Journal of Stored Products Research 90 (2021) 101758

Contents lists available at ScienceDirect

Journal of Stored Products Research

journal homepage: www.elsevier.com/locate/jspr

Impacts of Storicide II on internal feeders of Brown rice

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ARTICLE INFO

Article history:
Received 29 September 2020
Received in revised form
13 December 2020
Accepted 14 December 2020
Available online 26 December 2020

Keywords:
Chlopyrifos-methyl
Deltamethrin
Rhyzopertha dominica
Sitophilus oryzae
Sitotroga cerealella

ABSTRACT

Protectants applied to grain can reduce damage caused by insect feeding during storage. Although these protectants are effective against many external feeders, they may also reduce damage caused by internal feeders as they often interact with the exterior surface of the grain during their larval or adult stages to feed or oviposit. For this study, we investigated impacts of Storicide® II applied to brown rice on three different internal feeders: *Rhyzopertha dominica* (Fauvel), *Sitophilus oryzae* (L.), and *Sitotroga cerealella* (Olivier). We also investigated the effects of this protectant at three different temperatures and when it was combined with different percentages of untreated brown rice. Time-series clustering was also performed to determine whether treatments caused disruptions to the timing of progeny emergence. Overall, *R. dominica* was the most susceptible as mortality and knockdown were observed in mixtures containing 10% treated brown rice. In contrast, *S. cerealella* was the least susceptible as mixtures containing at least 50–75% treated brown rice were required to reduce progeny production. However, lowering the temperature to 22 °C did reduce the amount of treated brown rice required to reduce progeny emergence and also reduced the number of progeny that emerged synchronously, which would likely reduce mating and reduce population levels over time. Similar effects on progeny were observed for *S. oryzae* and *R. dominica*. Overall, these findings suggest that Storicide II can reduce population levels of internal feeders and that combining this protectant with cooler temperatures can provide additional protection.

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1. Introduction

Grain protectants are insecticides that are applied to raw grains as they are loaded into storage facilities (Arthur, 1996). They are expected to give residual control of stored product insects for extended periods of time of several months to one year; however, several biotic and abiotic factors can influence the efficacies of these grain protectants in integrated pest management (IPM) programs. For example, stored product insects can be broadly grouped into two major categories: (1) internal feeders that complete most of their life cycle inside the kernel, or (2) external feeders that spend their entire life cycle outside the kernel (Mason and McDonough, 2012). External feeders are vulnerable to the protectant residues throughout their entire life cycle. In contrast, internal feeders will contact these protectants in the adult stage after they complete development inside the kernels. Examples of internal feeders include members of the genus *Sitophilus*, which typically insert an egg directly into a grain kernel, allowing the larva to hatch and develop completely within the kernel. Adults eventually emerge from the kernels after pupation, where they will eventually contact the grain protectants applied to the exterior surface of the grain. In contrast, *Rhyzopertha dominica* (Fauvel) (Coleoptera: Bostrichidae), the lesser grain borer, and the Angoumois grain moth, *Sitotroga cerealella* (Oliver) (Lepidoptera: Gelechiidae), both lay eggs outside of grain kernel and a neonate larva will bore into the kernel where it completes development to the adult stage (Mason and McDonough, 2012). Thus, the neonate larvae will come into direct contact the grain protectants in these species as they bore into the kernels.

Movement and mixing of grains often occur after they are harvested from the field and loaded into storage, and thus, grains that have been treated with a protectant insecticide can be mixed with untreated grains. To simulate the effects of mixing treated and untreated grains on insecticide efficacy, previous studies in which

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[1] Retired 31 July 2020

https://doi.org/10.1016/j.jspr.2020.101758
0022-474X/Published by Elsevier Ltd.

Storicide® II and other grain protectants were applied to grains and mixed with different percentages of untreated grains and bioassays were conducted with different insect species to study their efficacies (Arthur, 1992a; Daglish and Nayak, 2010; Kavallieratos et al., 2012). The percentage of treated grain required for complete mortality varied with insect species, which in some cases can differ from the percentage of treated grain required to eliminate or reduce progeny production (Athanassiou et al., 2008; Vassilakos and Athanassiou, 2012).

Temperature and humidity during storage can also influence the efficacy of grain protectants. In the south-central US, rice is usually harvested and loaded into storage in early August in eastern Texas and western Louisiana, and in mid-late September in eastern Arkansas and southeastern Missouri (Arthur et al., 2011; Arthur and Siebenmorgen, 2005). Thus, temperature at the time of storage and grain protectant application varies, which may affect insecticidal efficacy. Historically, organophosphates were reported to be more effective at higher temperatures, but degradation also increases at temperatures higher than 37 °C (Arthur et al., 1992). Pyrethroids are more stable and generally have a negative temperature coefficient, in contrast to organophosphates (Sehgal et al., 2014). The half-life of deltamethrin applied to wheat is 35 weeks when stored at 35 °C and 15% moisture (Noble et al., 1982). In addition, the susceptibility of rice to insect feeding damage changes as it is processed. The hulls of rough rice offer some degree of protection against stored product insects, and *R. dominica* and *S. cereallella* larvae may need a crack or split in the pod to facilitate entry (Chanbang et al., 2008a, 2008b; Cogburn et al., 1983; Kavallieratos et al., 2015). When rough rice is milled to remove the husk, the resulting brown rice becomes extremely susceptible to damage by *R. dominica*, *S. oryzae*, and *S. cerealella*, compared to rough rice (Arthur, 2016). Milled rice is also more susceptible to stored product insect damage compared to rough rice (Hasaranga et al., 2018).

