety with the second most, PVL02, and nearly 18-fold more than Caffey which produced the fewest emerged adults per vial. Beetle development time was longest in Gemini, which was 11% longer than that of the fastest development occurring in Frontière. The progeny in Frontière were also the largest and were 16% greater in mass than the smallest (CLXL745).

Table 1. *Sitophilus oryzae* adult damage, larval damage, progeny counts, days to eclosion, and progeny mass by rice variety.

<table>
<thead>
<tr>
<th>Rice Variety</th>
<th>Adult Damage (mg/vial)</th>
<th>Larval Damage (mg/vial)</th>
<th>Total Progeny/vial</th>
<th>Days to Eclosion</th>
<th>Progeny Adult Mass (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PVL02</td>
<td>43.37a</td>
<td>27.79bc</td>
<td>12.47b</td>
<td>40.65cde</td>
<td>1.63b</td>
</tr>
<tr>
<td>Frontière</td>
<td>24.19b</td>
<td>NA</td>
<td>12.33b</td>
<td>38.97e</td>
<td>1.84a</td>
</tr>
<tr>
<td>CLXL745</td>
<td>15.03bc</td>
<td>14.23bc</td>
<td>3.47cd</td>
<td>41.32abcd</td>
<td>1.59b</td>
</tr>
<tr>
<td>CL153</td>
<td>14.67bc</td>
<td>39.00abc</td>
<td>11.34b</td>
<td>41.04bcd</td>
<td>1.70a</td>
</tr>
<tr>
<td>XP753</td>
<td>13.37bc</td>
<td>62.19a</td>
<td>23.75a</td>
<td>40.20de</td>
<td>1.70ab</td>
</tr>
<tr>
<td>Jazzman 2</td>
<td>11.52bc</td>
<td>24.67bc</td>
<td>9.78b</td>
<td>40.69cde</td>
<td>1.79a</td>
</tr>
<tr>
<td>Gemini - 214CL</td>
<td>11.48bc</td>
<td>22.68bc</td>
<td>3.27d</td>
<td>43.24a</td>
<td>1.71ab</td>
</tr>
<tr>
<td>CL111</td>
<td>9.71c</td>
<td>39.53ab</td>
<td>10.31b</td>
<td>41.92abc</td>
<td>1.81a</td>
</tr>
<tr>
<td>CL151</td>
<td>9.42c</td>
<td>23.20bc</td>
<td>11.59b</td>
<td>40.94cd</td>
<td>1.74ab</td>
</tr>
<tr>
<td>Cheniere</td>
<td>8.34c</td>
<td>10.65c</td>
<td>8.15bc</td>
<td>40.76cde</td>
<td>1.81a</td>
</tr>
<tr>
<td>Mermentau</td>
<td>8.14c</td>
<td>5.88bc</td>
<td>1.80d</td>
<td>41.31abcde</td>
<td>1.62ab</td>
</tr>
<tr>
<td>Caffey</td>
<td>5.73c</td>
<td>8.03bc</td>
<td>1.56d</td>
<td>42.61abcd</td>
<td>1.81ab</td>
</tr>
<tr>
<td>Jupiter</td>
<td>3.79c</td>
<td>11.21bc</td>
<td>4.15cd</td>
<td>42.91ab</td>
<td>1.81a</td>
</tr>
</tbody>
</table>

\[
F = 15.89 \\
\text{df} = 12, 225 \\
P = <0.001 \\
SE = 2.42
\]

<table>
<thead>
<tr>
<th>Rice Variety</th>
<th>Adult Damage (mg/vial)</th>
<th>Larval Damage (mg/vial)</th>
<th>Total Progeny/vial</th>
<th>Days to Eclosion</th>
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</tr>
<tr>
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<td>1.84a</td>
</tr>
<tr>
<td>CLXL745</td>
<td>15.03bc</td>
<td>14.23bc</td>
<td>3.47cd</td>
<td>41.32abcd</td>
<td>1.59b</td>
</tr>
<tr>
<td>CL153</td>
<td>14.67bc</td>
<td>39.00abc</td>
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</tr>
<tr>
<td>XP753</td>
<td>13.37bc</td>
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<td>Gemini - 214CL</td>
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<tr>
<td>CL111</td>
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<tr>
<td>CL151</td>
<td>9.42c</td>
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<td>8.14c</td>
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<td>Caffey</td>
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<td>3.79c</td>
<td>11.21bc</td>
<td>4.15cd</td>
<td>42.91ab</td>
<td>1.81a</td>
</tr>
</tbody>
</table>

\[
F = 15.89 \\
\text{df} = 12, 225 \\
P = <0.001 \\
SE = 2.42
\]

NA: Not available due to data loss.

2.3.2. Associational Resistance and Susceptibility

During this experiment another 1,000 beetles were produced, sexed, and weighed. There
were no differences in sex ratio among treatments ($\chi^2 = 4.13, P = 0.903$). The effect of sex on mass, seen in the previous experiment, was also demonstrated here ($F_{1,988} = 17.24, P < 0.001$). Males weighed $1.59 \pm 0.04$ mg (LSM ± MSE), and females weighed $1.76 \pm 0.04$ mg. There was no effect of treatment × sex on mass ($F_{9,985} = 0.84, P = 0.582$). Also consistent with the previous experiment, there was no effect of sex ($F_{1,988} = 2.43, P = 0.119$) or sex × treatment ($F_{9,975} = 0.51, P = 0.865$) on days to eclosion, which was $40.36 \pm 1.68$ across sexes and treatments.

Similar to the previous experiment, adult damage, larval damage, total progeny, development time, and progeny adult mass were influenced by treatment (Table 2). The damage mass produced by adults in C+P was >3-fold greater than Caffey; damage weight in M+P was >2-fold more than Mermentau; damage weight in X+P was almost 2-fold more than XP753. However, none of these mixes were different from their more susceptible component rice variety, PVL02. Conversely, adult damage to C+M was not different from Caffey or Mermentau. Stored rice treatment also had significant effects on larval damage. Larval damage in X+P was 2-fold greater than that of PVL02; larval damage in X+C was 3-fold greater than Caffey; however, larval damage in those mixes was not significantly different than that of the more susceptible component rice variety, XP753.

Progeny development to adulthood in Caffey took 5 days longer on average than in X+C. Progeny masses in Mermentau and PVL02 were 30% and 18% larger, respectively, than in M+P. Additionally, progeny mass in XP753 was 10% greater than in X+M and X+P.
Table 2. *Sitophilus oryzae* adult damage, larval damage, progeny counts, days to eclosion, and progeny mass by rice variety or mix.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Adult Damage (mg)/vial</th>
<th>Larval Damage (mg)/vial</th>
<th>Total Progeny/vial</th>
<th>Days to Eclosion</th>
<th>Progeny Adult Mass (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C+P</td>
<td>26.67a</td>
<td>11.56bc</td>
<td>4.44de</td>
<td>40.96abc</td>
<td>1.57bcd</td>
</tr>
<tr>
<td>X+P</td>
<td>26.41a</td>
<td>27.98a</td>
<td>9.28ab</td>
<td>40.21bc</td>
<td>1.58cd</td>
</tr>
<tr>
<td>PVL02</td>
<td>24.97a</td>
<td>14.02bc</td>
<td>7.94bc</td>
<td>41.74ab</td>
<td>1.66abc</td>
</tr>
<tr>
<td>M+P</td>
<td>21.41ab</td>
<td>10.78bc</td>
<td>4.28def</td>
<td>41.47abc</td>
<td>1.40d</td>
</tr>
<tr>
<td>X+C</td>
<td>20.37ab</td>
<td>19.66ab</td>
<td>6.50bcd</td>
<td>40.52bc</td>
<td>1.77ab</td>
</tr>
<tr>
<td>M+X</td>
<td>17.01bc</td>
<td>12.67bc</td>
<td>5.00cd</td>
<td>40.22bc</td>
<td>1.59cd</td>
</tr>
<tr>
<td>XP753</td>
<td>14.48bcd</td>
<td>28.37a</td>
<td>12.28a</td>
<td>39.28c</td>
<td>1.75ab</td>
</tr>
<tr>
<td>Mermentau</td>
<td>10.91cde</td>
<td>3.55c</td>
<td>0.78g</td>
<td>38.63bc</td>
<td>1.82abc</td>
</tr>
<tr>
<td>C+M</td>
<td>8.14de</td>
<td>3.49c</td>
<td>0.94fg</td>
<td>41.01abc</td>
<td>1.97a</td>
</tr>
<tr>
<td>Caffey</td>
<td>7.34e</td>
<td>6.68c</td>
<td>1.06efg</td>
<td>44.51a</td>
<td>1.65abcd</td>
</tr>
</tbody>
</table>

| F           | 22.06                  | 14.34                   | 25.98              | 4.19            | 8.03                    |
| df          | 9, 167                 | 9, 167                  | 9, 168             | 9, 131          | 9, 9                    |
| p           | <0.001                 | <0.001                  | <0.001             | <0.001          | 0.002                   |
| SE          | 0.93                   | 5.44                    | 1.44               | 1.60            | 0.04                    |

**2.4. Discussion**

This study is the first to document differential susceptibility to *S. oryzae* among a diverse assemblage of modern commercial cultivars produced in the USA. Varieties of stored rice showed differential resistance through our measures of adult damage, larval damage, progeny count, progeny development, and progeny weight. These findings agree with previous studies, in that stored rice varietal differences create differential resistances (Cogburn and Bollich 1990b; Rizwana et al. 2011; Arthur et al. 2013b; Chaudhary et al. 2021). However, unlike past studies, resistance toward adults was not necessarily the same as resistance towards larvae. Resistance based on life-stage has not been demonstrated in stored grain, but it is seen in other systems. For example, *Melitaea cinxia* (L.) (Lepidoptera: Nymphalidae) larvae prefer drought-exposed host plants, while adult females preferred to oviposit upon well-watered host plants (Salgado and
Saastamoinen 2019). Insects with life stage-dependent resistances may require additional consideration to effectively manage. In our study, XP753 and CL111 were intermediately susceptible to adult *S. oryzae* but were heavily damaged by larvae, which may be important for management. Control of initial adult infestations is of less priority than controlling subsequent larval generations in these rice varieties.

The susceptible rice varieties identified in this study may require additional monitoring and management inputs. The heavier damage seen in PVL02 and XP753 reported herein suggests growers will need to be more aggressive with insecticides or other management techniques. Although our larval damage data were lost for Frontière, the high number of progeny, high progeny mass, and low days to eclosion suggest this rice variety is highly suitable for larval development. Which is to say, it is another susceptible rice variety that may require more aggressive monitoring and management than other varieties of stored rice. The conventional inbred lines Mermentau, Jupiter, Caffey, Cheniere were among the most resistant rice varieties overall suggesting the similar genetic backgrounds of these rice varieties may impart some resistance to *S. oryzae*, and that they likely will require less intensive pest management. Other groups of rice varieties, including Clearfield lines (CL111, CL151, CL153) and hybrids (CLXL745, Gemini – 214CL, and XP753), had more varied responses.

Progeny mass is not strictly an indicator of rice susceptibility, nor is it necessarily an indicator of increased fitness, though it is commonly used as such (Honěk 1993). Many of the stored rice varieties we studied that were relatively resistant in other measures produced some of the largest adults in our dataset. For instance, the *S. oryzae* adults produced in Jupiter and Caffey are the second and third leading rice varieties for high progeny mass. Sex is a large factor influencing *S. oryzae* size. Previous research has found that female *S. oryzae* are larger than
males (Kiritani 1965), and our own results agree with that literature. However, this is unlikely to be the cause of the size differentiation we have seen across rice varieties, as we found no evidence of rice variety influencing sex ratio, and no effect of sex × variety on mass. Alternatively, nutritional content may provide an explanation for the discrepancies in beetle mass. Frontière’s susceptibility is likely the result of enhanced nutritional content relative to other rice varieties, as it is a high protein rice developed to help combat malnutrition in the developing world (Boyd, 2021). Protein is a key nutrient for insect growth and development and is especially sought after for herbivorous insects given its variable availability in plants (Behmer, 2009; Le Gall & Behmer, 2014).

Development time was also affected by rice variety, but like mass, it is difficult to say if changes in developmental rate are indicators of host resistance. A shorter development time could mean less time to acquire nutrients; however, some research has demonstrated that a focus on efficient nutrient utilization can be unnecessary or even harmful to fitness, i.e., reproductive output (Miller et al. 2009; Zehnder and Hunter 2009). Moreover, Frontière had the shortest developmental time, and rice varieties that were commonly susceptible in other measures also produced adults with shorter developmental cycles. These findings suggest that shorter developmental cycles are not harmful to S. oryzae, and thus, a longer developmental cycle is an indicator of host resistance in this system. In terms of management, over an extended storage period, the difference between a 39-day and a 43-day generation time can add up to another generation. Additionally, understanding a generation time in a particular rice variety can also assist growers with some control applications, like timing the application of insecticides.

Looking at the overall development time, S. oryzae eclosion occurred within its expected range of 5–6 weeks (Okram and Hath 2019). Our finding that sex did not affect development
time is in contrast to the findings of the existing literature. Some studies have found that male *S. oryzae* develop more quickly than females at 20°C and 25°C (Kiritani 1965). While other studies have found the opposite to be true at 30°C (Nishigaki 1958). Our experiments were run at 27°C, and we found no differences in development based on sex. Being between the temperatures at which males develop more quickly (25°C), and the temperature at which females develop more quickly (30°C), 27°C may be the inflection point where males and females develop at the same rate.

In our experiment mixing varieties, mixes of two varieties of rice usually resulted in associational susceptibility or no effect, as the susceptibility a mix was often between its two component effects. Our finding that susceptible rice varieties imparted associational susceptibility suggests these may require enhanced management even when stored with less susceptible rice. We could not distinguish which rice varieties the adults fed on, but because adults had the freedom to choose between rice varieties within a vial. Thus, the associational susceptibility may be the result of adults preferentially feeding on the susceptible rice in the mixes. In other studies diet choice has allowed herbivores to optimize their diets thereby minimizing negative effects of diet mixing or maximizing its positive effects (Waldbauer and Friedman 1991; Mody et al. 2007; Wetzel and Thaler 2018). In a mixed diet, if one food source is poor in a particular nutrient, it can be supplemented by another, allowing for an improved nutritional balanced and fitness (Waldbauer and Friedman 1991; Mody et al. 2007). Additionally, a harmful substance can be avoided if insects have the option to feed on other food sources (Waldbauer and Friedman 1991; Wetzel and Thaler 2018). The ability to choose could then produce results where any mix is as susceptible as its most susceptible rice variety. Only the mix of two resistant rice varieties did not result in associational susceptibility.
Larval damage and progeny counts were most often between the levels expected for individual rice varieties, suggesting that while adults chose where to feed, they may not have used the same decision-making process during oviposition. Thus, larvae may have been evenly distributed amongst the grain, which is unusual given that several studies have demonstrated that *S. oryzae* oviposition choice can be influenced by physical characteristics like grain hardness and texture (Russell 1968; Salunkhe and Jadhav 1982; Akhter et al. 2017). Those findings were done within stored sorghum systems, so it remains unclear if physical characteristics affect *S. oryzae* oviposition behavior in stored rice. It is worth noting that there were some minor instances of associational resistance among our findings, but further research is needed before it can be of use to growers. Overall, our results suggest that there are very few pest management applications for mixes of resistant and susceptible rice varieties where *S. oryzae* is concerned.

While our results provide some new information, they also raise new questions. For instance, the mechanisms that confer rice resistances to adults and larvae are poorly understood. The role of nutritional and physical characteristics in resistance are still unknown. It is also unknown if these rice resistance mechanisms are specific to *S. oryzae*, or also confer resistance to other pests. Some grain pest beetles, like *R. dominica*, lead similar life histories to *S. oryzae*, living inside the grain as larvae, only emerging as adults, while others have larval stages that are in whole or in part spent outside the grain, like *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) and *T. confusum* Jacquelin du Val (Coleoptera: Tenebrionidae) (USDA 2016). These life histories may interact with resistance mechanisms in different ways or completely circumvent them. The practical implications for growers have also not yet been studied. While growers are unlikely to select their rice varieties based upon its resistance towards stored grain insects, resistance information can be used to inform a grower’s stored grain pest management
practices. These studies aim to improve incorporation of varietal resistance into stored grain IPM programs.
Chapter 3. Examining factors influencing varietal resistance to *Rhyzopertha dominica* (F.) in stored rice

3.1. Introduction

Rice (*Oryza sativa* (L.)) is a staple crop of the world, and the lesser grain borer, *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae), is a global pest of postharvest rice. Globally, 5–30% of stored grain is destroyed by insects (Deshpande & Singh, 2001; USDA, 2005; Santos, 2006; Yigezu et al., 2010; Jiang, 2013; Sharon et al., 2014; Zhang et al., 2021). As a primary pest, *R. dominica* attacks sound and undamaged rice, and infestations can result in large financial costs for growers (USDA 2016). Damaged rice will weigh less, which reduces revenue, and heavily damaged rice may be designated as animal feed (Harein and Meronuck 1990; USDA 2016). If a load of rice is infested once it reaches the mill, it might be turned away, or the grower may be required to fumigate before the rice is accepted. Fumigation with phosphine gas is currently the dominant form of stored grain pest management, but there are concerns about the development of phosphine resistances. Phosphine-resistant populations of *R. dominica* have already been identified in several places around the world (Lorini et al. 2007; Opit et al. 2012; Nayak et al. 2019). As such, alternative control methods are important for the continuing management of these beetles.

Host-plant resistance is one method of control that may already be available to rice growers. Previous work has documented differential susceptibility to *R. dominica* among varieties of rice (Chanbang et al. 2008a,b; Astuti et al., 2013). Moreover, this research has shown that hull thickness can be a mechanism of resistance (Chanbang et al. 2008a). Additionally, examinations of amylose content, grain hardness, and rice type (i.e. long grain, short grain) determined that those traits were not indicators of resistance (Chanbang et al. 2008a,b).
However, questions remain about the mechanisms of stored rice resistance. Proteins and amino acids are essential components for insect development (House, 1961; Nation, 2022), and reductions in availability of amino acids can contribute to resistance (Reay-Jones et al. 2007). The role of these nutrients in stored rice resistance is unclear, as is the role of the physical characteristics of the grain such as grain dimensions and weight.