One grain protectant that is registered in the United States (US) is Storicide II®, which is a combination of 3 parts per million (ppm) of the organophosphate chlorpyrifos-methyl and 0.5 ppm of the pyrethroid deltamethrin (Arthur and Subramanyam, 2012). Several recent studies have shown that this insecticide formulation is effective against both *R. dominica* and *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae), the rice weevil, or both when it was applied to wheat, rough rice, or brown rice (Arthur, 1992a; Bajracharya et al., 2013; Daglish and Nayak, 2010; Sehgal et al., 2013). There are few new studies with Storicide II on *S. cerealella* as the test species, but studies with the older commercial product (Reldan®) containing 6 ppm of chlorpyrifos-methyl, which was replaced by Storicide II (Arthur and Subramanyam, 2012), did indicate effectiveness against this species when applied at the label rate (LaHue, 1975, 1976, 1977). However, other studies have shown that the efficacies of insecticides applied below label rates quickly taper off for this species (Arthur, 2019b).

Although grain protectants can reduce or prevent damage due to insect feeding during storage, there are few recent studies involving efficacy of Storicide II on brown rice at different temperatures, or with *S. cerealella* as the test species. Additional data regarding efficacy at different percentages of treated and untreated brown rice would be helpful for pest management programs involving bulk and bagged brown rice. Finally, in previous tests in which progeny production was assessed after adults were exposed to Storicide II, usually only one count was made for progeny. Thus, no data concerning how insecticide treatment affected the rate or onset of progeny production over time have been previously collected. The objectives of this study were to determine (1) the efficacy of Storicide II as a protectant of brown rice at different temperatures and mixture levels required for mortality of parental adult *R. dominica* and *S. oryzae*, (2) the mixture levels required for reduction of progeny production in *R. dominica*, *S. oryzae*, and *S. cerealella*, and (3) the impacts of Storicide II on the rate of progeny production over time for all three species.

2. Materials and methods

2.1. General information

This study was conducted at the USDA-ARS-Center for Grain and Animal Health Research (CGHAR), Manhattan, KS, US. The insects used in this study were obtained from pesticide-susceptible colonies that had been reared in the lab for more than 35 years. The *R. dominica* and *S. oryzae* colonies were originally reared on wheat but separate colonies have been maintained on brown rice for about five years. Similarly, the *S. cerealella* colony was originally reared on whole-kernel corn but a separate colony was established on brown rice and has been maintained on this commodity for about five years. All species were reared inside Percival incubators (Percival, Perry, IA, US) maintained at 27 °C and 60% relative humidity (RH) in complete darkness.

2.2. Treatment procedures

Storicide II was obtained from Bayer Corporation (Research Triangle Park, NC, US). The formulation contained 216 mg/mL active ingredient (AI)/mL of chlorpyrifos-methyl and 37.2 mg AI/mL of deltamethrin and label specifications indicated an application rate of 275 mL of formulation in 18.9L to treat 20,454 kg of brown rice. This same application rate was used for brown rice in this study. In our case, the total amount of treated brown rice per experimental block was 3 kg; hence, the amount of formulated insecticide needed per 3 kg of brown rice was 2.7 mL, which was formulated in a 25-mL volumetric flask. Three kg of brown rice were laid out on a piece of Kraft paper inside a 0.60 by 0.45 m cardboard box covered with plastic. The brown rice was sprayed using a Badger 100 artists airbrush (Badger Corporation, Franklin Park, IL, US) to mist the insecticide onto the brown rice. The brown rice was treated by spraying a partial amount of the required 2.7 mL onto the brown rice, then mixing the brown rice, and repeating the procedure several times until the entire amount of insecticide was dispensed. The brown rice was allowed to dry overnight, and the following day, the treated brown rice was mixed with the following amounts of untreated brown rice in a 60 mL vial: 95, 90, 75, 50, 25, or 0 g, which created mixtures containing the following percentages of treated brown rice: 5, 10, 25, 50, 75 and 100%, respectively. Controls consisting of 100% untreated brown rice were also included in each experimental block. Bioassays were performed at three different storage temperatures (22, 27, and 32 °C) maintained in separate Percival incubators at 65% RH. Each experimental block consisted of three species, three temperatures, and seven percentages of treated brown rice. The entire experimental block was replicated a total of five times, with insecticide formulated separately for each replicate separately on five different dates. Thus, the treatment design was a randomized complete block for each replicate.

2.3. Holding procedures

Ten 1-2-week old adult *R. dominica*, ten 1-2-week old adult *S. oryzae*, and ten newly-emerged adult *S. cerealella* were placed individually into separate 60 mL vials containing 100 g of one of the seven percentages of treated brown rice. Vials were held at one of the three temperatures listed above for one week. After one week, vials containing *R. dominica* and *S. oryzae* were removed from the incubator, the brown rice was sieved to remove the adults, and mortality was assessed. In addition, the number of beetles that

were still active or "running" were recorded as well as the number of beetles that were knocked down (on their backs or unable to sustain motor movement), but not dead, after one week. Insects were recorded as dead if no movement was observed after they were touched with a pipette tip and they were recorded as knocked down if movement was observed after a gentle touch stimulus. The adults were removed, the vials were returned to the incubator, and progeny production was recorded beginning several weeks after initial emergence. For progeny counts, live adults were removed and recorded until no more progeny were produced, as determined by multiple dates with repeated zeros for progeny counts. Mortality counts were not feasible for *S. cerealella* adults because they generally die within a week in control treatments and removing them may have also removed any eggs the adults had laid, so they were allowed to die inside the vials, and only progeny production was recorded. After progeny production was complete, the feeding damage in each vial was recorded by assessing a random sample of 100 kernels for emergence holes and under a stereo microscope. After the rice was sifted to remove adult progeny, the frass was weighed on an analytical balance (Mettler Toledo, Columbus, OH). Low frass weights were observed for *S. cerealella*, so frass weights were not measured for this species.

2.4. Statistical analysis

2.4.1. Univariate statistical analysis

Univariate data were analyzed using Proc Glimmix in SAS (version 9.4, SAS Institute, Cary, NC, US) with percent treated grain, temperature, and species as the explanatory variables and either percent mortality, percent knocked down, percent "running", percent damaged grain, total number of progeny produced, and frass weight as the dependent variables. Gaussian distributions were used for damage counts, frass weights, and percent running, and a negative binomial distribution with log-link function was used for progeny production. Type III effects of the two and three-way interactions among all the explanatory variables were also assessed and replicates were treated as random effects in the model. LS-means were calculated for the interaction effect with Tukey adjustment at a significance level of $\alpha = 0.05$.

2.4.2. Principal components analysis

Principal components analysis (PCA) was performed using the 'factoextra' package in R (version 3.5.3). Means for damage count, proportion of affected adults after 7 days, which included the number of dead and knocked down individuals summed together, the total number of progeny produced, kernel damage count, and frass weight were used to calculate loading scores using the 'prcomp' function. This analysis was performed only for *R. dominica* and *S. oryzae* since the proportions of affected adults and frass weights were not assessed for *S. cerealella*.

2.4.3. Progeny emergence over time

Progeny production over time was compared for curve fit using 'dtwclust' package in R (version 3.5.3) for time series clustering. For each temperature and replicate block, the first date that progeny were detected for any of the three species was designated as day 1 of emergence. Progeny were regularly counted every 2.8 ± 0.18 days at 22 °C, 2.4 ± 0.14 days at 27 °C, and 2.1 ± 0.13 days at 32 °C and emergence over time was calculated by taking the differences between the number of progeny counted on neighboring dates. Comparisons of progeny emergence curves over time were computed for each species individually as well as all species together. Hierarchical clustering using Ward's method in R (version 3.5.3) was employed (seed = 390) for the time series comparisons and clustering indices were calculated. The Silhouette score was used to determine the optimal number of clusters for each dataset. For all species analyzed together, 23 clusters were used while six, five, and nine clusters were optimal for *S. cerealella*, *S. oryzae*, and *R. dominica* time series data, respectively.

2.4.4. Risk thresholds with mortality and damage

To calculate lethal dose of 50% or 90% of the adults (LD_{50}, LD_{90}), we used Proc Probit analysis in SAS (version 9.4) accounting for ten adults in each original vial and the total number of dead adults after seven days. These analyses were only performed for *R. dominica* and *S. oryzae* since dead *S. cerealella* adults were not assessed. The probabilities for the LD_{50} and LD_{90} were calculated based on the percentages of treated brown rice mixed with untreated brown rice for *S. oryzae* and *R. dominica* mortality data combined for all temperatures, *R. dominica* mortality alone for all temperatures, *S. oryzae* alone for all temperatures, and then for each species and temperature combination individually. These nine individual calculations were performed to provide a comparison of variation in estimates when using specific species and temperatures or when all data is used as an estimate. We also calculated damage thresholds for LD_{50} and LD_{90} based on the counts of damaged kernels. We used 100 kernels as our total value since we evaluated damaged kernels based on a subset of 100. We performed the 9 separate analyses as described above for LD_{50} and LD_{90}.

3. Results

3.1. Impact on mortality and knockdown

The percentage of brown rice treated with Storicide II had a significant impact on mortality of both adult *R. dominica* and *S. oryzae* and the interaction of Storicide II concentration with species on mortality was also significant (Table 1). No differences were observed in mortality across the three temperature treatments and no other interactions were significant. For *R. dominica* adults, adding as little as 10% treated brown rice significantly increased mortality compared to the control and mortality was also higher compared to the control when 25, 50, 75, or 100% treated brown rice was used (Fig. 1a). The highest mortalities were observed in the mixtures that contained 75 and 100% treated brown rice. In contrast to *R. dominica*, significant adult mortality was observed in *S. oryzae* only in mixtures that contained ≥25% treated brown rice relative to the control (Fig. 1b). Overall, *R. dominica* responded more strongly to lower concentrations of Storicide II compared to *S. oryzae* as higher adult mortality of *R. dominica* was observed in mixtures containing 10% treated brown rice compared to *S. oryzae* (Fig. 1a–b). This finding suggests that *R. dominica* adults are likely more susceptible to lower concentrations of this grain protectant than adult *S. oryzae*. No other major differences in mortality were observed between the two species when exposed to mixtures containing the same percent of Storicide II treated brown rice.