Additionally, a recent study has shown that both associational resistance and susceptibility can be achieved in stored rice systems (Doherty et al. 2023). Associational resistance is resistance conferred to a target plant by its neighbor (Tahvanainen and Root 1972). Similarly, associational susceptibility is susceptibility of a target plant conferred to it by a neighbor. While these phenomena are typically observed due to interspecific diversity, they have also been demonstrated to occur through intraspecific diversity (Tooker and Frank 2012; Grettenberger and Tooker 2016, 2017; Doherty et al. 2019). While these relationships have been examined in stored rice for resistance toward the rice weevil (Sitophilus oryzae), it is unknown if the trends extend to other grain pests.

For these reasons, we sought to improve the understanding of varietal resistance towards *R. dominica* though the following objectives:

1. To identify which hybrid and inbred varieties of rice grown in the southern US are resistant to *R. dominica*.
2. To determine what physical and nutritional characteristics of stored rice are associated with resistance or susceptibility.
3. To determine if mixtures of rice varieties confer associational resistance or susceptibility toward *R. dominica*.

3.2. Methods
3.2.1 Insects

Initial colonies of *S. oryzae* and *R. dominica* were provided by the USDA-ARS, Manhattan, Kansas, who had maintained these colonies for over 40 years. These colonies were then supplemented with field collected beetles from Louisiana in 2019-2020. Beetles were reared on wheat (*Triticum aestivum*, 12% MC) in 24-32 oz deli containers inside a growth chamber (27°C, continual darkness). Fresh wheat was provided every 2 months or as needed.

3.2.2 Host Plants

Thirteen varieties of rough rice were acquired from the LSU Rice Research Station, including two long grain inbred varieties (Mermentau and Cheniere), a hybrid (XP753), two medium grain inbred varieties (Jupiter and Caffey), three imidazolinone herbicide-resistant “Clearfield” inbred varieties (CL111, CL151, and CL153), two imidazolinone herbicide-resistant hybrids (CLXL745 and Gemini 214CL), a quizalofop-p-ethyl (QPE)-resistant long grain (PVL02), an aromatic Jasmine-type variety (Jazzman 2), and the high protein, low-glycemic variety, Frontière (Wenefrida et al. 2017). Rice was sieved and tempered to 12% moisture content (MC) according to the following equation (AACC 2009):

\[
mL \text{ water} = ((100 - \text{original moisture} \, \%) / (100 - \text{desired moisture} \, \%) - 1) * \text{sample mass}
\]

3.2.3. Varietal Resistance Assay

Rice varieties were added to 10-dram plastic vials in 24 g portions with ten mixed sex *R. dominica* adults from a laboratory colony. Vials were kept in a growth chamber (27 ± 1°C, continual darkness). The sex ratio of introduced beetles was assumed to be approximately 1:1 based on prior research (Edde 2012) and sampling of our colony. After 2 weeks, the adult beetles were removed from the vials. We then sieved (#12 mesh) the contents of each vial to assess adult
feeding damage, weighing the rice fragments and frass. The intact rice was then returned to the vials and placed into the growth chamber for another 6 weeks. During the remainder of the experiment, we checked each vial every two days for emerging progeny. Emerged progeny were removed from the vials as they appeared, and their time until emergence and mass were recorded. At the end of the experiment, vial contents were sieved again to assess larval damage, by weighing the broken grains and frass which passed through a #12 mesh sieve. In a completely randomized design, there were 6 replicates of each rice variety in an experimental trial, and three experimental trials which were conducted from May to August of 2021 (n = 18 vials/variety).

3.2.4. Nutritional Assays

Nutritional tests were run for all 13 aforementioned rice varieties from March to April of 2022. Amino acid analyses were performed in triplicate using HPLC to test for alanine, arginine, aspartic acid, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tyrosine, valine, and cysteine. Samples were prepared by weighing 1 g of sample into a hydrolysis tube with 7 ml 6N HCl containing 0.25% phenol. Samples were then frozen and connected to vacuum for 2 min. After thawing, this process was repeated twice more. The samples were then placed on a heating block to hydrolyze for 24 hours at 110 °C. Twenty µl of the filtered hydrolysate was mixed with 20 µl of norleucine (2.5 umol/ml) and dried. One-hundred µl of phenylisothiocyanate (PITC) solution (EtOH:water:PITC:triethylamine = 7:1:1:1) was added to the residue and mixed for 30 min. Afterwards, samples were freeze dried. The derivatized residue was dissolved into 1 ml of buffer (140 mM sodium acetate, 0.05% triethylamine, titrated to pH 6.40 with glacial acetic acid with the addition of 60 ml/L acetonitrile) and filtered with 0.2 um filter to obtain the injection sample.

Starch, protein, and nitrogen analyses were also run in triplicate. Starch was analyzed using AOAC method 996.11 (AOAC 2005). Nitrogen was tested using dry combustion of ground grains (Dumas 1831). Protein was calculated from nitrogen using AOAC method 990.03 (AOAC 2005). To test for an effect of age on nutrients, two additional treatments were added to the starch, protein, and nitrogen assays. These treatments included additional batches of Cheniere and CL151 which were one year older than the other treatments.

3.2.5. Physical Characteristic Analysis

We obtained mean grain physical characteristics of each variety from their registrations (Linscombe et al. 2006; Sha et al. 2006, 2013; Blanche et al. 2011, 2012; Oard et al. 2014a, b; Famoso et al. 2017; Wenefrida et al. 2017). Measures were collected for rough, brown, and milled rice, and included: grain length, width, length/width ratio, thickness, and weight. Thickness can be differentiated from width by resting grain on a flat surface; the width is the dimension parallel to the surface, while the thickness is perpendicular. Hull parameters were estimated by subtracting the brown rice dimensions (i.e., length, width, and thickness) from the rough rice dimensions. Bran parameters were estimated by subtracting the milled rice dimensions from the brown rice dimensions.

3.2.6. Associational Resistance Assay

From the varietal resistance assay, we chose two susceptible (CL111 and CL151) and two resistant rice varieties (Jazzman 2 and PVL02). Six treatments were created from every unique mixture of two varieties from the four rice varieties, and an additional four treatments were made
from each individual rice variety, for a total of ten treatments. Rice varieties were tempered to 12% MC before use. Mixtures of two rice varieties were created by stirring 12 g of each variety together. Mixes of rice varieties were referred to by the initials of their component varieties (ex. Jazzman 2 and PVL02 is labeled as J+P). Single variety treatments were created with 24 g of the one rice variety. Following setup, methods were identical to the varietal resistance assay. There were 6 replicates of each treatment in a single experimental trial, and 3 experimental trials (n = 18 vials/treatment) run from November 2021 to February 2022 (n = 18 vials/variety).

3.2.7. Statistical Analyses

All statistical analyses were run in JMP Pro 16 (α = 0.05). For the varietal resistance assay, analyses of variance were run for total progeny, larval damage, and adult damage using variety as a fixed effect and experimental trial as a random effect. Additionally, we ran an analysis of variance examining factors that influenced days to eclosion and adult progeny mass, with variety as a fixed effect, while random effects included experimental trial and trial × variety × replicate. Very few progeny survived in PVL02, so data for that variety were excluded from the larval damage, total progeny, progeny mass and days to eclosion analyses. Tukey’s HSD were used for post-hoc mean separations.

Statistical analyses of the associational resistance assay were identical to those of the varietal resistance experiment but replacing variety with treatment. For the protein, nitrogen, amino acid, and starch assays, analyses of variance were run to examine the effects of variety, where variety was the only fixed effect and there were no random effects. Using the age data from the protein and nitrogen assays, we ran analyses of variance, where age was a fixed effect and variety was a random effect. Finally, using the means from our varietal resistance assay data,
we examined the relationships between our measures of susceptibility to *R. dominica* and the physical and chemical characteristics of varieties with a series of linear regression analyses.

3.3. Results

3.3.1. Varietal Resistance Assay

There was an effect of variety on adult damage (*F*<sub>12,218</sub> = 8.72, *P* < 0.001), larval damage (*F*<sub>12,217</sub> = 38.60, *P* < 0.001), progeny counts (*F*<sub>12,191</sub> = 39.53, *P* < 0.001), progeny mass (*F*<sub>12,165</sub> = 7.35, *P* < 0.001), and days to eclosion (*F*<sub>12,181</sub> = 5.38, *P* < 0.001). In terms of damage, Frontière saw the greatest amount of damage by adults, and the second most damage by larvae. There was a >5-fold difference in adult damage between Frontière and Jupiter, the most resistant variety of that measure (Figure 1). A >14-fold difference was observed in larval damage between CL111, the most susceptible variety, and XP753, the least susceptible variety (Figure 2). XP753 also had the fewest progeny emerge, which was 17-fold fewer progeny than emerged in Frontière (Figure 3). The few progeny that did emerge from XP753 were also the largest and had 1.3-fold more mass than those in Jazzman, which produced the smallest progeny (Figure 4). Progeny developed most quickly on Frontière and most slowly on Jupiter, with a 3-day difference between the two varieties (Figure 5).
Figure 1. The feeding damage mass by adult *R. dominica* among stored rice varieties in the varietal resistance assay (LS Means ± 1.67 [SE]). Bars that share a letter are not significantly different (Tukey’s HSD, \( \alpha = 0.05 \)).

Figure 2. The feeding damage mass by *R. dominica* larvae among stored rice varieties in the varietal resistance assay (LS Means ± 42.00[SE]). Bars that share a letter are not significantly different (Tukey’s HSD, \( \alpha = 0.05 \)).
Figure 3. Adult *R. dominica* progeny production among varieties in the varietal resistance assay (LS Means ± 4.43 [SE]). Bars that share a letter are not significantly different (Tukey’s HSD, \( \alpha = 0.05 \)).

Figure 4. *Rhyzopertha dominica* progeny mass among varieties in the varietal resistance assay (LS Means ± SE). Bars that share a letter are not significantly different (Tukey’s HSD, \( \alpha = 0.05 \)).
3.3.2. Nutritional Analyses

Concentrations of all amino acids differed ($P < 0.001$) among varieties (Table 1). Every amino acid was present in each variety of rice except for cysteine, which was only present in six of the 13 varieties. Glutamine had the largest range, with a minimum of 0.573 nmol/g of sample and a maximum of 0.792 nmol/g of sample. Varieties had differential nitrogen ($F_{12,26} = 15.88$, $P < 0.001$) and protein contents ($F_{12,26} = 15.99$, $P < 0.001$). There was a 25% difference between the varieties with the most protein and nitrogen, and those with the least (Table 2). Additionally, the age of a variety influenced nitrogen ($F_{1,9} = 25.54$, $P < 0.001$) and protein content ($F_{1,9} = 24.78$, $P < 0.001$). Over the course of 12 months, both protein and nitrogen decreased by about 5% (Figure 6). Varieties also had differential starch content ($F_{12,26} = 135.14$, $P < 0.001$). The varieties with the most starch had nearly two-fold that of those with the least starch (Table 3).

Figure 5. The days to eclosion for *R. dominica* progeny among varieties in the varietal resistance assay (LS Means ± SE). Bars that share a letter are not significantly different (Tukey’s HSD, $\alpha = 0.05$).
Table 1. The average concentration (nmol of amino acid per gram of sample) of each of the 17 tested amino acids within each stored rice variety. Means within a column that share a letter are not significantly different (Tukey’s HSD, α = 0.05).

<table>
<thead>
<tr>
<th>Variety</th>
<th>Tyr (nmol/g)</th>
<th>Val (nmol/g)</th>
<th>Met (nmol/g)</th>
<th>Cys (nmol/g)</th>
<th>lle (nmol/g)</th>
<th>Leu (nmol/g)</th>
<th>Phe (nmol/g)</th>
<th>Lys (nmol/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caffey</td>
<td>3.70E-02af</td>
<td>2.65E-01b</td>
<td>4.67E-02b</td>
<td>0.00E+00b</td>
<td>1.69E-01b</td>
<td>3.01E-01de</td>
<td>2.81E-01b</td>
<td>1.39E-01ab</td>
</tr>
<tr>
<td>Cheniere</td>
<td>4.97E-02a</td>
<td>2.81E-01a</td>
<td>4.03E-02c</td>
<td>0.00E+00b</td>
<td>1.63E-01c</td>
<td>3.10E-01c</td>
<td>2.51E-01d</td>
<td>1.27E-01ab</td>
</tr>
<tr>
<td>CL151</td>
<td>3.67E-02ef</td>
<td>2.20E-01e</td>
<td>3.73E-02cd</td>
<td>0.00E+00b</td>
<td>1.39E-01fg</td>
<td>2.71E-01ph</td>
<td>2.15E-01g</td>
<td>1.23E-01ab</td>
</tr>
<tr>
<td>CL153</td>
<td>3.50E-02f</td>
<td>2.19E-01e</td>
<td>3.77E-02cd</td>
<td>0.00E+00b</td>
<td>1.42E-01ef</td>
<td>2.72E-01g</td>
<td>2.27E-01f</td>
<td>1.21E-01bf</td>
</tr>
<tr>
<td>CL111</td>
<td>4.57E-02ab</td>
<td>2.52E-01c</td>
<td>4.80E-02b</td>
<td>6.67E-03a</td>
<td>1.68E-01b</td>
<td>3.50E-01a</td>
<td>2.96E-01a</td>
<td>1.53E-01a</td>
</tr>
<tr>
<td>CLXL1</td>
<td>4.10E-02cd</td>
<td>2.71E-01ab</td>
<td>4.80E-02b</td>
<td>0.00E+00b</td>
<td>1.75E-01a</td>
<td>3.36E-01b</td>
<td>2.95E-01a</td>
<td>1.43E-01ab</td>
</tr>
<tr>
<td>Frontiere</td>
<td>3.53E-02f</td>
<td>2.43E-01cd</td>
<td>4.57E-02b</td>
<td>0.00E+00b</td>
<td>1.62E-01c</td>
<td>2.98E-01e</td>
<td>2.87E-01b</td>
<td>1.49E-01ab</td>
</tr>
<tr>
<td>Gemini 214 CL</td>
<td>3.47E-02f</td>
<td>2.38E-01d</td>
<td>5.37E-02ac</td>
<td>6.67E-03a</td>
<td>1.56E-01d</td>
<td>3.10E-01c</td>
<td>2.85E-01b</td>
<td>1.43E-01ab</td>
</tr>
<tr>
<td>Jazzman 2</td>
<td>4.07E-02de</td>
<td>2.36E-01d</td>
<td>4.93E-02b</td>
<td>7.67E-03a</td>
<td>1.60E-01c</td>
<td>3.02E-01de</td>
<td>2.98E-01a</td>
<td>1.52E-01a</td>
</tr>
<tr>
<td>Jupiter</td>
<td>2.93E-02g</td>
<td>2.08E-01ef</td>
<td>3.17E-02g</td>
<td>0.00E+00b</td>
<td>1.38E-01fg</td>
<td>2.65E-01ph</td>
<td>2.16E-01g</td>
<td>1.18E-01bf</td>
</tr>
<tr>
<td>Mermentau</td>
<td>4.57E-02ab</td>
<td>2.11E-01ef</td>
<td>4.77E-02b</td>
<td>9.67E-03a</td>
<td>1.43E-01e</td>
<td>2.86E-01f</td>
<td>2.71E-01c</td>
<td>1.40E-01ab</td>
</tr>
<tr>
<td>PVLO2</td>
<td>3.40E-02f</td>
<td>1.90E-01g</td>
<td>3.57E-02dc</td>
<td>9.00E-03a</td>
<td>1.36E-01g</td>
<td>2.69E-01ph</td>
<td>2.35E-01e</td>
<td>1.25E-01ab</td>
</tr>
<tr>
<td>XP753</td>
<td>4.40E-02bc</td>
<td>2.01E-01fg</td>
<td>4.03E-02c</td>
<td>8.00E-03a</td>
<td>1.43E-01e</td>
<td>3.05E-01cd</td>
<td>2.53E-01d</td>
<td>1.28E-01ab</td>
</tr>
</tbody>
</table>

F = 50.0104 124.2188 63.632 18.3973 243.8633 419.982 549.9634 4.2154

df = 12, 26, 12, 26, 12, 26, 12, 26

p = < 0.001 < 0.001 < 0.001 < 0.001 < 0.001 < 0.001 < 0.001

SEM = 8.27E-04 2.54E-03 8.11E-04 9.78E-04 8.72E-04 1.26E-03 1.32E-03 5.95E-03
Table 2. Percent nitrogen and protein among varieties of rough rice (LS Means ± SE; nitrogen SE = 0.02; protein SE = 0.14). Means within a column that share a letter are not significantly different (Tukey’s HSD, α = 0.05).

<table>
<thead>
<tr>
<th>Variety</th>
<th>Mean % N</th>
<th>Mean % Protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>CLXL745</td>
<td>1.29a</td>
<td>8.09a</td>
</tr>
<tr>
<td>CL111</td>
<td>1.28ab</td>
<td>7.99ab</td>
</tr>
<tr>
<td>Jazzman 2</td>
<td>1.23abc</td>
<td>7.72abc</td>
</tr>
<tr>
<td>Frontiere</td>
<td>1.18abed</td>
<td>7.37abcd</td>
</tr>
<tr>
<td>XP753</td>
<td>1.18bcd</td>
<td>7.37bcd</td>
</tr>
<tr>
<td>CL153</td>
<td>1.17bcd</td>
<td>7.34bcd</td>
</tr>
<tr>
<td>Cheniere</td>
<td>1.16bcd</td>
<td>7.27bcd</td>
</tr>
<tr>
<td>Caffey</td>
<td>1.15cd</td>
<td>7.21cd</td>
</tr>
<tr>
<td>Jupiter</td>
<td>1.11de</td>
<td>6.94de</td>
</tr>
<tr>
<td>Mermentau</td>
<td>1.10def</td>
<td>6.90de</td>
</tr>
<tr>
<td>CL151</td>
<td>1.10def</td>
<td>6.88def</td>
</tr>
<tr>
<td>PVL02</td>
<td>1.02ef</td>
<td>6.37ef</td>
</tr>
<tr>
<td>Gemini 214 CL</td>
<td>0.99f</td>
<td>6.18f</td>
</tr>
</tbody>
</table>
Figure 6. The impact of time postharvest on percent protein (A) and nitrogen (B) in stored rough rice (LS Means ± SE). Bars that share a letter are not significantly different (Tukey’s HSD, α = 0.05).
Table 3. Percent starch by weight of stored rice among varieties of rough rice (LS Means ± 0.44 [SE]). Means within a column that share a letter are not significantly different (Tukey’s HSD, α = 0.05).