In addition, knockdown effects in the insecticide treatments were also observed for *R. dominica* adults that were not directly killed (Table 2 & Fig. 2a). Species, Storicide II treatment, and temperature all had significant effects on the number of insects that were observed in knockdown and the interaction between species and Storicide II treatment was also significant (Table 2). Adding as little as 5% treated grain increased the proportion of adult *R. dominica* that were knocked down compared to the control (Fig. 2a). This finding suggests that even though adult mortality did not differ between the control and treatments containing 5% treated grain (Fig. 1a), the abundance of Storicide II-treated kernels in the 5% treatment was sufficient to cause negative impacts to *R. dominica*. The knockdown effects in *R. dominica* were

Table 1
Type III effects for proportion of adult *Rhyzopertha dominica* and *Sitophilus oryzae* that were dead after exposure to Storicide II for 1 week. The effects of species, temperature, and percent treated grain on the proportion of adults that were dead were determined one week after exposure to Storicide II. DF = degrees of freedom.

Effect	Numerator DF	Denominator DF	F-value	p-value
Species	1	163	1.04	0.3089
Percent treated grain	6	163	202.89	<0.0001
Temperature	2	163	1.87	0.1572
Species x percent treated grain	6	163	4.87	0.0001
Species x temperature	2	163	1.34	0.2655
Percent treated grain x temperature	12	163	0.69	0.7581
Species x percent treated grain x temperature	12	163	0.72	0.7294

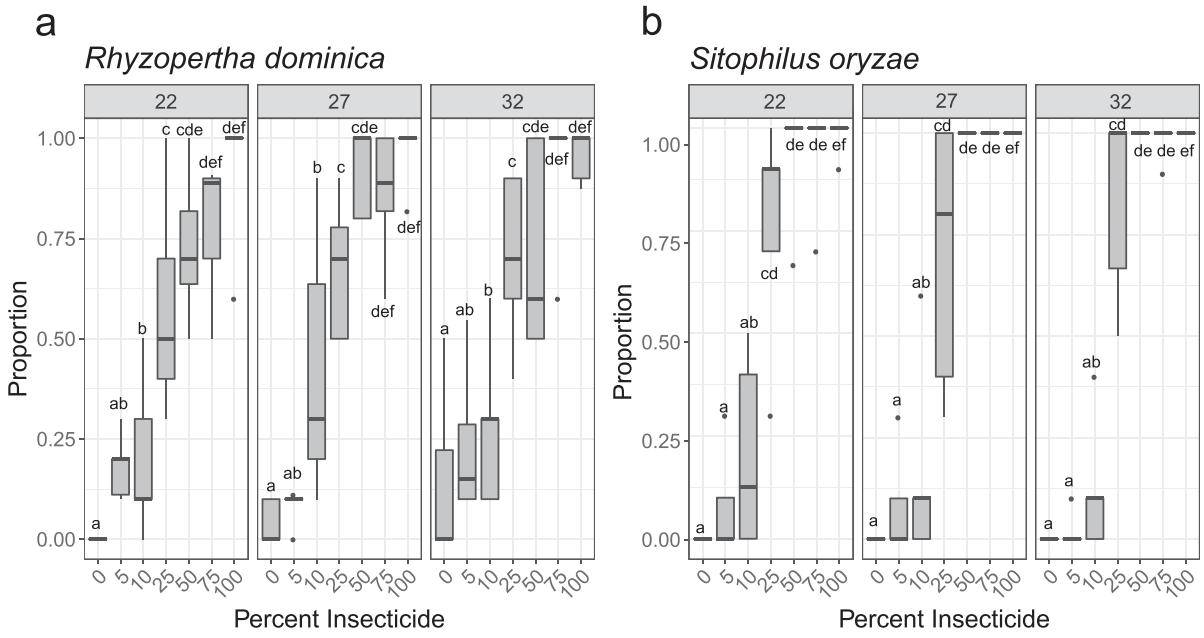

Fig. 1. Impact of Storicide II and temperature on mortality of adult (a) *Rhyzopertha dominca* and (b) *Sitophilus oryzae*. Mortality was assessed after a one-week exposure to brown rice containing different percentages of grain that had been treated with Storicide II at three different temperatures. Letters indicate significant differences for species and treatment interactions only at a significance threshold of $\alpha = 0.05$. Since the three-way interaction of temperature, species, and Storicide II treatment was not significant, no differences were observed between the three temperatures. Edges of the boxes represent the 25 and 75% distribution of the data (interquartile range or IQR) and the error bars represent $\pm 1.58 \times (\mathrm{IQR})/\sqrt{(n)}$. Outliers are represented by dots.

Table 2
Type III effects for proportion of adult *Rhyzopertha dominica* and *Sitophilus oryzae* that were knocked down after exposure to Storicide II for 1 week. The effects of species, temperature, and percent treated grain on the proportion of adults that were knocked down one week after exposure to Storicide II were measured. Individuals in knockdown were able to move their legs in response to touch, but were unable to right themselves and walk around. DF = degrees of freedom.

Effect	Numerator DF	Denominator DF	F-value	p-value
Species	1	163	99.66	<0.0001
Percent treated grain	6	163	7.20	<0.0001
Temperature	2	163	3.46	0.0337
Species x percent treated grain	6	163	5.47	<0.0001
Species x temperature	2	163	1.40	0.2508
Percent treated grain x temperature	12	163	1.03	0.4272
Species x percent treated grain x temperature	12	163	1.38	0.1826

significantly higher than the controls in the mixtures that contained 10%, 25%, and 50% treated brown rice, but there were no differences in knockdown between the 75%, 100%, and control treatments (Fig. 2). The low number of survivors observed at ≥75% treated brown rice was the reason why the number of insects in knockdown did not differ from the controls in these treatments for *R. dominica* (Fig. 1a). Unlike mortality, knockdown was also influenced by temperature with higher numbers of insects observed in the knockdown state at 22 °C compared to the other two temperatures (Fig. 2a). However, the interactions between species and temperature and species and Storicide II treatment were not significant (Table 2). No differences between any other treatment combinations were observed and no major differences in knockdown were observed among any of the Storicide II or temperature treatments for *S. oryzae* (Fig. 2b).

Similarly, the proportions of individuals that were running after a one-week exposure to Storicide II were impacted by the treatments. These impacts largely followed the same trends as mortality

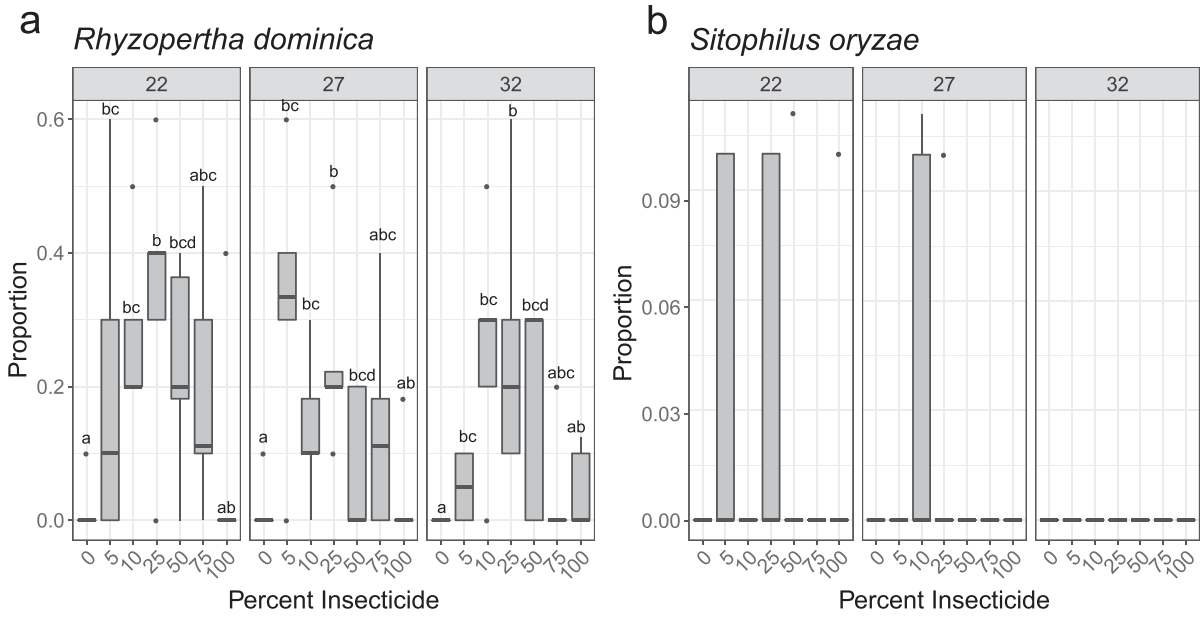

Fig. 2. Impact of Storicide II on knockdown of adult (a) *Rhyzopertha dominca* and (b) *Sitophilus oryzae*. The number of insects that were knocked down after a one-week exposure to brown rice that contained different percentages of grain that had been treated with Storicide II at three different temperatures was recorded Letters indicate significant differences for species × treatment interactions only at $\alpha = 0.05$. Since the three-way interaction of temperature x species x Storicide II treatment was not significant, no differences were observed between the three temperatures. Edges of the boxes represent the IQR and error bars represent $\pm 1.58 \times (\text{IQR})/\sqrt{(n)}$. Outliers are represented by dots.

Table 3
Type III effects for proportion of adult *Rhyzopertha dominica* and *Sitophilus oryzae* running after exposure to Storicide II for 1 week. The effects of species, temperature, and percent treated grain on the proportion of adults that were still actively moving around one week after exposure to Storicide II were assessed. DF = degrees of freedom.

Effect	Numerator DF	Denominator DF	F-value	p-value
Species	1	163	41.37	<0.0001
Percent treated grain	6	163	272.73	<0.0001
Temperature	2	163	0.29	0.7502
Species x percent treated grain	6	163	9.24	<0.0001
Species x temperature	2	163	0.29	0.7462
Percent treated grain x temperature	12	163	0.58	0.8565
Species x percent treated grain x temperature	12	163	0.35	0.9776

with species, Storicide II treatment, and the interaction between these two factors having a significant impact (Table 3). Temperature alone did not influence the proportion of individuals that were running (Table 3). However, the proportion of the insects that were running were significantly reduced in all mixtures that contained ≥10% treated grain for both adult *R. dominica* and *S. oryzae* compared to the control (Fig. 3a–b). In *R. dominica*, proportion of individuals that were running was also impacted in mixtures containing as little as 5% treated grain compared to the control (Fig. 3a). In contrast, no effects on *S. oryzae* were observed in the 5% mixture (Fig. 3b).