<table>
<thead>
<tr>
<th>Variety</th>
<th>Starch (g/100g of rice)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jupiter</td>
<td>39.99a</td>
</tr>
<tr>
<td>Jazzman 2</td>
<td>38.35a</td>
</tr>
<tr>
<td>XP753</td>
<td>33.91b</td>
</tr>
<tr>
<td>Caffey</td>
<td>33.57b</td>
</tr>
<tr>
<td>Gemini 214 CL</td>
<td>31.67bc</td>
</tr>
<tr>
<td>CL153</td>
<td>30.52cd</td>
</tr>
<tr>
<td>CLXL745</td>
<td>29.05de</td>
</tr>
<tr>
<td>Frontiere</td>
<td>27.14ef</td>
</tr>
<tr>
<td>PVLO2</td>
<td>27.11ef</td>
</tr>
<tr>
<td>CL151</td>
<td>26.38fg</td>
</tr>
<tr>
<td>Mermenau</td>
<td>26.37fg</td>
</tr>
<tr>
<td>Cheniere</td>
<td>24.12gh</td>
</tr>
<tr>
<td>CL111</td>
<td>23.87h</td>
</tr>
</tbody>
</table>

3.3.3. Character Analyses

No nutritional measures (amino acids, protein, nitrogen, starch) were correlated with any measures of susceptibility ($P > 0.05$). On the other hand, several physical characteristics were correlated with measures of susceptibility (Table 5). We found that damage by adult beetles was positively correlated the grain length and length/width ratio. Adult damage was positively correlated with thickness of brown and milled rice, but not rough rice. There was also a trend for damage by adults to be negatively correlated with rice width. Of all the measures, progeny counts and progeny damage were only ones affected by milled rice thickness; they were positively correlated. Progeny mass was positively correlated with grain width and grain weight, but negatively correlated with length/width ratio. Hull thickness along grain width was positively correlated with progeny mass. Bran weight was positively correlated progeny mass.
Table 4. Grain physical characteristics obtained from the available registrations of rice varieties.

<table>
<thead>
<tr>
<th>Measure</th>
<th>Caffey</th>
<th>Cheniere</th>
<th>CL151</th>
<th>CL111</th>
<th>CL153</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rough Length (mm)</td>
<td>8.08</td>
<td>9.33</td>
<td>8.74</td>
<td>9.34</td>
<td>9.51</td>
</tr>
<tr>
<td>Brown Length (mm)</td>
<td>5.97</td>
<td>7.14</td>
<td>6.68</td>
<td>7.31</td>
<td>7.26</td>
</tr>
<tr>
<td>Milled Length (mm)</td>
<td>5.68</td>
<td>6.92</td>
<td>6.36</td>
<td>6.46</td>
<td>6.95</td>
</tr>
<tr>
<td>Rough Width (mm)</td>
<td>3.19</td>
<td>2.36</td>
<td>2.61</td>
<td>2.61</td>
<td>2.42</td>
</tr>
<tr>
<td>Brown Width (mm)</td>
<td>2.76</td>
<td>2.16</td>
<td>2.22</td>
<td>2.27</td>
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<td>1.7</td>
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References

(Blanche et al., 2012) (Linscombe et al., 2006) (Blanche et al., 2011) (Oard et al., 2014) (Famoso et al., 2017)

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References

(Wenefrida et al., 2017) (Sha et al., 2013) (Sha et al., 2006) (Oard et al., 2014b)
Table 5. Measures of susceptibility examined as functions of rice physical characteristics. Only significant or near significant correlations are displayed ($\alpha = 0.05$).

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</table>

3.3.4. Associational Resistance Assay

Treatment affected both adult damage ($F_{9,109} = 2.73$, $P = 0.007$, Figure 7) and larval damage ($F_{9,108} = 4.12$, $P < 0.001$, Figure 8). Moreover, there was one instance of associational resistance in those measures. J+CL151 received about 30% less larval damage and adult damage than CL151. Treatment also affected progeny production ($F_{9,2448} = 3.52$, $P < 0.001$, Figure 9), and progeny mass ($F_{9,102} = 6.01$, $P < 0.001$, Figure 10), but there were no instances of associational resistance or susceptibility in either measure. Days to eclosion was not affected by treatment ($F_{9,83} = 0.43$, $P = 0.916$).
Figure 7. The feeding damage mass by adult *R. dominica* among stored rice varieties and varietal mixes in the associational resistance assay (LS Means ± 1.67 [SE]). Varietal mix names abbreviate Jazzaman 2 to J, and PVL02 to P. Bars that share a letter are not significantly different (Tukey’s HSD, α = 0.05).

Figure 8. The feeding damage mass by *R. dominica* larvae among stored rice varieties and varietal mixes in the associational resistance assay (LS Means ± 44.09 [SE]). Varietal mix names abbreviate Jazzaman 2 to J, and PVL02 to P. Bars that share a letter are not significantly different (Tukey’s HSD, α = 0.05).
Figure 9. Adult *R. dominica* progeny production among varieties in the associational resistance assay (LS Means ± 0.52 [SE]). Bars that share a letter are not significantly different (Tukey’s HSD, α = 0.05).

Figure 10. *Rhyzopertha dominica* progeny mass among varieties in the associational resistance assay (LS Means ± SE). Bars that share a letter are not significantly different (Tukey’s HSD, α = 0.05).

### 3.4. Discussion

This is the most comprehensive examination of varietal resistance to a stored insect pest and its mechanisms among modern US rice varieties. We examined resistance among 13
varieties of stored rice, 20 different nutritional characteristics, and 21 different physical characteristics through measuring the growth and development of over 15,000 beetles. As seen in prior studies, rice varieties were differentially susceptible to *Rhyzopertha dominica* (Cogburn, 1977; Chanbang et al. 2008b; Arthur et al., 2013; Asiwaju-Bello et al., 2019). While studies by Chanbang et al. 2008a (2008) and Asiwaju-Bello et al. (2019) had previously found differential susceptibility of rice varieties towards *Rhyzopertha dominica*, this study is the first to identify the relative susceptibility of modern rice varieties grown in the southern US. This is also the first documentation of pest susceptibility in Clearfield varieties, as CL111, CL151, and CL153 were among the more susceptible varieties in terms of damage by *R. dominica* adults, damage by progeny, and progeny production. Alongside those varieties was Frontière, which had previously been shown to highly susceptible to *S. oryzae* (Doherty et al. 2023). In knowing the relative susceptibility of these varieties, growers can make more informed pest management decisions, and implement additional control methods when necessary.

Frontière was recently developed as a high-protein, low-glycemic rice variety (Wenefrida et al. 2017), and while we had initially expected its susceptibility to be due to its high protein, that appears to not be the case. The registration notes the protein content of the variety to be around 11%. However, in our analyses of starch and protein content, Frontière had moderate levels of both protein and starch. It is currently unclear where this discrepancy comes from, but one possibility may be due to differences in methods. Our methods for calculating protein marginally differed from those of Wenefrida et al. (2017). While they also analyzed nitrogen and protein through combustion of ground grains, they verified their analyses using the Bradford method. A similar verification could have strengthened our own data. Another possibility for the discrepancy in protein content between studies may be due to a decline in protein content over
time. In our protein analyses, we found that protein content declined with time. Prior research has also found that rice may lose α-helix and β-sheet structures during storage (Zhao et al. 2021). While we did not specifically test the decline of protein content in Frontière, if it experiences a similar rate of decline as the other varieties, we would expect to find its protein content at 9-10% after a year. Assuming our rice began at 11%, protein content in Frontière seems to decline several times faster than other varieties. It is also possible that protein varied due to field conditions (Saruta et al. 2013). While we had thought the susceptibility of Frontière came from its nutritional content, given the existing data, it seems more likely that its susceptibility stems from its physical features. Its greater than average length and thickness, as well as its smaller width, denote it as a more susceptible variety at a glance.

Similar to previous research, the difference between the most susceptible and most resistant varieties equaled days-worth of development time in R. dominica, and over 10-fold differences in progeny counts and damage (Chanbang et al. 2008a; Arthur et al., 2013; Asiwaju-Bello et al., 2019). Mermentau, XP753, and Jazzman 2 were among the more resistant varieties in this study. While we had found PVL02 to be highly resistant to progeny development in the varietal resistance assay, this was not consistent with our PVL02 results in the associational resistance assay. Thus, we excluded its data from much varietal resistance analyses. Different batches of rice were utilized between these experiments, but it is unclear what differences may have existed between these batches to create that change.

Previous research has found Mermentau to also be resistant to S. oryzae, but XP753 was especially susceptible to S. oryzae (Doherty et al. 2023). While some other studies have found that the stored rice varieties susceptible to R. dominica are also susceptible to S. oryzae, others have found that to not be the case (Sayed and Saad 2018; Asiwaju-Bello et al. 2019). Though
both species are primary pests of stored grain, there are many differences in which modern US varieties are susceptible/resistant, as well as differences in the degree to which those varieties are resistant/susceptible. In these varieties, resistance toward *S. oryzae* is not as impactful as resistance to *R. dominica*, as *S. oryzae* is less damaging and less reproductively active (Doherty et al. 2023). This information may allow rice growers to better prepare for storage as they will be able to better anticipate potentially problematic pests and prepare additional controls accordingly.

When analyzing the characteristics of stored rice that correlate with resistance, we found that physical characteristics were more relevant than nutritional characteristics. Previous work has also found physical defenses to be effective mechanisms of resistance in stored rice (Breese, 1963; Chanbang et al. 2008a; Kavallieratos et al., 2011; Asiwaju-Bello et al., 2019). Antunes et al. (2016) found susceptibility toward the maize weevil, *Sitophilus zeamais* Motschulsky, increased with grain length but was inversely correlated with width and hardness of milled rice. These findings align with our own, as damage by adult *R. dominica* was positively correlated with grain length and negatively correlated with grain width. It is unclear why grain shape affects resistance, but it is possible that long and narrow grain are associated with increased handling time, or the time it takes a beetle to find a spot to begin feeding. In systems outside of stored grain, seed mass has been found to slow seed predation through increased handling time, particularly relative to body size (Tamura and Hayashi 2008; Muñoz and Bonal 2008). While these studies focus on rodents, seed size and shape may also be a factor influencing insect handling time.

We also found grain thickness affected *R. dominica* reproductive success and damage by progeny. Among the literature, it is one of a myriad of physical characteristics that have been
found to influence granivore reproduction. In beans (Phaseolus vulgaris L.), Schoonhoven et al. (1983) found that seed size influenced progeny counts, development time, and progeny mass of the bean weevil, Acanthoscelides obtectus (Say), and the Mexican bean weevil, Zabrotes subfasciatus (Boheman). Other studies have found that R. dominica oviposition was facilitated by textured surfaces, as adults preferred to oviposit upon rough surfaces rather than smooth ones (Breese 1963; Kavallieratos et al. 2011). More directly related to our work, Asiwaju-Bello et al. (2019) found length/width ratio of stored rice was positively correlated with progeny emergence of S. oryzae and R. dominica. We found length/width ratio was not related to progeny emergence, but instead was negatively correlated with progeny mass. Interestingly, while our research used both medium and long grain, Asiwaju-Bello et al. (2019) made use of exclusively medium grain rice. Further research is necessary to identify why there is such a discrepancy between these two studies. It may be an indication that grain shape can be associated with mechanisms of resistance and may not be the mechanism itself, or perhaps the relationship between grain dimensions and susceptibility is more complex than previously assumed.

Chanbang et al. (2008a) found hull thickness to be an effective mechanism of resistance of against R. dominica. In our study, the hull had minimal influence; however, our measurements of the hull are approximations based on other measures. Assuming our hull measurements are valid, it appears that while hull thickness may have contributed towards resistance of R. dominica in the varieties studied by Chanbang et al. (2008a), that mechanism of resistance is not present in the varieties grown in the southern US today. However, it is possible that our power to detect an effect of the hull was diminished by the error introduced through our approximations. Jupiter can be used as a point of comparison, as this was the only variety their study had in common with our own. They found Jupiter’s hull to vary from 46 µm at the thinnest point, to 94
μm at the thickest point. Our approximations ranged from 170 μm along the width of the grain, to 1010 μm along its length. The other varieties in our study had comparable hull approximations. Our measures are several times larger, leading us believe that our approximations may include other parts of the grain beyond the hull. Thus, more direct measures of the hull would be prudent before conclusions can be drawn about its role in the resistance of modern stored rice varieties.

While physical characteristics of grain correlated with aspects of *R. dominica* development and feeding, the nutritional aspects of rice varieties studied here were not. Neither amino acid, nitrogen, protein, nor starch content correlated with any measure of susceptibility. Even though nutritional content varies between varieties, and by age, it did not affect susceptibility to *R. dominica*. This leads us to believe that all these varieties meet the protein and starch requirements of *R. dominica*, and that these nutrients are not factors limiting growth and reproduction. C

Previous studies have demonstrated stored grain beetles may benefit from additional increased amino acid content, but only up to a point (Taylor and Medici 1966). Prior work has also found that protein content did not affect *R. dominica* susceptibility, but instead susceptibility was associated with mineral content (Asiwaju-Bello et al. 2019). While the mineral requirements of grain beetles are low relative to their protein requirements, they may be a limiting factor in stored grain beetle development, and worth exploring further as the minerals which provide resistance have not been characterized (Taylor and Medici 1966; Asiwaju-Bello et al. 2019).

Understanding that physical characteristics are more relevant to *R. dominica* resistance than nutritional characteristics could mean that breeders may invest their efforts into improving the nutritional content of rice without worrying about increased pressure from stored grain pests.
Instead, additional attention can be placed on the physical characteristics of stored grain. While stored grain pest susceptibility is not assessed as part of breeding programs, the easily observable characteristics of rice grain could allow breeders to anticipate stored rice pest susceptibility without the need for a biological assay. The same can be said for growers; the visual traits denoting susceptibility may allow growers know the susceptibility of their varieties even without prior evaluations.

In our associational resistance study, we found that mixes of rice varieties may provide associational resistance towards *R. dominica*. J+CL151 received less damage by adults than either of its component varieties. This contrasts with a similar study in which mixes of rough rice varieties typically resulted in associational susceptibility to *S. oryzae* (Doherty et al. 2023). This trend with *S. oryzae* was attributed to the feeding and ovipositional choices made by *S. oryzae* adults. Based on our results, adult *R. dominica* do not seem to have the same level of preferences; instead, their feeding behavior is heavily reduced when resistant varieties are present. Furthermore, J+CL151 only received as much larval damage as its most resistant component variety, Jazzman 2. Given that *R. dominica* adults select which grains are accessible to larvae through their feeding choice, larval choice and larval feeding damage are likely intertwined with the adult damage (Edde 2012). It is unclear how associational resistance in J+CL151 arose, but it is certainly an avenue of research worth pursuing in the future. Ultimately, these relationships will be better understood with further exploration of the mechanisms of resistance underlying them. If the relationships between store grain beetles and varietal mixes of grain can be better understood, it may become another useful tool for growers against stored grain pests.
Chapter 4. Efficacy of stored grain insecticides against two pest beetles in rough rice

4.1. Introduction

The lesser grain borer, *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae), and the rice weevil, *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae), are two of the most damaging pests in stored grain systems. Rice (*Oryza sativa* (L.)), wheat (*Triticum aestivum* L.), and other grains can be damaged by larvae feeding on the grain internally, and adults feeding externally or internally. Damage to rice can result in a significant decrease in value due to weight loss or designation as animal feed (Harein and Meronuck 1990, USDA 2016). Fumigant insecticides, namely phosphine gas, are the primary method of controlling these pests. Fumigants are broad-spectrum, fast-acting, and cost-effective when applied correctly (Hagstrum et al. 2012). However, there are populations of stored grain pests in the US and Brazil that are resistant to phosphine (Lorini et al. 2007; Opit et al. 2012). Alternative chemical control strategies are needed to reduce reliance on fumigants and enhance sustainability of stored grain pest management.

Where fumigants are used reactively to control an existing insect infestation, grain protectants are typically applied during binning as a preventative measure. These insecticides can be effective against several major pests for periods up to one year (Ghimire et al. 2016; Arthur 2018, 2019a, b). DDT and organophosphates were once used as grain protectants, but they since have been phased out due to the development of resistances and safety concerns (Boyer et al. 2012; Edde 2012). In the US, the pyrethroids deltamethrin and β-cyfluthrin have since filled their role as neuroinhibitors (Williams et al. 1982; Athanassiou et al. 2004b).

In addition to pyrethroids, several other insecticidal products are registered for stored
grain protection including diatomaceous earth, s-methoprene, and s-methoprene + deltamethrin formulations. S-methoprene is a juvenile hormone analogue which can be an effective control against larval insects in stored grain that are exposed to the chemical during development (Arthur 2016). Moreover, s-methoprene can remain effective for several years and be used in combination with deltamethrin (Arthur 2019a). Diatomaceous earth acts by disrupting the insects’ spiracles and wax layers, thereby resulting in desiccation (Webb 1946; Mewis and Ulrichs 2001; Athanassiou et al. 2005; Wakil et al. 2010). Like s-methoprene, diatomaceous earth can also be used in combination with other insecticides (Athanassiou 2006).

A variety of studies have examined the efficacy of these insecticides with stored grain pests (Lorini and Galley 1999, Arthur 2002, 2016, 2019a, Athanassiou et al. 2004, Athanassiou et al. 2008, 2011, Chanbang et al. 2008, Daglish 2008, Islam et al. 2010, Wakil et al. 2010, Paudyal et al. 2016). However, many of these studies were done in the laboratory using small vials, which may not be relevant to in situ management. Further, efficacy of these products has not been compared in rough rice. Rough rice is the form of rice that is stored by growers, and without direct comparisons of insecticide efficacy, it is difficult for growers to make informed pest management decisions. Thus, our research aims to evaluate and compare stored grain protectants alone and in combination for S. oryzae and R. dominica control on rough rice in metal containers similar to grain bins.

4.2. Methods

4.2.1 Insects

Initial colonies of S. oryzae and R. dominica were provided by the USDA-ARS Manhattan, Kansas, who had maintained these colonies for over 40 years. These colonies were
then supplemented with field collected beetles from Louisiana in 2019-2020. Beetles were reared on wheat (*T. aestivum*, 12% MC) in 24-32 oz deli containers inside a growth chamber (27°C, continual darkness). Fresh wheat was provided every 2 months or as needed. No quiescent periods were observed, and physiological status appeared consistent under these conditions.

### 4.2.2. Insecticide Trials

Insecticides were evaluated across two trials at the LSU AgCenter (Baton Rouge, Louisiana, USA) from 2020–2022. All insecticides were applied at the label rate to 22.7 liters of rough rice (Var: Mermentau) (Oard et al. 2014b). In the first trial, conducted from September 2020 to May 2021, twenty-five 22.7-liter metal containers were assigned one of five different insecticide treatments: 1) diatomaceous earth (DE) at a rate of 6.57 grams per container (0.25 g ai/L rice) (Crawling Insect Killer®, Garden Safe, Bridgeton, MO), 2) s-methoprene at a rate of 2.63 grams per container (0.93 mg ai/L rice) (Diacon®-D IGR, Central Life Sciences, Schaumburg, IL), 3) deltamethrin at a rate of 0.03 ml per container (1.32 mg ai/L rice) (Centynal®, Central Life Sciences, Schaumburg, IL), 4) a premixed formulation of s-methoprene and deltamethrin (M+D) at a rate of 0.6 ml per container (deltamethrin: 1.30 mg ai/L rice; s-methoprene: 3.17 mg ai/L rice) (Diacon® IGR PLUS, Central Life Sciences, Schaumburg, IL), and 5) a control group without insecticides. The deltamethrin + s-methoprene formulation also included 0.0005 ml/L of piperonyl butoxide (PBO) synergist as recommended on the product label.

Diatomaceous earth and s-methoprene dust were mixed in by hand during the transfer of rice into the container. Deltamethrin and the deltamethrin + s-methoprene formulation were applied by spreading out the grain over a plastic tarp and using a CO₂-pressurized backpack sprayer calibrated to deliver 1.13 mL per L of rice. After the insecticide applications and rice
binning, containers remained capped and stored together indoors. Room temperature was monitored through the experiment (21°C), and rice moisture content was checked at the beginning and end of the experiments (12% MC and 4% MC respectively).

Fifty mixed-sex *R. dominica* adults and 50 *S. oryzae* adults were both added to each container following treatment and every month thereafter. At the end of six months, the entire contents of the containers were sieved (#10, 1.9 mm mesh). The total beetle biomass and the total damage (rice fragments and frass) was weighed.

A second insecticide trial was run from September 2021 to April 2022 using the same methods, but the diatomaceous earth treatment was replaced with β-cyfluthrin (Tempo SC Ultra®, Pasadena Blvd, Pasadena, TX) which was applied to the interior surfaces of the containers at a rate of 1.04 mL per container (0.24 g AI/m²) prior to the addition of rice. Additionally, the rice variety used was changed from Mermentau to CL111, as Mermentau was found to be resistant to *S. oryzae* (Oard et al. 2014a; Doherty et al. 2023).

### 4.2.3. Vial Assays

Alongside each insecticide trial, at 0.5, 1, 2, 3, 4, 5, and 6 months after treatment, 24 g of rice were sampled from the surface grain of each container in the insecticide trials using 50 mL vials. Either 10 adult *R. dominica* or 10 adult *S. oryzae* were then introduced into each vial and stored in a growth chamber (27°C, continual darkness). After two weeks, adults were removed from vials and counted as alive or dead to calculate the proportion of survival. After another six weeks, the number of living and dead progeny were counted for each vial.

Because beetles were introduced into the insecticide trial containers each month and allowed to proliferate, they could not be separated from the rice sampled for the vial assays.
Thus, the rice collected for the vial assays may have contained both living and dead individuals of *R. dominica* and *S. oryzae* before the vial assays began. The abundance of the beetles already present in the rice was assessed by counting beetles present in the vials where they were not introduced. *Rhizopertha dominica* presence was assessed by counting the live *R. dominica* in the vials with introduced *S. oryzae* at the two-week mark, when *S. oryzae* mortality was assessed. Similarly, the presence of *S. oryzae* in the vials was assessed by counting *S. oryzae* in the vials with introduced *R. dominica*. In the *S. oryzae* vial assay, *R. dominica* per 24 g of rice was assessed, and used to correct the results of the *R. dominica* vial assay. *Sitophilus oryzae* presence within the *R. dominica* vial assay was also assessed, but only two live *S. oryzae* were found among the 300 vial samples used to study *R. dominica*, so the impact of *S. oryzae* per 24 g of rice was deemed negligible. From the *R. dominica* within the *S. oryzae* assay vials were calculated total *R. dominica* per 24 g of rice, dead beetles per 24 g of rice, and percent surviving beetles. 

Given the large presence of *R. dominica* in the rice samples of the *S. oryzae* vial assay, the following adjustment was made to data collected from the *R. dominica* vial assays:

\[
\text{LiveRd} - ((\text{Rd}_{\text{total}} - 10) \times \text{CF}) = \text{CorrectedLiveRd}
\]

Where \(\text{LiveRd}\) is the number of living *R. dominica* in each vial after two weeks, \(\text{Rd}_{\text{total}}\) is the number of living and dead *R. dominica* in each vial after two weeks, and \(\text{CF}\) is a correction factor equaling the average percentage of *R. dominica* that were living in the rice samples used for the *S. oryzae* vials that same month from the same treatment. This correction was made for the *R. dominica* adult survival data.

Similarly, progeny counts were adjusted into progeny per adult. For *S. oryzae*, progeny was adjusted to progeny per introduced adult (progeny/10), as the presence of *S. oryzae* collected from the containers was minimal. However, for *R. dominica*, progeny was adjusted to progeny
per surviving *R. dominica* adult two weeks after vial collections, to account for both adults and larvae already present in the rice at the start of the assay.

### 4.2.4. Statistical Analyses

Statistical analyses were run in JMP Pro 16 (α = 0.05) from both trials together, except for the *S. oryzae* vial data, which was split by trial due to differences in sampling frequency. Sampling at the 2-week timepoint was missed in the second trial. Normally-distributed residuals were confirmed through histograms, as well as analyses of skewness and kurtosis. Skewness was considered normal for values between -2 and +2, while kurtosis was considered normal for values between -7 and +7 (Byrne 2010; Hair et al. 2010). Homogeneity of variances were confirmed using the DHARMa package in R 4.2. In the linear mixed effects models used to assess beetle mass per container and damage mass per container, treatment was a fixed effect, while trial and trial × rep were random effects. For the analyses of data from the vial assays, fixed effects included: treatment, month, and treatment × month. The random effects associated with the vial assays were trial, trial × replicate, and trial × replicate × treatment. Analyses of the Tukey’s HSD was used for all post hoc mean separations.

### 4.3. Results

#### 4.3.1. Insecticide Trials

Damage mass per container was affected by treatment (F = 6.84; df = 5, 35; P < 0.001). Treatments containing s-methoprene received less damage than the non-treated control group, with s-methoprene and M+D receiving 40% and 32% less damage, respectively (Figure 1A). Additionally, damage in s-methoprene and M+D was approximately 51% and 47% that of DE
treated rice, respectively. The total mass of beetles in the containers also varied by treatment (F = 13.76; df = 5, 35; P < 0.001). Again, s-methoprene and M+D had lower beetle masses than the non-treated control group, as did deltamethrin. Beetle mass in the DE and the non-treated control group was approximately 2-fold greater than that in deltamethrin, s-methoprene, and D+M treatments (Figure 1B).

Figure 1. The damage mass (A) and beetle mass (B) as influenced by insecticide treatments 6-months after treatment (LS Means ± SE). Bars that share the same letter are not significantly different (Tukey’s HSD, α = 0.05).
4.3.2. First Trial *S. oryzae* Vial Assays

In the first trial of the *S. oryzae* vial assay, we found that both treatment (F = 2.96; df = 4, 133; P = 0.022) and month (F = 5.34; df = 6, 120; P < 0.001) affected *S. oryzae* survival, but not the treatment × month interaction (F = 1.27; df = 24, 120; P = 0.197). Adult survival was 20-fold greater in the non-treated control than in DE-treated containers (Figure 2). Adult survival in the non-treated control group was also nearly 2-fold that of s-methoprene and deltamethrin and 4-fold that of M+D, however, these differences were not statistically significant. Survival also decreased with month; survival rates were nearly 5-fold greater in the first month than the last (Figure 3A). *Sitophilus oryzae* progeny production per adult in the first trial was not affected by treatment (F = 0.28; df = 4, 119; P = 0.892) as progeny counts were all close to 0. However, there was an effect of month (F = 3.26; df = 6, 120; P = 0.005) where progeny production at the sixth month was 4–5-fold greater than progeny production at the beginning (Figure 3B). There was also an effect of treatment × month (F = 1.88; df = 24, 120; P = 0.014), but pairwise comparisons using Tukey’s HSD showed no significant differences. In the second trial, *S. oryzae* mortality was high across treatments including non-treated controls, and therefore the *S. oryzae* data of the second trial was not analyzed.

*Rhyzopertha dominica* were also present in the *S. oryzae* assay vials. From the populations collected from the containers during the first trial, *R. dominica* per 24 g of rice was affected by treatment (F = 2.93; df = 4, 102; P = 0.025) and treatment × month (F = 1.74; df = 24, 120; P = 0.027), but not month (F = 1.69; df = 6, 120; P = 0.129). There were almost 3-fold more beetles in the diatomaceous earth treatment than in the deltamethrin or s-methoprene treatments (Figure 4A). In the treatments over time, *R. dominica* per 24 g of rice in the non-treated control and the diatomaceous earth treatment increased over the course of the first few
months, whereas beetle counts in the other treatments were lower and remained relatively
contant across time (Figure 4B). Dead beetle counts per 24 g of rice in the first trial were also
affected by treatment ($F = 3.60; df = 4, 105; P = 0.009$), month ($F = 3.47; df = 6, 120; P =
0.003$), and treatment × month ($F = 1.96; df = 24, 120; P = 0.009$). There were over 12-fold more
dead beetles in the M+D treated rice than in the non-treated rice (Figure 5A). Dead beetles also
increased with time, as there were almost 70% more by the end of the experiment than there
were in the beginning (Figure 5B). The increase of dead beetles over time was most prominent in
the rice treated with diatomaceous earth or M+D (Figure 5C).

The percent of surviving adults was affected by treatment ($F = 6.81; df = 4, 65; P <
0.001$), month ($F = 8.77; df = 6, 120; P < 0.001$), and treatment × month ($F = 2.18; df = 24, 120;
P = 0.003$). Survival in the non-treated control group was over 3-fold higher than treatments
containing deltamethrin, including deltamethrin alone and deltamethrin with methoprene (Figure
6A). Survival rates decreased with time, as survival in the first two months was higher than that
of the later months (Figure 6B). While survival in most treatments decreased with time, survival
in deltamethrin and diatomaceous earth increased in the first few months of the experiment
before declining (Figure 6C). When examining progeny per adult, we found an effect of
treatment ($F = 6.91; df = 4, 122; P < 0.001$) and month ($F = 2.99; df = 6, 120; P = 0.009$). Both s-
methoprene- and M+D-treated containers had fewer progeny per adult than the non-treated
control and diatomaceous earth containers where progeny per adult counts were about 6-fold
higher (Figure 7A). Progeny per adult counts were also lower in the middle months of the
experiment than at the start (Figure 7B). There was no treatment × month interaction ($F = 0.93;
df = 24, 120; P = 0.566$).
Figure 2. Percent *S. oryzae* adult survival as influenced by insecticide treatments in the trial 1 *S. oryzae* vial assays (LS Means ± SE). Bars that share the same letter are not significantly different (Tukey’s HSD, α = 0.05).
Figure 3. Percent *S. oryzae* adult survival (A) and progeny per adult (B) as influenced by months across treatments in the trial 1 *S. oryzae* vial assays (LS Means ± SE). Means that share the same letter are not significantly different (Tukey’s HSD, α = 0.05).
Figure 4. *Rhyzopertha dominica* adults per 24g of rice as influenced by treatments (A), and the treatment × month interaction (LS Means ± 3.52 [SE]) (B) in trial 1 of the *S. oryzae* vial assay (Means ± SE). Means that share the same letter are not significantly different (Tukey’s HSD, α = 0.05).
Figure 5. Dead adult *R. dominica* collected per 24g of rice as influenced by treatments (LS Means ± SE) (A), months (LS Means ± SE) (B), and the treatment × month interaction (LS Means ± 2.04 [SE]) (C) in trial 1 of the *S. oryzae* vial assay. Means that share the same letter are not significantly different (Tukey’s HSD, \( \alpha = 0.05 \)).
Figure 6. Percent living *R. dominica* adults as influenced by treatments (LS Means ± SE) (A), months (LS Means ± SE) (B), and the treatment × month interaction (LS Means ± 10.31 [SE]) (C) in trial 1 of the *S. oryzae* vial assay. Means that share the same letter are not significantly different (Tukey’s HSD, \( \alpha = 0.05 \)).
Figure 7. Percent living *R. dominica* adults as influenced by treatments (A), as well as their progeny counts over time (B) in trial 1 of the *S. oryzae* vial assay (LS Means ± SE). Means that share the same letter are not significantly different (Tukey’s HSD, α = 0.05).

4.3.3. Second Trial *S. oryzae* Vial Assays

In the second trial, *R. dominica* per 24 g of rice was not affected by treatment (*F* = 0.34; df = 4, 81; *P* = 0.849), month (*F* = 1.45; df = 4, 79; *P* = 0.224), or treatment × month (*F* = 0.65; df = 16, 79; *P* = 0.833). Moreover, dead *R. dominica* counts were affected by month (*F* = 3.39; df
= 4, 80; P = 0.013), but not treatment (F_{4,81} = 0.27; df = 4, 81; P = 0.895) or treatment × month
(F = 0.75; df = 16, 80; P = 0.73). There was over a 40% increase in dead beetles from the first
month of the experiment to the last (Figure 8). *Rhyzopertha dominica* survival was affected by
treatment (F = 6.15; df = 4, 89; P < 0.001), and month (F = 11.60; df = 4, 80; P < 0.001), but not
treatment × month (F = 1.17; df = 16, 80; P = 0.308). The survival rate in the non-treated control
group was approximately 15-fold greater than the survival rate of M+D (Figure 9A). The
proportion of living adults dropped sharply after the first month of the experiment, but there was
no change among the later months (Figure 9B). *Rhyzopertha dominica* progeny in the second
trial was not affected by treatment (F = 1.26; df = 4, 95; P = 0.290), month (F = 0.74; df = 4, 80;
P = 0.567), or the interaction (F = 0.68; df = 16, 80; P = 0.808).

![Figure 8. Dead *R. dominica* collected per 24g of rice as over time in trial 2 of the *S. oryzae* vial
assay (LS Means ± SE). Means that share the same letter are not significantly different (Tukey’s
HSD, α = 0.05).](image)
4.3.4. Rhyzopertha dominica Vial Assays

From the vials with introduced *R. dominica*, after adjusting for beetles already present, survival was affected by treatment ($F = 4.90; \text{df} = 5, 208; P < 0.001$), month ($F = 4.73; \text{df} = 5, 220; P < 0.001$), and the treatment × month interaction ($F = 1.66; \text{df} = 25, 220; P = 0.028$).

Survival in M+D was nearly 28-fold lower than survival in the non-treated control group and
significantly lower than all other treatments except deltamethrin (Figure 10A). Survival in month 2 was almost half of that in the other months (Figure 10B). Adult survival in diatomaceous earth rose to 100% at month 3 and remained high afterwards (Figure 10C). *Rhizopertha dominica* progeny counts were also affected by treatment ($F = 10.65; df = 5, 274; P < 0.001$). There were over 2-fold more progeny per adult in diatomaceous earth than in the non-treated control group, while the control had 4-fold more progeny per adult than M+D (Figure 11A). There was also an effect of month ($F = 6.60; df = 5, 220; P < 0.001$), where progeny counts were 10-fold higher in the first month than the fifth (Figure 11B). Finally, there was an interaction effect of treatment × month ($F = 3.82; df = 25, 220; P < 0.001$), but it is not presented here as there were no clear trends.

Figure 10. From both trials of the *R. dominica* vial assays, the percent survival of adults among treatments (LS Means ± SE) (A), months (LS Means ± SE) (B), and the treatment × month interaction (LS Means ± 0.12 [SE]) (C). Means that share the same letter are not significantly different (Tukey’s HSD, $\alpha = 0.05$).
Figure 11. From both trials of the *R. dominica* vial assays, the progeny counts per adult among treatments (A) and months (B) (LS Means ± SE). Means that share the same letter are not significantly different (Tukey’s HSD, α = 0.05).

4.4. Discussion

This study is the first to compare efficacy of insecticidal grain protectants for control of *S. oryzae* and *R. dominica* in rough rice in conditions similar to a grain bin. When results across trials and species are considered collectively, the combination of s-methoprene and deltamethrin...
was generally the most effective control of stored grain beetles. The effects of s-methoprene and deltamethrin were generally additive, thereby providing a high level of control in nearly all measures, even when deltamethrin or s-methoprene alone may have only provided an intermediate level of control.