3.2. Impact on progeny production

As an initial assessment of the impacts of Storicide II treatment and temperature on progeny production, the total number of progeny produced across all timepoints were summed together for each of the three insect species. Both *S. cerealella* and *S. oryzae* produced progeny at all three temperatures when reared on Storicide II-free brown rice; however, no progeny were found in the majority of the controls or the treated samples at 22 °C for *R. dominica*, indicating that temperature conditions were not suitable for progeny development in this species (Fig. 4). When species, Storicide II treatment, and temperature were tested together as factors influencing progeny production, none of the individual factors alone were significant; however, the two-way interaction of Storicide II treatment and species and the three-way interaction of temperature, species, and Storicide II treatment were both significant (Table 4). Temperature had definitive impacts on progeny production in Storicide II-treated samples in both *R. dominica* and *S. cerealella* (Fig. 4a–c). For *S. cerealella*, reductions in progeny production relative to controls were observed at ≥50% treated brown rice at both 22 and 32 °C; however, reductions in progeny were not observed until ≥75% treated brown rice was used at 27 °C, indicating that a larger amount of insecticide-treated brown rice was needed to suppress progeny production by this species at this temperature (Fig. 4c). For *R. dominica*, higher amounts of treated brown rice were needed to suppress progeny production when insects were reared at 32 °C (Fig. 4a). Specifically, suppression of progeny production was observed in treatments with ≥5% treated brown rice at 27 °C, but suppression of progeny production at 32 °C required ≥10% treated brown rice (Fig. 4a). In contrast, temperature had no impacts on the efficacy of Storicide II treatments on *S. oryzae* as ≥50% treated brown rice was required to suppress progeny production at all three temperatures (Fig. 4b).

When Storicide II treatment and temperature were analyzed as factors for each species individually, some additional trends for *S. cerealella* and *S. oryzae* emerged beyond those discovered when

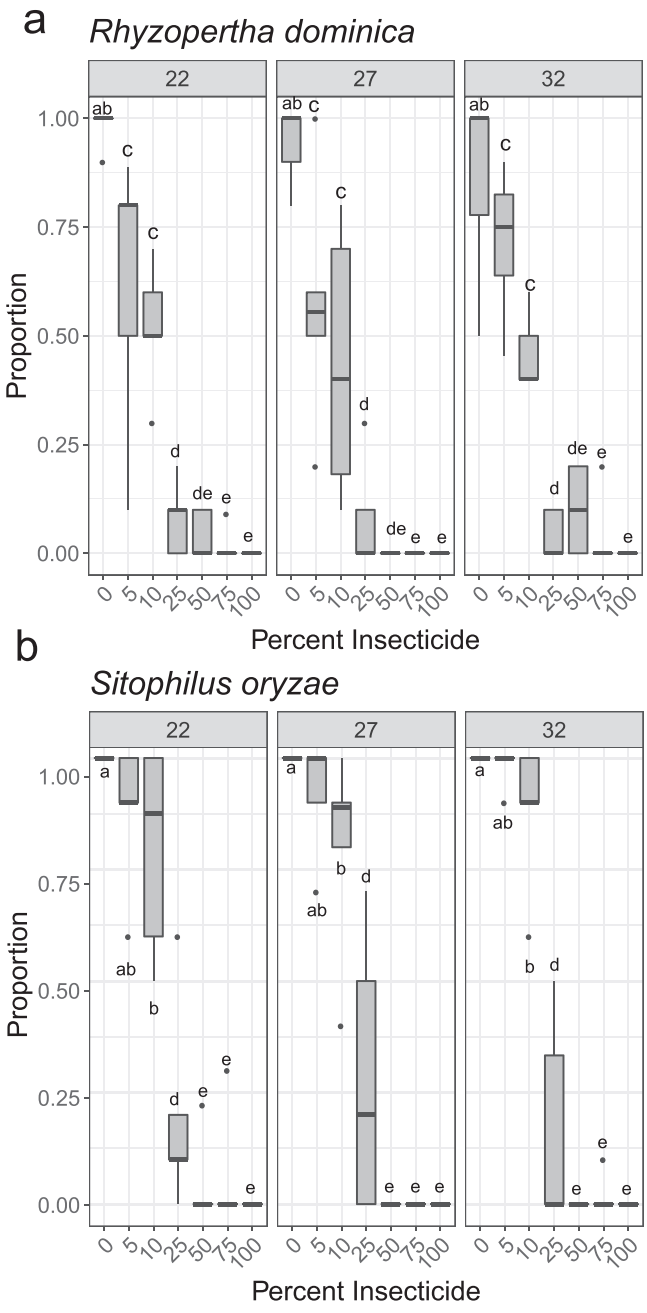

Fig. 3. Impact of Storicide II and temperature on proportion of adult (a) *Rhyzopertha dominca* and (b) *Sitophilus oryzae* that were active. The number of insects that were actively moving around or "running" was recorded after a one-week exposure to Storicide II at three different temperatures. Letters indicate significant differences for species × treatment interactions only at $\alpha = 0.05$. Since the three-way interaction of temperature, species, and Storicide II treatment was not significant, no differences were observed between the three temperatures. Edges of the boxes represent the IQR and the error bars represent $\pm 1.58 \times (\text{IQR})/\sqrt{(n)}$. Outliers are represented by dots.

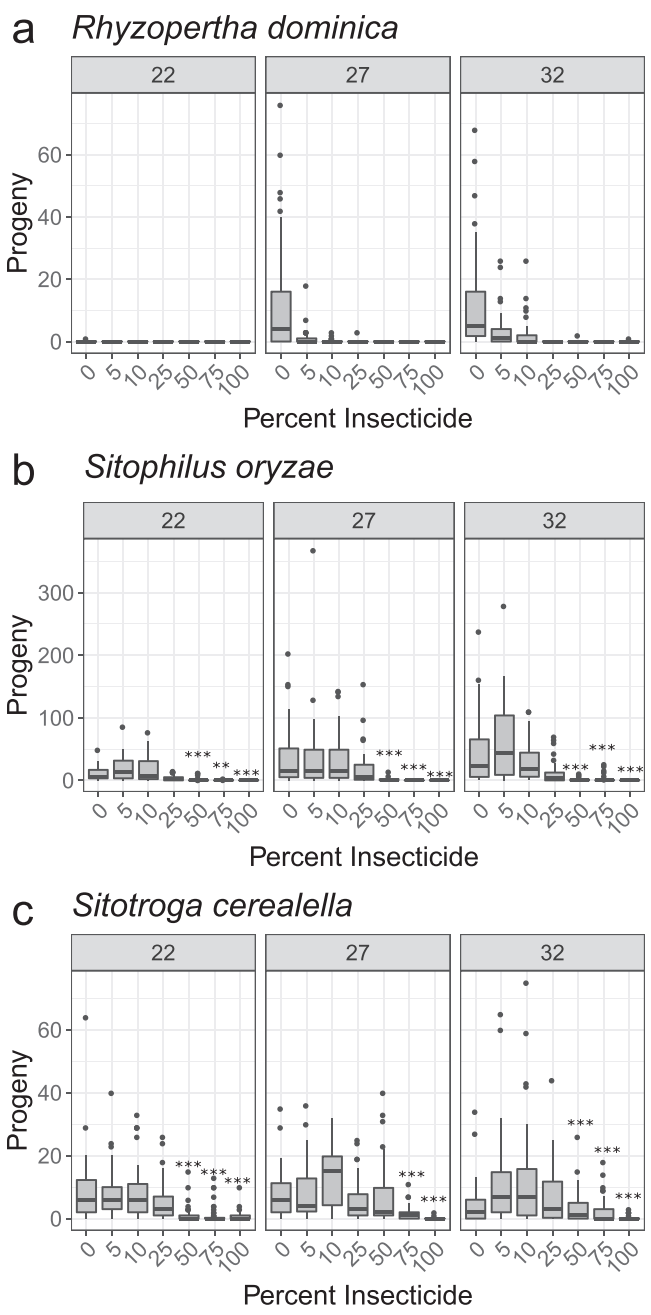

Fig. 4. Impact of Storcide and temperature on total number of progeny produced by (a) *Rhyzopertha dominica*, (b) *Sitophilus oryzae*, and (c) *Sitotroga cerealella*. The total number of progeny produced over a 50-day period were recorded after exposure to Storicide II at three different temperatures. Asterisks indicate significant differences between control and Storicide II treatment within each temperature treatment: ** adjusted p-value <0.01 and ***adjusted p-value <0.001. No post-hoc comparisons were significant for *R. dominica*, so no letters are displayed. Edges of the boxes represent the IQR and the error bars represent $\pm 1.58 \times (\text{IQR})/\sqrt{(n)}$. Outliers are represented by dots.

all three species were assessed together in the same model (Table 4). No other major trends were observed when *R. dominica* was analyzed separately (Table 4 and Fig. 4a). For *S. cerealella*, the effect of Storicide II treatment and the interaction of Storicide II treatment with temperature on progeny production were both significant, but temperature alone was not (Table 4). Specifically, there were no major differences in progeny emergence in the control treatments at any temperature and thus, all temperatures were suitable for progeny production. In addition, there were no major differences in progeny emergence at 22, 27, or 32 °C when 100% treated brown rice was used (Fig. 4c). At both 27 and 32 °C, progeny emergence was lower when 100% treated brown rice was used compared to 75% treated brown rice; however, no differences were observed between these two Storicide II treatments at 22 °C (Fig. 4c). Thus, when temperature was lower, mixing in smaller

Table 4
Type III effects for progeny production after exposure to Storicide II for 1 week. The effects of species, temperature, and percent treated grain on the total number of progeny produced by *Rhyzopertha dominca*, *Sitotroga cerealella*, and *Sitophilus oryzae* were measured DF = degrees of freedom.