When examining the total damage and beetle mass of the containers, the effect of an insecticide on total beetle mass was typically mirrored in its effect on total damage mass. The high similarity between these suggests that a reduction in infestation provides a corresponding level of protection of grain. In these measures, s-methoprene alone was as effective a control as the combination of s-methoprene and deltamethrin. S-methoprene, as an insect growth regulator, targets insect larvae and typically has minimal effect upon adults (Henrick 2007). In the *R. dominica* samples from the containers in the first trial (*S. oryzae* vial assay), s-methoprene did not affect survival of adults, but it did reduce progeny counts as expected based on its mode of action. Additionally, though not statistically significant, s-methoprene trended towards having fewer dead *R. dominica* adults than the other insecticide treatments, leaning closer to the non-treated control. However, these trends were not seen in the *R. dominica* vial assay, where the progeny per adult counts in s-methoprene were similar to those in the non-treated control group. Only in combination with deltamethrin were progeny counts significantly lower than those of the non-treated control group. The *R. dominica* vial assays suggest that in the course of a single generation, s-methoprene provides an intermediate level of control, but by examining the entirety of the containers over the 6-month interval and multiple generations, s-methoprene provides a high level of control.

Looking at the beetles sampled per 24 g of rice over time, we can see that deltamethrin, s-methoprene, and the combination of the two all provided control throughout the experiment,
suggesting that their residual efficacy did not decline over the course of the experiment. In this measure, diatomaceous earth provided very little control to begin with and its beetle populations steadily increased from there. In the non-treated rice, beetle populations quickly increased, but then decreased halfway through the experiment. There was also a drop in beetle populations within the diatomaceous earth-treated rice, but only in the final month of the experiment. Interestingly, both populations peaked at the same beetle density before sample beetle densities declined. While the cause of these declines in beetle density is unclear, one possible explanation may have to do with density-dependent dispersal behaviors. Previous research has found that certain densities of *R. dominica* are more likely to initiate flight behaviors (Barrer et al. 1993). While very few beetles were observed leaving the containers, a flight response may have driven some beetles out of the rice towards the lid of the container. Other possible explanations for the declines in beetle density include sampling biases or the spread of pathogens. We sampled from the surface of the container, so over time *R. dominica* may have dispersed to lower strata of the container; however, previous work has found that *R. dominica*, unlike some other stored grain pests, tend to be randomly distributed among strata such that sampling is unaffected (Subramanyam and Harein 2014). Where pathogens are concerned, higher densities of insects tend to increase pathogen transmission (Moerbeek and Van Den Bosch 1997), but if this were the issue, we would expect to see dead beetles per 24 g of rice increase in those treatment around those beetle density peaks. Future research might explore density-dependent factors affecting *R. dominica* population dynamics.

β-Cyfluthrin was the only treatment not applied to the rice itself, and instead to the containers. Despite this, it still generally provided an intermediate level of control in several measures. β-cyfluthrin generally appeared more efficacious when assessed by measures of what
is occurring inside the grain containers, such as total beetle mass, damage mass, and surviving *R. dominica* in the *S. oryzae* vials. Measures from the vial assays are less closely associated with the containers, and so we would expect β-cyfluthrin to have a reduced impact on those measures. In the *R. dominica* vial assay, β-cyfluthrin did not affect adult survival relative to the non-treated control group. This lack of difference is likely because the rice extracted from containers did not have insecticide directly applied to it. However, there was a trend for it to reduce *R. dominica* progeny per adult. Due to the small diameter of the containers, much of the rice may have been in contact with the container, and therefore some residue of β-cyfluthrin was likely on the collected rice. It is possible that the residue on the rice was not enough to be lethal to adults, but was enough to kill *R. dominica* eggs, which are oviposited outside the grain, and first instar larvae, which must crawl upon the rice before finding a kernel to develop inside (Edde 2012). Another possibility is that some β-cyfluthrin was able to volatilize (Panger and Hetrick 2013). This would allow the pyrethroid to reach the larvae inside the kernel, while still likely remaining below the lethal dose intended for adult beetles. Finally, it is possible, that any residues on the collected rice were sublethal for adult beetles, but still enough to alter their reproductive behavior, resulting in lower progeny counts. Sublethal doses of β-cyfluthrin have been shown to reduce mobility in other stored product pests (Guedes et al. 2008). Before conclusions can be drawn though, additional work should investigate the sublethal effects of β-cyfluthrin on *R. dominica*, volatilization, and residues transferred to rice.

The total beetle mass measure combines the mass of all the *S. oryzae* and *R. dominica* collected from the containers. While the exact proportion of total beetle mass made up by each species was not recorded, we can gain a bit more insight into the likely composition using the vial samples. Nearly every sample (99.3%) contained at least one *R. dominica*, with several
containing more than 40. Only three vials contained any *S. oryzae*. Given this large discrepancy among our samples, the total beetle mass is likely almost completely comprised of *R. dominica*. The minimal presence of *S. oryzae* within the containers is also reflected in their vial assays. Even in our non-treated control group, *S. oryzae* survival was unusually low. The reason for their high mortality is unknown, but some evidence suggests it may be related to the moisture content (MC) of the rice. Moisture content was not monitored throughout the experiment, but a couple of tests were made. At the start of the experiments, moisture content was around 12% as expected; however, at the end of the second trial moisture content was found to be around 4%. Previous work has shown that *S. oryzae* struggle to survive on hosts below 11% MC (Birch 1953). If a fluctuating moisture content was an intervening factor in our trials, *R. dominica* seemed to be more tolerant of it than *S. oryzae*. This is in line with prior research, which has shown that *R. dominica* is more capable of surviving on low moisture content hosts than *S. oryzae* (Birch 1953). If *R. dominica* are relatively resistant to desiccation, that may help explain why they are less affected by rice moisture content, as well as why diatomaceous earth was particularly ineffective against them. Diatomaceous earth acts by disrupting the wax layer of the insect, leading to desiccation (Mewis and Ulrichs 2001). On the contrary, the large reduction in *S. oryzae* adult survival in diatomaceous earth-treated vials relative to non-treated controls suggests that *S. oryzae* is highly susceptible to desiccation.

Overall, while diatomaceous earth may have impacted *S. oryzae* in the vial assays, it provided minimal to no appreciable control of *R. dominica*. Unusually, in the *R. dominica* vial assay, diatomaceous earth had a positive impact on *R. dominica* progeny production and survival rates across time. It was likely these changes resulted in the trend for diatomaceous earth to have the highest damage mass and total beetle mass. Though it is unclear why, one possible
explanation may have to do with the presence *S. oryzae*. *Sitophilus oryzae* was negatively impacted by diatomaceous earth, and their survival rates were dramatically reduced in the diatomaceous earth treatment. The removal of a competitor may mean that a higher proportion of the rice was untouched by *S. oryzae* feeding, and therefore more suitable for *R. dominica* oviposition. However, given the minimal presence of *S. oryzae* in the experiments, it is unclear if they were ever a noteworthy competitor. Another possibility is that the diatomaceous caused abrasions on the rice hull, allowing adults to bore into the rice more easily. There are a great variety factors which influence diatomaceous earth efficacy including insect species, host plant, grain type, grain moisture, temperature, and particle size (Dowdy 1999, Arthur 2000, McGaughey 1972, Fields and Korunic 2000, Athanassiou et al. 2005, Vayias et al. 2009). Given the variability in diatomaceous earth efficacy and the breadth of factors that affect it, additional research may be needed to explain why diatomaceous earth seemed to assist *R. dominica*.

Future work may also wish to look at these insecticides while applied to larger grain bins, as any effects seen from the surrounding environment would be more relevant. While our experiment is similar to larger, commercial scale grain bins, we introduced insects to the system, rather than letting them naturally colonize our containers. We also did not account for changes in moisture content, which may have impacted our results. Additionally, some of these results may appear differently at a larger scale. Grain type and processing may also effect insecticide efficacy (McGaughey 1972, Arthur 2016). While some studies have directly compared efficacy across rice types, there are still gaps in the knowledge base. Finally, our containers were stored indoors at 21°C, while commercial grain bins will experience more dynamic temperatures across the year. At warmer temperatures, increases in insect reproduction and development would be expected, so evaluations at higher temperatures may provide information about efficacy with
increased pest pressure. Based on the research done here and the majority of our measures, a combination of s-methoprene and deltamethrin with a PBO synergist may be the most effective choice for growers. The efficacy of the other treatments was more variable, and thus it is difficult to predict the level of protection they may provide.
Chapter 5. Entomopathogenic fungi as biological control agents of beetle pests in stored rice

5.1. Introduction

Lesser grain borers, *Rhyzopertha dominica* (Bostrichidae: Coleoptera), and rice weevils, *Sitophilus oryzae* (Curculionidae: Coleoptera), attack rice worldwide (Koehler 1994; Edde 2012). Damage to rice can translate to economic loss in several ways. Growers might earn less from damaged kernels due to the weight loss, or grain damaged at a high enough rate might be designated sample grade, restricting the product to sale as animal feed (Harein and Meronuck 1990; USDA 2016). Additionally, if a grower’s rice is infested once it reaches the mill, they might be asked to fumigate immediately, or their shipment might be rejected entirely.

Management of these pests in the US has relied heavily on fumigant insecticides (Hagstrum et al. 2012). While fumigants are effective and can be used reactively, there are problems associated with them. Phosphine gas is now the sole registered fumigant for stored grain, creating concerns that phosphine-resistant pests may become widespread. Phosphine-resistant populations of stored grain beetles already exists in the United States, Brazil, and other countries (Lorini et al. 2007; Opit et al. 2012; Nayak et al. 2019). As such, there is an urgent need for alternative management tactics that are environmentally sustainable and financially prudent.

Integrated pest management combines management strategies to reduce the economic impact of pests, while reducing the environmental impact of pest management strategies. In stored rice growers have access to tools like chemical controls, cultural controls, and host-plant resistances (Arthur 2016; Atungulu et al. 2018; Doherty et al. 2023). However, there are no commercially-available biological control agents for stored rice. Previous studies have identified
several entomopathogenic fungi to provide control of rice weevils and lesser grain borers in stored wheat, including *Beauveria bassiana* (Bals.) Vuill. and *Cordyceps fumosorosea* (Wize) (Sheeba et al. 2001; Vassilakos et al. 2006; Riasat et al. 2011; Kavallieratos et al. 2014). Yet none of the fungal strains used in these studies are commercially-available for any agricultural system. The commercially-available strains of *Beauveria bassiana* and *Cordyceps fumosorosea* (*Bb* GHA and *Cfr* FE9901, respectively) have not been studied or evaluated in stored grain systems. Moreover, no research has examined control of these beetles through fungi in stored rice, all the previous work has been done in stored wheat.

Diatomaceous earth (DE) is a silica dust that can be used as an insecticide in stored grain. Some research has found that entomopathogenic fungi can work synergistically with diatomaceous earth (Riasat et al. 2011; Shafighi et al. 2014; Rizwan et al. 2019; Wakil et al. 2021). Studies have proposed that the synergism may be due diatomaceous earth increasing fungal attachment rates, or perhaps the drier condition improve fungal viability (Lord 2001, 2005). But once again, these studies have not been done in stored rice or with commercially-available entomopathogens. Moreover, it is unknown how these fungi would interact with each other within stored grain systems. When combining fungal species, research has shown that their effects on control can be additive or antagonistic as they compete for nutrients (Roy and Pell 2000; Kavallieratos et al. 2014). Additionally, no studies have examined the behavioral effects of entomopathogenic fungi upon stored grain pests. There are many open questions about the efficacy of *Bb* GHA and *Cfr* FE9901 in stored rice, how they integrate with other control methods, and how they influence their hosts. Here, we address these questions through the following objectives:
1. To determine the efficacy of *Beauveria bassiana* GHA and *Cordyceps fumosorosea* FE9901 as fungal spores and formulated products against *R. dominica* and *S. oryzae* in stored rice.

2. To determine the efficacy of *Beauveria bassiana* GHA and *Cordyceps fumosorosea* FE9901 in combination with each other, and in combination with diatomaceous earth.

3. To determine how the presence of *Beauveria bassiana* GHA and *Cordyceps fumosorosea* FE9901 may modify *R. dominica* and *S. oryzae* behavior.

5.2. Methods

5.2.1 Biological Materials

Initial colonies of *R. dominica* and *S. oryzae* were provided by the USDA-ARS Manhattan, Kansas, who had maintained these colonies for over 40 years. These colonies were then supplemented with field collected beetles from Louisiana in 2019–2020. Beetles were reared on wheat (*T. aestivum*, 12% MC) in 24–32 oz deli containers inside a growth chamber (27°C, continual darkness). Fresh wheat was provided every 2 months or as needed.

Rough rice (Var: CL111) was acquired from the LSU Rice Research Station. CL111 was chosen for its availability and relative susceptibility to both *R. dominica* and *S. oryzae* (Doherty et al., 2023a, b). Rice was sieved and tempered to 12% moisture content (MC) according to the following equation (AACC 2009):

\[
ml\text{ water} = ((100 - \text{original moisture (‰)}) / (100 - \text{desired moisture (‰)}) - 1) \times \text{sample mass}
\]

BotaniGard® 22WP and NoFly® WP are commercially-available products containing *Bb* GHA and *Cfr* FE9901 respectively. Isolates of *Bb* GHA and *Cfr* FE9901 were collected from fungus killed rice weevils and cultured on potato dextrose agar (PDA) media. Isolate viability
was checked by first creating suspensions in fungi in 0.01% Tween-80 and estimating conidia with a hemocytometer. Isolate viability was confirmed to be above 95% before usage in the experiments described below.

5.2.2. Biological Control Efficacy Assay

Laboratory studies were conducted which applied *Bb* GHA and *Cfr* FE9901 to stored rice. Deli containers containing 220 g of stored rice were assigned one of five different treatments:

1. BotaniGard® 22WP
2. NoFly® WP
3. *Bb* GHA spores
4. *Cfr* FE9901 spores
5. Non-treated control

BotaniGard® 22WP was applied according to the label rate, as an aqueous solution, at a rate of 2.14 mg /L rice (approximately 1 × 10^{11} conidia per kg rice). NoFly® WP was also applied according to the label, as an aqueous solution, with a rate of 6.15 mg /L rice (approximately 1 × 10^{8} conidia per kg rice), while spore collections were applied at a rate of 1 × 10^{7} conidia per kg rice. Isolate concentrations were chosen based on availability and parity with prior research (Kavallieratos et al. 2014; Mantzoukas et al. 2019). Treatments and rice were mixed in glass jars and allowed to dry to ensure even application.

Alongside those treatments, an additional 5 treatments were setup to examine how treatments interact with each other.

1. BotaniGard® 22WP + NoFly® WP
2. *Bb* GHA + *Cfr* FE9901 spores
3. Diatomaceous earth
4. BotaniGard® 22WP + diatomaceous earth
5. NoFly® WP + diatomaceous earth
Treatments were applied at the rates as previously described. Diatomaceous earth was applied at the label rate of 0.25 g/L rice.

After applying each treatment, 50 adult *R. dominica* were added to each container. Containers were then stored in a growth chamber (27°C, 40–70% RH, continual darkness). After 2 weeks, beetles were removed from the containers to assess survival rates. Rates of fungal infection were assessed by inducing sporulation in beetle cadavers by placing them in diet cups with cotton balls saturated with water (Furlong and Pell 2001). Fungal species were confirmed through morphology (Humber 2005; Avery et al. 2013; Gao et al. 2017; Norjmaa et al. 2019). Containers of rice were then returned to the growth chamber. After an additional 4 weeks, the numbers of progeny reaching adulthood in each container were recorded. Additionally, we assessed survival of adult progeny and progeny mass. Then, the rice was sieved (#12 mesh) to assess damage mass by weighing the resulting rice fragments and frass (Doherty et al., 2023a, b). Each treatment was replicated 5 times. After completing the experiment with *R. dominica*, the entire experiment was repeated with *S. oryzae*.

Residual activity of treatments was assessed through washes during these experiments. Ten grains of rice were collected from each container after 3 days, 2 weeks, 6 weeks after treatment. Rice samples were washed in 1 mL of 0.01% Tween-80 and vortexed for 20 sec. The resulting solution was then plated and cultured. Colony formation units were counted after 4 days.

5.2.3. Fungal Competition Assay

Studies examining fungal growth in the presence and absence of competitors were conducted by transferring 3mm cylindrical cores of the *Bb* and *Cfr* isolates from their cultures on
PDA to a clean plate of PDA on a 6.5 × 6.5 square Petri dish. A core of each fungus was placed 4 cm apart in the dish. Control dishes were set up by placing one core fungus in the dish. Thirty competition assays were set up, as well as seven of repetitions of each control. Photos of the dishes were taken of every 2–3 days for 14 days. Photos were taken straight on, from 38 cm away. Measurements of fungal area were taken using ImageJ (National Institutes of Health, Bethesda, Maryland). From these analyses we collected data on fungal growth over time, and area covered by each fungus with and without a competitor present.

5.2.4. Behavioral Assays

A behavioral experiment examining *R. dominica* space-usage was conducted using EthoVision XT 16. Two half-circles of filter paper were placed to cover the base of Petri dishes. One half-circle was saturated with 0.01% Tween-80, while the other half-circle was saturated with one of three different treatments: *Bb* GHA (1 × 10⁷ conidia/mL), *Cfr* FE9901 (1 × 10⁷ conidia/mL), or a control (0.01% Tween-80). Both fungi were applied in suspensions of 0.01% Tween-80, and so other applications of 0.01% Tween-80 alone acted as controls. In each dish, one *R. dominica* was placed on the 0.01% Tween-80 side of the Petri dish. EthoVision XT 16 is a program which records and tracks the movement of subjects between predetermined zones. Using this software, we determined the number of times a beetle crossed to the treated side of the Petri dish, time spent on each side of the dish, total distance traveled, time spent moving, and time spent not moving across a one-hour period. This entire experiment was repeated 12 times using *R. dominica* as subjects, and then 12 more times using *S. oryzae* as subjects.

5.2.5. Statistical Analyses
All statistical analyses were performed using JMP 17 with $\alpha$ set to 0.05. The normality of residuals was tested using histograms and Q-Q plots. Post-hoc Tukey’s HSD were used following ANOVAs when appropriate.

For measures from biological control efficacy assays, data were analyzed using linear mixed models. Treatment was a fixed effect, while replicate was a random effect. When analyzing the percent of dead beetles that sporulated with $Bb$ or $Cf\tilde{r}$, treatments which did not have the fungus of interest applied were excluded from analyses, after confirming there was not contamination. The residual activity data of both species’ trials were analyzed together in a linear mixed model. Fixed effects included treatment, time, and treatment $\times$ time, while random effects included replicate and beetle species $\times$ replicate.

Data of the competition assay were also analyzed through three analyses. An ANOVA was used to examine fungal area over time, where fixed effects included fungus, time, and fungus $\times$ time. Additionally, two student’s t-tests were run examining the area covered by each fungus by the end of the experiment in the presence and absence of a competitor. Behavioral data were determined to be non-normal, and so were analyzed using Kruskal-Wallis tests.

5.3. Results

5.3.1. Biological Control Efficacy Assay

5.3.1.1. *Rhizopertha dominica* trial

In the *R. dominica* efficacy trial, treatment influenced the majority of measures ($P < 0.05$; Table 1). Regarding the percent mortality of introduced beetles, DE and Botanigard+DE had a 3-fold increase in mortality over the non-treated control group. The percent of introduced adult beetles which sporulated with $Bb$ differed by treatment, as all treatments containing Botanigard
had more sporulation than the non-treated controls and other treatments not inoculated with Bb. Moreover, the Botanigard treatments, exceeded the sporulation counts of the isolates, and Botanigard+DE had about a 75% increase in Bb sporulation over Botanigard+NoFly and Botanigard alone. Additionally, the percentage of dead beetles which sporulated with Bb differed by treatment, as all treatments containing Botanigard had a higher infection rate than Bb+Cfr (F_{4,16} = 13.92, P < 0.001; Figure 1). Additionally, Botanigard+DE and Botanigard alone had higher rates of sporulation than Bb alone. In terms of Cfr sporulation, treatment affected both the number of introduced adult beetles sporulating with Cfr (Table 1) and the percentage of dead beetles sporulating with Cfr (F_{4,16} = 5.39, P = 0.006; Figure 2). The rate of Cfr infection was around 3-fold higher in NoFly+DE than in treatments containing the Cfr isolate (Table 1). Treatments containing the Cfr isolate, as well as Botanigard+NoFly, were not statistically different from the non-treated control. Examining the percent of dead introduced beetles that sporulated with Cfr, NoFly+DE had a sporulation rate 3- to 8-fold higher than treatments containing both Bb and Cfr (Figure 2).

Treatment also influenced (P < 0.05) all parameters recorded in the R. dominica assay relating to the adult progeny apart from those relating to the percentage of progeny infected with Cfr (Table 1). The non-treated control group had 70% more progeny than Botanigard+DE. Percent mortality of adult progeny in DE was 2- to 9-fold higher than in the other treatments, and no other treatments differed from non-treated controls. When it came to the percentage of the progeny which sporulated with Bb, Botanigard+DE was the only treatment different from the non-treated control. In the percent of dead progeny sporulating with Bb, Botanigard+DE and Botanigard alone were significantly different from Bb+Cfr, which had no progeny sporulate with Cfr (F_{4,16} = 6.16, P = 0.003; Figure 3). Treatment had no effect on the number of progeny
infected with *Cfr* (Table 1), nor did affect the percentage of dead beetles which sporulated with *Cfr* \((F_{4,16} = 1.17, P = 0.183)\). Damage to the rice was similar in all treatments, except in DE which received about 40\% less damage than the non-treated, but no other treatments differed from controls.

Figure 1. The percent of dead introduced *R. dominica* that sporulated with *Bb* after 2 weeks by treatment (LS Means ± 6.42 [SE]). Bars that share the same letter are not significantly different (Tukey’s HSD, \(\alpha = 0.05\)).
Figure 2. The percent of dead introduced *R. dominica* that sporulated with *Cfr* after 2 weeks by treatment (LS Means ± 10.83 [SE]). Bars that share the same letter are not significantly different (Tukey’s HSD, α = 0.05).
Figure 3. The percent of dead *R. dominica* progeny that sporulated with *Bb* after 2 weeks by treatment (LS Means ± 12.66 [SE]). Bars that share the same letter are not significantly different (Tukey’s HSD, α = 0.05).
Table 1. *Rhyzopertha dominica* mortality, progeny production, infection rates, and damage as influenced by treatments applied to stored rice (LS means).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Adult % mortality</th>
<th>% Adult Bb infection</th>
<th>% Adult Cfr infection</th>
<th>Adult progeny per vial</th>
<th>% Adult progeny mortality</th>
<th>% Progeny Bb infection</th>
<th>% Progeny Cfr infection</th>
<th>Damaged rice (g/container)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DE</td>
<td>42.67a</td>
<td>0.00c</td>
<td>0.00c</td>
<td>157.60ab</td>
<td>11.26a</td>
<td>0.00b</td>
<td>0.00</td>
<td>3.11b</td>
</tr>
<tr>
<td>Botanigard + DE</td>
<td>40.51ab</td>
<td>30.51a</td>
<td>0.00c</td>
<td>138.80b</td>
<td>4.89b</td>
<td>2.57a</td>
<td>0.00</td>
<td>3.98ab</td>
</tr>
<tr>
<td>Botanigard + NoFly</td>
<td>31.00abc</td>
<td>18.71b</td>
<td>3.29bc</td>
<td>231.20ab</td>
<td>2.19b</td>
<td>0.35b</td>
<td>0.10</td>
<td>5.13a</td>
</tr>
<tr>
<td>Botanigard</td>
<td>26.46abc</td>
<td>17.05b</td>
<td>0.00c</td>
<td>182.40ab</td>
<td>2.92b</td>
<td>1.56ab</td>
<td>0.00</td>
<td>4.68ab</td>
</tr>
<tr>
<td>NoFly + DE</td>
<td>17.31bc</td>
<td>0.00c</td>
<td>11.57a</td>
<td>202.60ab</td>
<td>4.60b</td>
<td>0.00b</td>
<td>0.80</td>
<td>4.71ab</td>
</tr>
<tr>
<td>NoFly</td>
<td>19.62abc</td>
<td>0.00c</td>
<td>7.64ab</td>
<td>180.40ab</td>
<td>1.51b</td>
<td>0.00b</td>
<td>0.00</td>
<td>4.54ab</td>
</tr>
<tr>
<td>Bb + Cfr</td>
<td>24.04abc</td>
<td>2.88c</td>
<td>4.01bc</td>
<td>203.60ab</td>
<td>2.04b</td>
<td>0.00b</td>
<td>0.12</td>
<td>5.09a</td>
</tr>
<tr>
<td>Bb</td>
<td>15.21c</td>
<td>3.51c</td>
<td>0.00c</td>
<td>230.80ab</td>
<td>1.28b</td>
<td>0.29b</td>
<td>0.00</td>
<td>5.47a</td>
</tr>
<tr>
<td>Cfr</td>
<td>11.65c</td>
<td>0.00c</td>
<td>3.50bc</td>
<td>203.40ab</td>
<td>1.64b</td>
<td>0.00b</td>
<td>0.08</td>
<td>5.41a</td>
</tr>
<tr>
<td>Non-treated</td>
<td>10.03c</td>
<td>0.00c</td>
<td>0.00c</td>
<td>237.20a</td>
<td>1.44b</td>
<td>0.00b</td>
<td>0.00</td>
<td>5.04a</td>
</tr>
</tbody>
</table>

SE = 6.07  
$F_{9, 36} = 5.35$  
$P = <0.001$  

*Means within a column that share a letter are not significantly different (Tukey’s HSD, $\alpha = 0.05$).
5.3.1.2. *Sitophilus oryzae* trial

In the *S. oryzae* efficacy trial, treatment influenced (*P* < 0.05) all parameters recorded in the *R. dominica* assay relating to the introduced adults except for the percent sporulation of *Bb* in dead beetles (Table 2). DE and Botanigard+DE were both over 75% mortality while other treatments did not differ from non-treated controls in which all introduced adults survived. Additionally, the percentage of beetles which sporulated with *Bb* differed by treatment; only Botanigard+DE was significantly different from the non-treated control. In terms of *Cfr* sporulation, only NoFly+DE was significantly different from the non-treated control. Treatments did not differ in the percent of dead introduced adults that sporulated with *Bb* (*F*<sub>4,16</sub> = 2.21, *P* = 0.114), but they did differ for *Cfr* (*F*<sub>4,16</sub> = 3.19, *P* = 0.042; Figure 4). NoFly had a greater rate of sporulation than the *Cfr* isolate.

Treatment influenced (*P* < 0.05) several more parameters, including adult progeny per vial, adult progeny mortality, and damage to the rice (Table 2). The non-treated control group had 50% more progeny than Botanigard+DE, and over 2-fold more progeny than DE alone. Conversely, Botanigard+NoFly and NoFly alone had nearly 2-fold more progeny than the non-treated control. Percent mortality in DE and Botanigard+DE was over 4-fold higher than in NoFly+DE, which, again, was over 4-fold higher than the non-treated control group. There was no difference among treatments for the percentage of the progeny which sporulated with *Bb*, the percentage of progeny which sporulated with *Cfr*, percentage of dead progeny which sporulated with *Bb* (*F*<sub>4,16</sub> = 1.99, *P* = 0.145), nor percentage of dead progeny which sporulated with *Cfr* (*F*<sub>4,16</sub> = 0.75, *P* = 0.575). Damage to the rice was differed among treatments with DE receiving over 2-fold less damage than treatments containing NoFly, but no treatments differed from non-treated controls (Table 2).
Table 2. *Sitophilus oryzae* mortality, progeny production, infection rates, and damage as influenced by treatments (LS means)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Adult % mortality</th>
<th>% Adult Bb infection</th>
<th>% Adult Cfr infection</th>
<th>Adult progeny per vial</th>
<th>% Adult progeny mortality</th>
<th>% Progeny Bb infection</th>
<th>% Progeny Cfr infection</th>
<th>Damaged rice (g/container)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DE</td>
<td>78.45ab</td>
<td>0.00b</td>
<td>0.00b</td>
<td>61.60e</td>
<td>97.51a</td>
<td>0.00</td>
<td>0.00</td>
<td>34.52b</td>
</tr>
<tr>
<td>Botanigard + DE</td>
<td>86.28a</td>
<td>34.34a</td>
<td>0.00b</td>
<td>86.00de</td>
<td>87.26a</td>
<td>0.85</td>
<td>0.00</td>
<td>49.40ab</td>
</tr>
<tr>
<td>Botanigard + NoFly</td>
<td>5.43c</td>
<td>2.86b</td>
<td>0.80b</td>
<td>229.80a</td>
<td>3.41c</td>
<td>0.08</td>
<td>0.10</td>
<td>83.46a</td>
</tr>
<tr>
<td>Botanigard</td>
<td>3.84c</td>
<td>3.84b</td>
<td>0.00b</td>
<td>169.00abc</td>
<td>2.08c</td>
<td>0.23</td>
<td>0.00</td>
<td>68.80ab</td>
</tr>
<tr>
<td>NoFly + DE</td>
<td>33.86bc</td>
<td>0.00b</td>
<td>5.81a</td>
<td>146.40cd</td>
<td>21.63b</td>
<td>0.00</td>
<td>0.00</td>
<td>80.89a</td>
</tr>
<tr>
<td>NoFly</td>
<td>1.58c</td>
<td>0.00b</td>
<td>1.58ab</td>
<td>214.60ab</td>
<td>0.83c</td>
<td>0.00</td>
<td>0.00</td>
<td>76.11a</td>
</tr>
<tr>
<td>Bb + Cfr</td>
<td>28.00bc</td>
<td>0.40b</td>
<td>0.80b</td>
<td>140.32cd</td>
<td>2.63c</td>
<td>0.00</td>
<td>0.00</td>
<td>65.07ab</td>
</tr>
<tr>
<td>Bb</td>
<td>2.04c</td>
<td>0.82b</td>
<td>0.00b</td>
<td>145.40cd</td>
<td>4.96c</td>
<td>0.00</td>
<td>0.00</td>
<td>58.77ab</td>
</tr>
<tr>
<td>Cfr</td>
<td>2.92c</td>
<td>0.00b</td>
<td>0.00b</td>
<td>161.20bc</td>
<td>5.66c</td>
<td>0.00</td>
<td>0.14</td>
<td>75.30a</td>
</tr>
<tr>
<td>Nontreated</td>
<td>0.40c</td>
<td>0.00b</td>
<td>0.00b</td>
<td>134.40cd</td>
<td>4.76c</td>
<td>0.00</td>
<td>0.00</td>
<td>67.66ab</td>
</tr>
<tr>
<td>SE =</td>
<td>10.91</td>
<td>3.29</td>
<td>95.95</td>
<td>14.29</td>
<td>3.10</td>
<td>0.27</td>
<td>0.06</td>
<td>0.41</td>
</tr>
<tr>
<td>$F_{9, 36}$ =</td>
<td>9.01</td>
<td>10.36</td>
<td>3.74</td>
<td>13.76</td>
<td>134.89</td>
<td>0.94</td>
<td>0.87</td>
<td>4.34</td>
</tr>
<tr>
<td>$P =$</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>0.002</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>0.502</td>
<td>0.557</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

*Means within a column that share a letter are not significantly different (Tukey’s HSD, α = 0.05).*
Figure 4. The percent of dead introduced *S. oryzae* that sporulated with *Cfr* after 2 weeks by treatment (LS Means ± 1.34 [SE]). Bars that share the same letter are not significantly different (Tukey’s HSD, α = 0.05).

5.3.1.3 Residual Activity Washes

Examining the *Bb* GHA colony formation units per plate from the washes of the collected rice samples, there was an effect of treatment ($F_{9,261} = 5.77$, $P < 0.001$). Only Botanigard (20.07 ± 5.65) and *Bb+Cfr* (17.10 ± 5.56) were significantly different from treatments without *Bb* GHA (Figure 5). There was also an effect of time ($F_{2,261} = 13.47$, $P < 0.001$). The presence of *Bb* sharply declined with time. While there was an average of 14.75 ± 3.11 CFUs at the start of the
experiment, there were only 7.04 ± 1.99 CFUs after two weeks, and almost none (0.32 ± 0.18) after six weeks. Finally, there was an effect of treatment × time ($F_{18,261} = 2.32, P = 0.002$). While all treatments ended with nearly 0 CFUs, Botanigard+NoFly dropped sharply after only 2 weeks, where other treatments declined more steadily (Figure 21). Similarly, when examining the presence of $Cfr$ from the washes of rice samples, there was an effect of treatment ($F_{9,261} = 2.866, P = 0.003$). However, when a post-hoc Tukey’s HSD was run, no significant differences were seen among the treatments. The amount of $Cfr$ present in treatments where it was applied was no different than in treatments without any $Cfr$ applied. When examining the effect of time, $Cfr$ presence began at 5.67 ± 1.52 CFUs, but had dropped to 1.4 ± 0.44 after 2 weeks ($F_{2,261} = 11.00, P < 0.001$). There was no effect of treatment × time ($F_{18,261} = 1.50, P = 0.090$).

Figure 5. Colony formation units of $Bb$ by treatment (LS Means ± 1.89 [SE]). Bars with the same letter are not significantly different (Tukey’s HSD, $\alpha = 0.05$).
5.3.2. Competition Assay

In the competition assay, there were differences between fungi ($F_{1,274} = 47.56$, $P < 0.001$), as *C. fumosorosea* covered an average area 2-fold greater than that of *B. bassiana* (8.94 ± 0.74 cm$^2$ and 4.00 ± 0.41 cm$^2$, respectively; Figure 7). There were also differences based upon time after treatment, as area covered by fungi was 12-fold greater at the end of the experiment than the beginning ($F_{7,274} = 25.93$, $P < 0.001$; Figure 8). Moreover, there was an interaction effect of days after treatment × fungi ($F_{7,274} = 47.56$, $P = 0.005$). While area coverage of both fungi increased with time, the rate of growth in *C. fumosorosea* was much greater than that of *B. bassiana* (Figure 9). Comparing the final area coverage of *B. bassiana* alone and in the presence of *C. fumosorosea*, *B. bassiana* growth was reduced by 3-fold when in the presence of the competitor ($t = 10.65$, $df = 13$, $P < 0.001$; Figure 10). Additionally, in the presence of *B.
**bassiana, C. fumosorosea** growth was reduced by nearly half \( t = 3.53, \ df = 9, \ P < 0.001; \) Figure 11).

Figure 7. Area covered by each fungi in shared Petri dishes across time (LS Means ± 0.51 [SE]). Bars that share the same letter are not significantly different (Tukey’s HSD, \( \alpha = 0.05 \)).
Figure 8. Area covered by fungi through time (LS Means ± SE). Timepoints with the same letter are not significantly different (Tukey’s HSD, α = 0.05).
Figure 9. Area covered by each fungi over time (LS Means ± SE).
5.3.3. Behavioral Assay

There was no effect of treatment on any measure of S. oryzae behavior. Regardless of
treatment, there was no difference in distance moved ($H(2) = 1.05, P = 0.59$), time spent moving ($H(2) = 1.73, P = 0.42$), time spent not moving ($H(2) = 1.65, P = 0.44$), number of instances moving into the treated area ($H(2) = 3.68, P = 0.16$), time spent in the treated area ($H(2) = 0.24, P = 0.88$), time until first moving into the treated area ($H(2) = 0.11, P = 0.94$), number of instances moving into the non-treated area ($H(2) = 0.30, P = 0.86$), and time spent in the non-treated area ($H(2) = 0.43, P = 0.80$).

Additionally, there was no effect of treatment on any measures of $R. dominica$ behavior; not for distance moved ($H(2) = 0.16, P = 0.92$), time spent moving ($H(2) = 0.51, P = 0.77$), time spent not moving ($H(2) = 0.21, P = 0.90$), number of instances moving into the treated area ($H(2) = 1.28, P = 0.53$), time spent in the treated area ($H(2) = 1.00, P = 0.61$), time until first moving into the treated area ($H(2) = 2.90, P = 0.23$), number of instances moving into the non-treated area ($H(2) = 0.94, P = 0.62$), nor for time spent in the non-treated area ($H(2) = 0.32, P = 0.85$).

We did not detect behavioral differences between species either. Neither species travel further than the other ($H(1) = 0.28, P = 0.59$), and neither species spent more time moving than the other ($H(2) = 0.02, P = 0.88$).

5.4. Discussion

This study is the first to evaluate $Bb$ GHA and $Cfr$ FE9901 for biological control of $R. dominica$ and $S. oryzae$ in stored rice. We found that both strains of fungi could infect both beetle species, but efficacy was variable. While our higher rate of $B. bassiana$ provided some short-term control of stored rice pests, it had very little effect on the following generation of beetles and the resulting damage to the rice. This may be due in part to the quickly declining residual activity of all treatments. Lord (2005) found that residual activity was longer lasting in
low humidity conditions, however, activity did not generally extend beyond 9 weeks. Perhaps in more stable and less humid conditions, the residual activity of our study species would have been extended and affected the progeny that emerged from the first generation. However, future research would be necessary to determine if a couple more weeks of residual activity is enough to impact efficacy. It is also possible that control of progeny may have been marginally greater if the cadavers of the infected introduced beetles were not removed, as cadaver density can greatly influence infection rates and mortality (Long et al. 2000).

Short residual activity alone does not fully explain the lack of impact our fungal treatments had on beetle progeny. The studied fungi neither prevented the introduced beetles from reproducing, nor impacted the development and survival of those progeny. In our behavioral study, the presence of fungi did not affect beetle space usage or locomotion. It may then also be true that beetle reproductive behavior is unaffected by entomopathogens. While some insects can detect entomopathogenic fungi and modify their oviposition behavior, it appears these beetles do not (Rännbäck et al. 2015). Other studies examining the effect of B. bassiana on stored grain beetles have found a reduction in progeny production, but the degree of efficacy was conditional on application rate (Khashaveh et al. 2011). While that study was done with a different host plant, with different stored grain beetles, and a different strain of B. bassiana, it seems likely that higher application rates may be more efficacious here as well.

Mantzoukas et al. (2019) found that applications of B. bassiana and C. fumosorosea resulted in over 60% mortality at $1 \times 10^8$ conidia/mL, and mortality decreased at lower rates; however, this study sprayed fungi directly onto the beetles. Previous work has shown a decline fungal viability when applied to stored products (Lord 2005). Thus, while $1 \times 10^8$ conidia/mL might be applied to a stored product, beetles will likely encounter fewer viable conidia than
expected. Even higher amounts of conidia may be required for any degree of control. Of all the fungal treatments Botanigard was the most effective, but the reason is likely due to the product’s high concentration of *B. bassiana*, which applied 10,000-fold more conidia than the *Bb* isolate treatment. While applying *B. bassiana* at $1 \times 10^{11}$ conidia/mL provided some noticeable effects, it did not provide a significant reduction in damage. Moreover, applying at the label rate reduces the concentration to $1 \times 10^{11}$ conidia/mL, but the product itself contains an even higher concentration of conidia and could be applied at that higher concentration. Further experiments could explore if a higher rate might provide a greater degree of control.

Based on the competition assay and a few measures of the efficacy assay, *C. fumosorosea* and *B. bassiana* had an antagonistic relationship. Previous research has tried applying both fungi directly to *S. oryzae* and found their effects to be additive (Mantzoukas et al. 2019). However, that appears to not be the case here. These differences may be due to the strains of fungi studied; while we used *Bb* GHA and *Cfr* FE9901, Mantzoukas et al. (2019) used *Bb* GBBSTTS and *Cfr* RHZ4RAS. However, it is also possible that these differences are due to the medium and methods by which these experiments were run. Fungi applied directly to a beetle may interact differently than when it is applied to a stored product or PDA media.

We did see some synergism between the fungi and diatomaceous earth. As seen in prior studies, desiccants can increase the efficacy of fungal entomopathogens (Lord 2001, 2005, 2007). Diatomaceous earth increased infection rates of fungi in some instances. However, the combination of diatomaceous earth and fungi did not increase control of the beetles beyond that of diatomaceous earth alone. While some research has proposed that diatomaceous earth facilitates fungal attachments, other papers have suggested that synergism may be due to other mechanisms, like improvements to viability (Lord 2001; Akbar et al. 2004). Based on our trials,
it seems likely that diatomaceous earth increased attachment, as we would have expected greater residual activity in DE-treated groups if it improved viability. However, in terms of pest control and damage prevention, diatomaceous earth alone was the most reliable treatment.

Prior work has also shown that wet applications of fungi may be negligible when combined with diatomaceous earth (Dal Bello et al. 2006). However, the effect of dry applications of fungal pathogens can be additive with diatomaceous earth. We applied fungal products as aqueous suspensions to adhere to product labels as closely as possible and applied the isolates in a similar manner. A dry application may have been more effective alongside diatomaceous earth. In fact, there were some instances of antagonism, where the inclusion of the fungi lessened the impact of diatomaceous earth. It is unclear why, but it is possible that an inert ingredient in the products (e.g. sucrose) may have assisted beetle development and survival. While fungal entomopathogens show some promise as integrated pest management tool in stored rice, there are still a number of questions that need answering before recommendations can be made to growers.
Chapter 6. Olfactory cues influencing attraction of two grain beetles to stored rice

6.1. Introduction

Responding to semiochemicals is an important component of insect host-finding. The volatiles of a host plant may directly attract an insect, or they may act synergistically with pheromones to attract insects (Reddy and Guerrero 2004). However, in stored grain ecosystems, the role of semiochemicals is less clear. Stored grain pests are well-adapted to human-mediated systems. Evidence suggests that humans may have been storing grain as far back as 23,000 years ago, and archeological evidence shows stored grain being infested by insects as far back as 4,500 years ago (Linsley 1944; Solomon 1965; Weiss et al. 2004). Some of the most damaging stored grain pests, like lesser grain borers, *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae), and rice weevils, *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae), are primary pests, which attack undamaged grain, as opposed to secondary pests which only attack damaged grain. Both *R. dominica* and *S. oryzae* can complete their development within a single grain of rice (Koehler 1994; Edde 2012). Infesting grain bins containing thousands of kilograms of rice, these insects are surrounded with an effectively unlimited source of food. Moreover, dispersal to new sources of grain is often facilitated by human-mediated transport (Cordeiro et al. 2019). Thus, the selective pressure for these pests to find hosts may be relatively weak compared to other herbivorous insects.

Prior studies have failed to demonstrate clear trends in host finding behavior among stored grain pests. When looking at plant volatiles, Nguyen et al. (2008) found *R. dominica* showed no preference between wheat volatiles and clean air. The beetles also had no preference among wheat, brown rice, and maize. Conversely, Edde and Phillips (2006) showed *R. dominica*
responded to the volatiles of many different grains, including wheat. However, this study mechanically damaged the plant material, which may have altered the volatile profile. Similarly, Phillips et al. (1993) reported that *S. oryzae* was attracted to cues of some hosts but did not respond to the volatiles of other hosts. Other studies have shown *S. oryzae* prefers sorghum over other hosts, but this preference was dependent on the hosts the colonies were reared upon (Vijay and Bhuvaneswari 2018). The variability among these studies suggests that the relevance of host plant volatiles to stored grain pests may be a nuanced issue.

Both *S. oryzae* and *R. dominica* produce aggregation pheromones, which attract conspecifics of both sexes (Phillips et al. 1985; Edde 2012). It then stands to reason, that an infested host would be more attractive than an uninfested host, but research into this topic is scarce. Only a couple of studies have examined how infestation by conspecifics affects responses to host volatiles. Nguyen et al. (2008) found that *R. dominica* discerned between clean wheat and wheat infested by conspecifics, preferring wheat infested by conspecifics. Vijay and Bhuvaneswari (2018) found that *S. oryzae* preferred some host plants over others, and that this preference was unchanged by infestation status, but they did not directly compare infested and uninfested grain. It is also unclear how infestation from a competitor may affect host-finding. Finally, very little work has been done to examine the influence of host plant volatiles in rough rice systems, and no studies have attempted to differentiate among volatiles from clean grain, damaged grain, and grain infested with live beetles. Thus, we set forth the following objectives: (1) to examine how *S. oryzae* and *R. dominica* host selection behavior is affected by the semiochemicals produced by damaged rice, rice infested with conspecifics, and rice infested by another herbivore, as well as (2) to identify the semiochemicals which may be responsible for any observed behavioral changes.
6.2. Methods

6.2.1 Biological Materials

The USDA-ARS Manhattan, Kansas provided the initial colonies of *R. dominica* and *S. oryzae*. These colonies were then supplemented with field collected beetles from Louisiana in 2019–2020. Beetles were reared on wheat grains (12% MC) in 24–32 oz deli containers inside a growth chamber (27°C, continual darkness). Fresh wheat was provided every 2 months or as needed (Doherty, 2023). Beetles were starved for 24 hours before experiments.

Rough rice (Var: XP753) was acquired from the LSU Rice Research Station. XP753 was chosen for its availability and relative susceptibility to *S. oryzae* (Doherty et al. 2023). Rice was sieved and tempered to 12% moisture content (MC) according to the following equation (AACC 2009):

\[
\text{mL water} = \left(\frac{100 - \text{original moisture} \text{ (%)}}{100 - \text{desired moisture} \text{ (%)}} - 1\right) \times \text{sample mass}
\]

6.2.2. Olfactometer

Two-choice olfactometer tests were arranged to assess *S. oryzae* and *R. dominica* preferences as they relate to damage and the presence of other organisms. Preferences for six different odor sources were tested:

1. Undamaged rice
2. Rice infested with conspecifics
3. Rice infested with a different beetle species
4. Damaged rice only
5. Conspecific beetles only
6. Empty container

For the undamaged rice treatment, 200 g of rice were tempered to 12% MC. For
treatments that required infestation by *S. oryzae* or *R. dominica*, 100 of the specified beetles were added to 200 g of rice and allowed to feed for 4 weeks. Damaged rice was created by introducing 100 of the specified beetles to 200 g of rice, allowing them to feed for 4 weeks, and then sieving the sample (#12 mesh), making use of the collected frass and fragments. The conspecifics treatment consisted of 100 beetles of test species after 24-hour starvation. Beetle preferences for these treatments were examined in 5 different pairings:

1. Empty container - Empty container (negative control)
2. Undamaged rice - Empty container
3. Rice infested with conspecifics - Undamaged rice
4. Rice infested with conspecifics - Damaged rice
5. Rice infested with conspecifics - Conspecifics
6. Rice infested with conspecifics - Rice infested with a different beetle species

For *S. oryzae*, treatments pairings were arranged in a four-arm olfactometer with two arms closed off (Figure 1A; airflow: 1.1 L/min). Treatments were kept in glass jars, which could be affixed to the olfactometer and changed as needed. After setting up treatments, 50 *S. oryzae* were introduced in the center of the olfactometer. Multiple beetles were tested at a time to allow for more beetles to be tested, to allow more time for beetles to make decisions, and to observe their decision-making among conspecifics. After 24 hours, the number of *S. oryzae* choosing each treatment was recorded. Beetles were considered to have chosen a treatment if they were found at least 9 cm from the center towards a treatment. Each pairing was tested with *S. oryzae* 18 times, with each treatment tested 9 times on each side of the arena to avoid biases that may have been introduced by the arena. The number of beetles present in each treatment was analyzed using paired t-tests, after confirming approximate normality through Q-Q plots and homogeneity of variances through Levene’s test in JMP Pro 17.

A similar experiment was conducted using *R. dominica*; however, because *R. dominica*
have limited mobility on slick surfaces, a custom arena was created (Figure 1B). Petri dishes were modified into pitfall arenas by attaching 25 mL vials to holes drilled into the dish. Vials were arranged to mimic Y-tube designs; the dishes had designated zone for introducing *R. dominica* on one end of the arena, and treatment vials were affixed to the other end of the arena, equidistant from the starting zone. Filter paper lining was used to enable movement. The treatments were modified to fit into these vials. For treatments containing rice (i.e., uninfested rice, rice infested with *R. dominica*, rice infested with *S. oryzae*), 10 g portions of the treatment were used in trials. The damaged rice treatment was comprised of 15 mg portions of damaged rice, which exceeded the amount of damage present in the *R. dominica*-infested treatment in order to force a positive response to the treatment if damage were the primary attractive component. Similarly, the beetle only treatment was comprised of 50 *R. dominica*, which exceeded the number of beetles that would be present in the *R. dominica*-infested treatment. Similar to the *S. oryzae* test, 50 *R. dominica* were tested at a time, and given 24 hours to move into a treatment. Beetles were starved for 24 hours before introduction to the arena. The number of beetles present in each treatment were counted and then adjusted to account for beetles that were present as part of a treatment. The pairings tested were identical to those of the *S. oryzae* study, and each pairing was tested 18 times (9 times on each side of the arena). The corrected number of beetles present in each treatment was analyzed using paired t-tests in JMP 16.
6.2.3. Gas chromatography - Mass spectrometry

We identified the compounds present in treatments using gas chromatography-mass spectrometry (GCMS). HayeSep Q volatile organic compound traps (Volatile Assay Systems, Rensselaer, NY) were used to sample air from treatments. Olfactometer air pumps (1.0 L/min) and vacuums (0.9 L/min) were used to collect volatiles over a 24-hour period. From each treatment, 6-7 samples were collected, and empty container control samples were collected concurrently. Traps were then washed with 360 µL of dichloromethane (DCM), and 100 µL samples of the wash were placed in the GCMS. Additionally, 100 µL samples of pure DCM were placed in the GCMS as another control. The GCMS drew 1 µL of the provided samples for analysis with Chromeleon 7 (GC: Trace 1310 GC in splitless mode; MS: ISQ 7000; column: TG-5MS, 30 m × 0.25 mm × 0.25 µm; Thermo Scientific, Waltham, MA). Helium acted as the carrier gas (1.0 ml/min). Column temperature was programmed to increase from 40°C to 280°C.
at 8°C/min. Injection temperature was 280°C, MS source temperature was 280°C, and transfer line was 300°C. The MS was set to scan a mass range from 45 to 550 m/z.

Only a select few GCMS peaks were chosen for further analyses. Focal peaks had to have met all the following criteria:

1. Peaks appeared in treatments that beetles were attracted to or deterred from in the olfactometer analyses.
2. Peaks appeared in at least 3 samples of those treatments in an abundance >1% of the total sample area.
3. Peaks did not appear in the Empty Container or DCM control groups.

Focal peaks were identified using the National Institute of Standards and Technology (NIST) libraries and recorded as percentage of the total sample area. We used % area for statistical analyses, as internal standards were not added to samples before processing. Additionally, because peaks were identified through libraries without confirmations from standards, they are considered tentative identifications. Descriptive statistics were reported for each focal peak.

6.3. Results

6.3.1. Olfactometer

No differences were seen in the S. oryzae negative control, the Empty container - Empty container comparison ($t = -0.96$, $df = 9$, $P = 0.361$), indicating that there was no bias for a particular side of the arena. Sitophilus oryzae preferred the clean rice to the empty container ($t = -3.19$, $df = 17$, $P = 0.005$; Figure 2). The rice had 50% more S. oryzae present than the empty container. In every other comparison, S. oryzae showed no preference. No preference was shown
between *S. oryzae*-infested rice and clean rice \((t = 0.57, df = 17, P = 0.579)\), *S. oryzae*-infested rice and damaged rice \((t = -1.08, df = 17, P = 0.297)\), *S. oryzae*-infested rice and *S. oryzae* beetles \((t = 1.00, df = 17, P = 0.329)\), or between *S. oryzae*-infested rice and *R. dominica*-infested rice \((t = -1.48, df = 17, P = 0.156)\).

No differences were seen in the *R. dominica* Empty container + Empty container comparison \((t = -0.08, df = 6, P = 0.939)\). *Rhyzopertha dominica* preferred clean rice to an empty container \((t = -3.27, df = 17, P = 0.005\); Figure 3), as there were 75% fewer beetles present in the empty container. *Rhyzopertha dominica* showed no preference between *R. dominica*-infested rice and clean rice \((t = -0.20, df = 17, P = 0.845)\). They also showed no preference for *R. dominica*-infested rice or *S. oryzae*-infested rice \((t = -0.98, df = 17, P = 0.339)\). However, *R. dominica* did prefer *R. dominica*-infested rice to *R. dominica* beetles only, as there were over 330% more beetles present in the *R. dominica*-infested rice \((t = 3.99, df = 17, P < 0.001\); Figure 4).

*Rhyzopertha dominica* also preferred *R. dominica*-infested rice to only the damaged rice \((t = 3.00, df = 17, P = 0.008\); Figure 5). There were 40% more beetles among the *R. dominica*-infested rice than the damaged rice.
Figure 2. The mean number of introduced *S. oryzae* present by the clean rice or empty container (LS Means ± 2.96 [SE])

Figure 3. The mean number of introduced *R. dominica* present in the clean rice or empty container (LS Means ± 3.82 [SE])
Figure 4. The mean number of introduced *R. dominica* present in the rice infested with *R. dominica* or *R. dominica* only (LS Means ± 6.44 [SE])

Figure 5. The mean number of introduced *R. dominica* present in rice infested with *R. dominica* or damage from *R. dominica* infested rice (LS Means ± 2.50 [SE])
6.3.2. Gas chromatography - Mass spectrometry

Comparisons were made among the *R. dominica* infested rice (n=6), damaged rice only (n=6), and *R. dominica* beetles only (n=7) treatments. Only volatiles from these three treatments were compared as there were not preferences among the other treatments. One volatile, 2-pentenoic acid, 2,3-dimethyl-, was unique to the beetles only treatment (Table 1; Figure 6). One volatile, nerol, was unique to the damage only treatment (Table 1; Figure 7). Six different volatiles were unique to the infested rice treatment, with the most dominant being 4-ethylbenzoic acid within the samples that contained it (Table 1; Figure 8). However, dodecane and tetradecane were present more consistently, appearing in nearly all of the infested rice volatile samples (Table 1). The infested rice, beetles only, and damage only treatments also shared 20 volatiles (Table 2). Eicosanebioic acid, dimethyl ester appeared most frequently and among all three treatments. Palmitoyl chloride was the other most common volatile, and it also appeared in the greatest relative amounts (Table 2).
Table 1. Unique chemicals found in the Beetles Only, Damage Only, and Infested Rice treatments of the *R. dominica* experiments, along with their average percent area and standard error.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Tentative Identification</th>
<th>Retention Time (min)</th>
<th>ID #</th>
<th>N</th>
<th>Mean Area (%)</th>
<th>Std Err (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beetles Only</td>
<td>2-Pentenoic acid, 2,3-dimethyl-</td>
<td>16.83</td>
<td>B1</td>
<td>3</td>
<td>19.55</td>
<td>13.75</td>
</tr>
<tr>
<td>Damage Only</td>
<td>Neral</td>
<td>17.09</td>
<td>D1</td>
<td>4</td>
<td>1.17</td>
<td>0.50</td>
</tr>
<tr>
<td>Infested Rice</td>
<td>Decane</td>
<td>11.63</td>
<td>R1</td>
<td>4</td>
<td>4.98</td>
<td>0.69</td>
</tr>
<tr>
<td>Infested Rice</td>
<td>Octanal</td>
<td>11.77</td>
<td>R2</td>
<td>4</td>
<td>2.53</td>
<td>1.39</td>
</tr>
<tr>
<td>Infested Rice</td>
<td>Formic acid, octyl ester</td>
<td>14.37</td>
<td>R3</td>
<td>4</td>
<td>11.40</td>
<td>2.52</td>
</tr>
<tr>
<td>Infested Rice</td>
<td>Dodecane</td>
<td>15.70</td>
<td>R4</td>
<td>6</td>
<td>10.10</td>
<td>1.75</td>
</tr>
<tr>
<td>Infested Rice</td>
<td>4-Ethylbenzoic acid</td>
<td>18.53</td>
<td>R5</td>
<td>3</td>
<td>12.55</td>
<td>1.33</td>
</tr>
<tr>
<td>Infested Rice</td>
<td>Tetradecane</td>
<td>19.17</td>
<td>R6</td>
<td>6</td>
<td>11.67</td>
<td>1.32</td>
</tr>
</tbody>
</table>
Table 2. Chemicals found in more than one of the LGB Only, Damage Only, and Infested Rice treatments, along with their average percent area and standard error.

<table>
<thead>
<tr>
<th>Tentative Identification</th>
<th>Retention Time (min)</th>
<th>ID #</th>
<th>N</th>
<th>Beetles Only (% area)</th>
<th>Damage Only (% area)</th>
<th>Infested Rice (% area)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-Cyclohexen-1-one</td>
<td>10.23</td>
<td>1</td>
<td>7</td>
<td>4.65 ± 1.06</td>
<td>8.95 ± 4.48</td>
<td></td>
</tr>
<tr>
<td>1,8-Nonadien-3-ol</td>
<td>12.32</td>
<td>2</td>
<td>4</td>
<td>4.38 ± 0.70</td>
<td>0.42 ± 0.00</td>
<td></td>
</tr>
<tr>
<td>1-Octanol</td>
<td>13.19</td>
<td>3</td>
<td>4</td>
<td>15.71 ± 0.00</td>
<td>28.26 ± 11.43</td>
<td></td>
</tr>
<tr>
<td>Nonanial</td>
<td>13.90</td>
<td>4</td>
<td>7</td>
<td>5.55 ± 1.48</td>
<td>6.51 ± 0.25</td>
<td></td>
</tr>
<tr>
<td>Benzaldehyde, 4-ethyl-</td>
<td>15.17</td>
<td>5</td>
<td>5</td>
<td>6.51 ± 0.00</td>
<td>3.39 ± 0.00</td>
<td>4.05 ± 1.36</td>
</tr>
<tr>
<td>Phenol, 4-(2-propenyl)-</td>
<td>15.17</td>
<td>6</td>
<td>4</td>
<td>4.30 ± 0.00</td>
<td>11.87 ± 7.16</td>
<td></td>
</tr>
<tr>
<td>1H-Indene, 1-methylene-</td>
<td>15.63</td>
<td>7</td>
<td>4</td>
<td>3.64 ± 0.00</td>
<td>3.40 ± 1.29</td>
<td></td>
</tr>
<tr>
<td>Benzaldehyde, 2-hydroxy-6-methyl-</td>
<td>15.82</td>
<td>8</td>
<td>7</td>
<td>14.89 ± 5.94</td>
<td>2.88 ± 0.00</td>
<td></td>
</tr>
<tr>
<td>2-Hexenoic acid, 5-hydroxy-3,4,4-trimethyl-,(E)-</td>
<td>16.82</td>
<td>9</td>
<td>5</td>
<td>8.67 ± 1.95</td>
<td>9.24 ± 0.68</td>
<td></td>
</tr>
<tr>
<td>m-Ethylacetophenone</td>
<td>17.03</td>
<td>10</td>
<td>8</td>
<td>6.77 ± 0.38</td>
<td>6.18 ± 0.91</td>
<td>28.33 ± 5.78</td>
</tr>
<tr>
<td>Hexadecane</td>
<td>22.24</td>
<td>11</td>
<td>7</td>
<td>3.56 ± 0.81</td>
<td>8.41 ± 1.24</td>
<td></td>
</tr>
<tr>
<td>Cyclooctasiloxane, hexadecamethyl-</td>
<td>23.13</td>
<td>12</td>
<td>3</td>
<td>3.91 ± 1.53</td>
<td>6.16 ± 0.00</td>
<td></td>
</tr>
<tr>
<td>2-Cyclohexene-1-carboxylic acid, 2-(7-hydroxy-3-methyl-1,3-octad</td>
<td>26.89</td>
<td>13</td>
<td>4</td>
<td>7.63 ± 3.09</td>
<td>3.07 ± 0.00</td>
<td></td>
</tr>
<tr>
<td>5-Octadecenal</td>
<td>29.67</td>
<td>14</td>
<td>8</td>
<td>11.70 ± 2.34</td>
<td>6.23 ± 1.59</td>
<td></td>
</tr>
<tr>
<td>Octadecane, 3-ethyl-5-(2-ethylbutyl)-1H-2,8a-Methanocyclopenta[a]cyclopropa[e]cyclodecen-11-one, 1a,2</td>
<td>29.74</td>
<td>15</td>
<td>4</td>
<td>0.32 ± 0.05</td>
<td>0.33 ± 0.06</td>
<td></td>
</tr>
<tr>
<td>Eicosanebioic acid, dimethyl ester</td>
<td>30.16</td>
<td>16</td>
<td>5</td>
<td>2.49 ± 1.62</td>
<td>3.01 ± 0.11</td>
<td></td>
</tr>
<tr>
<td>Tricosanoic acid, 2-methoxy-, methyl ester</td>
<td>31.69</td>
<td>17</td>
<td>13</td>
<td>38.91 ± 12.85</td>
<td>36.24 ± 5.25</td>
<td>9.83 ± 4.26</td>
</tr>
<tr>
<td>Palmitoyl chloride</td>
<td>33.56</td>
<td>19</td>
<td>10</td>
<td>48.41 ± 5.78</td>
<td>41.33 ± 8.97</td>
<td>30.76 ± 15.05</td>
</tr>
<tr>
<td>5,8-(4-Phenyl-3,5-dioxo[1,2,4]triazolidin-1,2-diyl)pregn-6-ene-2</td>
<td>35.16</td>
<td>20</td>
<td>3</td>
<td>12.07 ± 5.09</td>
<td>11.69 ± 0.00</td>
<td></td>
</tr>
</tbody>
</table>
Figure 6. Chromatogram from a sample of the *R. dominica* beetles only treatment. Peak IDs correspond to IDs in Tables 1 and 2.
Figure 7. Chromatogram from a sample of the *R. dominica* damage only treatment. Peak IDs correspond to IDs in Tables 1 and 2.
Figure 8. Chromatogram from a sample of the *R. dominica* infested rice treatment. Peak IDs correspond to IDs in Tables 1 and 2.
6.4. Discussion

This study is the first to compare both *R. dominica* and *S. oryzae* host finding preferences among rice of varying infestation status. While *R. dominica* distinguished between some treatments, *S. oryzae* only distinguished between clean rice and the empty container. The ability of *S. oryzae* to distinguish rice from the empty container stands in contrast to Phillips et al. (1993), which found that *S. oryzae* did not respond to rice volatiles. There were no differences in *S. oryzae* response among the other comparisons, suggesting that while the beetles preferred a host to nothing, they did not respond differently to a clean host, infested host, or conspecifics. Vijay and Bhuvaneswari (2018) showed that *S. oryzae* preferences may change depending on the grain they were reared within. Because our colony insects were reared on wheat, it is possible that our subjects were not primed to respond to rice volatiles as a desirable host, and instead treated it similarly to every other treatment. Their relative lack of response to the semiochemicals in the tested treatments may be indicative of *S. oryzae* possessing weaker host-finding abilities than *R. dominica*. Further research is needed to clarify why such a difference exists, but this may be due to evolutionary selective pressures. Wild populations of *R. dominica* can feed on seeds outside of stored grain systems, often inhabit forests, and can travel between forests and stored grain systems (Jia et al. 2008b; Mahroof et al. 2010b; Edde 2012). On the other hand, Togola et al. (2014) surveyed over 50 field sites for *S. oryzae* and found that they were almost completely absent from wild vegetation. *Sitophilus oryzae* predominantly live within human-mediated stored grain systems, thus, host finding may not be as relevant.

The preference for clean rice over the empty container by *R. dominica* reported herein falls in line with previous research, which found *R. dominica* responded to a myriad of host plants, regardless of species (Edde and Phillips 2006). We also found that *R. dominica* did not
distinguish between clean rice, rice infested with conspecifics, or rice infested by another beetle. When presented with whole grains, infestation type did not matter. Prior studies demonstrated that *R. dominica* pheromones can work synergistically with host plants against some stored grain pests, including *R. dominica* (Bashir et al. 2001; Athanassiou et al. 2006). Thus, we had expected beetles to prefer infested hosts to clean host. It is unclear why this turned out to not be the case, but it may be due to our methodology. Many volatile studies observe the behavior of one insect at a time; here, we studied beetles in groups of 50. An attraction to the volatiles of conspecifics is likely mitigated or more difficult to observe when test subjects are already surrounded by conspecifics. Another possibility may have to do with the production of pheromones. The male-produced *R. dominica* aggregation pheromones are comprised of two esters, (S)-(+)1-methylbutyl (E)-2-methyl-2-pentenoate (Dominicalure-1) and (S)-(+)1-methylbutyl (E)-2,4-dimethyl-2-pentenoate (Dominicalure-2) (Williams et al. 1981). While the NIST libraries in our GCMS analyses identified one chemical with similar components to the aggregation pheromones in the *R. dominica* beetles only treatment, 2-pentenoic acid, 2,3-dimethyl-, no pheromones were identified in any treatment. The expression of these pheromones can vary depending on the presence of other beetles and the quality of hosts (Bashir 2000; Bashir et al. 2001). *Rhyzopertha dominica* aggregation pheromones were not detected in our treatments, which may have made the rice infested with conspecifics and the beetle only treatments less attractive than expected. Pheromone production is negatively correlated with population density (Mayhew and Phillips 1994). It is possible that pheromones were not detected due to the density of beetles in the treatments.

There were several chemicals identified through the GCMS analyses that appeared in the infested rice treatment, but not the beetles only treatment nor the damage only treatment. None
of them resemble *R. dominica* aggregation pheromones, but these were presumably the
semiochemicals which attracted the beetles to infested rice treatment over the other treatments.
Many of these volatiles are similar, but not the same as the rice volatile profiles analyzed by
Bryant and McClung (2011). Collectively, these results add to a growing body of research
examining host finding among grain pests. Papers have frequently reported conflicting evidence,
and clear trends in host finding behavior of *R. dominica* and *S. oryzae* remain elusive. Due to the
variation seen in the research, future studies might consider a more holistic approach. Studies
conducted using grain bins and examining factors that facilitate colonization, rather than host
finding, may add some context to the existing body of literature.
Chapter 7. Summary and Conclusions

Rice is a staple food, with over 50% of the world population consuming rice daily, and the US is among its largest exporters (Zeigler 2010; USDA 2023). Insects are the leading cause of postharvest grain loss and are responsible for 5-30% decreases in value worldwide (Deshpande & Singh, 2001; USDA, 2005; Santos, 2006; Yigezu et al., 2010; Jiang, 2013; Sharon et al., 2014; Zhang et al., 2021). Improvements to the sustainability of Louisiana rice production and management will not only benefit local growers and communities, but also make an impact worldwide. Despite their deceptive simplicity, stored rice systems are like any other cropping system, with are a range of potential tools available for integrated control. However, only a few of these tools have made it into the repertoires of growers. Currently over-reliance on phosphine fumigation has led to concerns about resistance and there is a need for diversified management strategies stored rice pests. This research investigates alternative management strategies including varietal resistance, grain protectants, and bio-pesticides as well as examining host finding behavior of both R. dominica and S. oryzae.

Chapters 2 and 3 explore the effects of host plant resistance against both S. oryzae and R. dominica. Rice varieties were differentially susceptible to both pests. Moreover, a variety susceptible to one pest was not necessarily susceptible to the other. While Clearfield varieties were susceptible to R. dominica, their susceptibility to S. oryzae differed by variety. XP753 was susceptible to S. oryzae, but resistant to R. dominica. There were a few similarities in that Mermentau was resistant and Frontière was susceptible to both species. By examining mixes of these varieties, we found that some mixes resulted in associational susceptibility while others resulted in associational resistance. The susceptibility of a mix also varied by beetle species. While mixes infested by S. oryzae typically resulted in associational susceptibility, mixes
infested by *R. dominica* typically resulted in associational susceptibility. In knowing these interactions, growers may be able to better anticipate pest pressure in a bin containing multiple varieties.

We also explored the characteristics of rice grain which confer resistance to *R. dominica*, and contrary to our expectations, nutritional components were not correlated with susceptibility. Physical characteristics were more important in predicting susceptibility. Specifically, the shape of rice grain allows for the anticipation of adult damage, progeny counts, larval damage, and progeny mass. Even if a variety of rice has not had its susceptibility evaluated, a grow can better anticipate pest pressures by observing its shape. Longer grains may incur more damage from adults, while thicker grains may incur more damage by larvae. Overall, growers ought to remain aware of the susceptibilities and resistances of their varieties. This will allow them to better anticipate which species may infest their grain, the degree of infestation, and which developmental stage of the insect will likely be the most damaging. In turn, that information allows growers to make more informed decisions about the quality and quantity of the other control methods they may wish to implement.

Growers have access to a variety of commercial insecticides for use in stored grain. However, as we demonstrate in Chapter 4, there is nuance to choosing the most appropriate insecticide. Deltamethrin has some acute toxicity, but it is best used in tandem with methoprene. The combination of deltamethrin and methoprene provide more consistent control of stored rice pests than either insecticide alone; however, over a long enough period of time, methoprene provides a comparable level of control as the combination. Thus, over a long storage period, it may be more cost-effective apply only methoprene. If the length of time the grain will be stored is unclear, the combination of deltamethrin and methoprene may be more reliable.
Biological controls, including biopesticides, have been underutilized in stored rice pest management programs. That is not without reason, as there are no commercially-available biopesticides registered for stored rice. However, as we demonstrate in Chapter 5, these products are not without promise. Both *Cordyceps fumosorosea* FE9901 and *Beauveria bassiana* GHA were capable of infecting both *Sitophilus oryzae* and *Rhyzopertha dominica*. Longevity of these fungi was relatively brief, so before recommendations can be made to growers, further work may be needed to optimize applications. Further research may also clarify the recommended rates of these fungi, though it appears a higher rate will provide greater control. Additional experiments were done to observe how these entomopathogenic fungi integrated with diatomaceous earth and each other. There were a variety of interactions between controls, including synergistic, antagonistic, and additive interactions. Further research is needed to fully understand how these interactions occurred, but with that understanding, will come the knowledge of how to maximize the efficacy of each control. At this time, a combination of *B. bassiana* and diatomaceous earth appears to be the most efficacious of the tested controls. *Beauveria bassiana* had higher efficacy than *C. fumosorosea*, and synergized with diatomaceous earth in some measures. However, these biopesticides do not approach the levels of control provided by methoprene or methoprene with deltamethrin. Biopesticides should be considered supplementary to traditional insecticides here, and not as replacements.

Finally, Chapter 6 investigated the host finding abilities and volatile preferences of *S. oryzae* and *R. dominica*. We found that *Rhyzopertha dominica* were able to distinguish infested rice from only damaged rice and only beetles. Though they did not have a preference between infested rice and clean rice. Thus, it appears as if the beetles were attracted by rice, regardless of its status. On the other hand, *S. oryzae* did not discern between most of the tested treatments. The
only distinction they made was their preference for clean rice over an empty container, as did *R. dominica*. While the applications of this information are more tangential than those of the other chapters, it does reinforce the importance of a common cultural control—cleaning a grain bin after it has been emptied. If there are no hosts, damage, or beetles in the grain bin, it is less likely to attract potential pests.

Collectively, these studies represent steps towards improving stored grain integrated pest management on several fronts. We have provided evaluations of varietal resistance, identified mechanisms of resistance, made the first identifications of associational resistance in the system, evaluated and compared commercial insecticides, assessed efficacy of potential biological control agents, identified numerous factors influencing stored grain pest behavior, and improved our understanding of how these systems integrate. These and future studies into stored rice integrated pest management are imperative to developing pest management recommendations for growers, thereby improving their financial prospects, the environmental impact of post-harvest agriculture, and the distribution of food to a growing population.
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Vita

Ethan grew up in Chapel Hill, North Carolina. He received his B.A. in Biology from the College of Wooster in 2013, with a minor in psychology. His undergraduate thesis explored the effects intraguild predation on the biological control of invasive soybean aphids. In 2013, he joined the Duke Lemur Center as a researcher exploring scent-marking and foraging behavior of endangered lemurs. He later joined the University of Florida’s Department of Entomology and Nematology in 2016 as a master’s student. His thesis explored the effects of turfgrass cultivar diversity upon development and behavior of fall armyworm. In 2018, he joined the Indian River Research and Education Center to research entomopathogenic fungi as endophytes of citrus rootstock. In 2019, he moved to Louisiana State University as a PhD student studying integrated pest management and behavior of stored grain beetles, with a minor in experimental statistics. After finishing his PhD in 2023, he intends to continue studying integrated pest management and behavior of agricultural insect pests.