Effect	Numerator DF	Denominator DF	F-value	p-value
Species	2	2211	0.00	0.9959
Percent treated grain	6	2211	0.00	1.0000
Temperature	2	2211	0.29	0.9959
Species x percent treated grain	6	2211	6.17	<0.0001
Species x temperature	4	2211	0.00	1.0000
Percent treated grain x temperature	12	2211	1.04	0.4118
Species x percent treated grain x temperature	12	2211	2.23	0.0007
Sitotroga cerealella				
Percent treated grain	6	819	50.89	<0.0001
Temperature	2	819	1.93	0.1455
Percent treated grain x temperature	12	819	5.18	<0.0001
Rhyzopertha dominica				
Percent treated grain	6	721	0.00	1.0000
Temperature	2	721	0.00	0.9998
Percent treated grain x temperature	12	721	1.37	0.1881
Sitophilus oryzae				
Percent treated grain	6	671	46.09	<0.0001
Temperature	2	671	0.00	0.9999
Percent treated grain x temperature	12	671	2.15	0.0127

amounts of untreated brown rice with treated brown rice may have been sufficient to suppress progeny emergence in *S. cerealella*. Furthermore, although progeny production was highly variable in many of the treatment and temperature combinations, it was less variable at 22 °C in the 5% and 10% Storicide II treatments compared to the same Storicide II treatments at 27 °C and 32 °C, indicating that lowering temperature in combination with adding grain protectant can lead to more consistent control of progeny (Fig. 4c). There was also a small reduction in progeny numbers at 22 °C in the 25% Storicide II treatment compared with the same Storicide II treatment at 27 and 32 °C (Fig. 4c). Overall, the lowest progeny counts were observed in 100% treated grain at 27 and 32 °C, followed by the 50, 75, and 100% treatments at 22 °C, the 75% treatment at 27 °C, and finally, the 75% and 50% treatments at 32 °C.

The same factors and interactions were also significant for *S. oryzae*. Overall, progeny production was lowest at 22 °C even in the controls and in the 5% Storicide II treatment at this temperature (Fig. 4b). No major differences in progeny production were observed in the 100% Storicide II treatment at all three temperatures, indicating that temperature had a minimal effect on progeny production when all grain had been treated with the grain protectant. Likewise, no differences were observed in progeny emergence in the 75% Storicide II treatments at 22 and 27 °C; however, progeny emergence was slightly higher in this treatment at 32 °C compared to 22 and 27 °C (Fig. 4b). The effect of temperature on Storicide II efficacy was also apparent in the 25% and 5% Storicide II treatments as lower progeny numbers were observed in these treatments at 22 °C compared to both 27 °C and 32 °C (Fig. 4b).

3.3. Damaged kernels and frass production

In addition to impacts on mortality and progeny production, the application of grain protectants and storage temperature can also impact feeding activity levels, which can directly impact the level of damage to the grain and the abundance of frass. Thus, damage estimates and frass weights were collected at the completion of the experiment as indicators of feeding activity. For damage estimates, species, Storicide II treatment, and temperature were all significant factors and the interaction between Storicide II treatment and species was also significant (Table 5). No other interactions were significant (Table 5). For *S. cerealella*, damage counts were generally low and no major differences in damage counts were observed in any of the treatments or temperatures (Fig. 5c). Additionally, damage counts were significantly lower in this species compared to both *R. dominica* and *S. oryzae*, so damage results for *S. cerealella* are not discussed further (Fig. 5).

For *R. dominica* and *S. oryzae*, damage counts increased at higher temperatures, with lower numbers of damaged kernels found at 22 °C compared to both 27 °C and 32 °C (Fig. 5a–b). No differences in damaged kernels were observed between 27 and 32 °C. Damage counts were also low for *R. dominica* across all Storicide II treatments, especially at 22 °C (Fig. 5a). Although steady downward trends in damage counts were observed at 27 and 32 °C with increasing percentages of Storicide II treated grain for *R. dominica*, none of the damage counts differed significantly compared to their respective controls (Fig. 5a). Among the three species, the highest number of damaged kernels were associated with feeding by *S. oryzae* and reductions in damage counts were observed in several of the Storicide II treatments relative to the controls (Fig. 5b). In this species, at least 50% treated grain was required to reduce damage counts relative to the controls and no further reductions in mean numbers of damaged kernels were observed in the 75% or 100% treatments compared to 50% (Fig. 5b) Furthermore, the damage counts for *S. oryzae* in treatments that contained ≥25% treated brown rice were similar to damage counts observed in *R. dominica* (Fig. 5b).

In terms of frass weights, temperature and Storicide II treatment had significant impacts and the two-way interactions between temperature and Storicide II treatment and Storicide II treatment and species were also significant (Table 6). For *R. dominica*, adding as little as 5% treated grain resulted in significantly lower frass weights relative to the controls; however, no further reductions in frass weights were observed at treatments containing ≥10% treated grain compared to the 5% treatment (Fig. 6a). In contrast, no reductions in frass weights were observed in any of the Storicide II treatments in *S. oryzae* (Fig. 6b). Effects of the interaction between temperature and species were also apparent as frass weights were lowest at 22 °C and highest at 32 °C for *R. dominica*; however, no differences were observed between frass weights at 22 and 27 °C or 27 and 32 °C in this species (Fig. 6a and Table 6). Although higher frass weights were observed in some of the *S. oryzae* replicates in the 32 °C control treatments, variation in frass weights were high in both the control and the 5% Storicide II treatments and no major differences were found in any of the three temperature (Fig. 6b).

Table 5
Type III effects for proportion damaged kernels. The effects of species, temperature, and percent treated grain on the proportion of damaged kernels were assessed after adult insects were exposed to different percentages of Storicide II treated grain for one week. DF = degrees of freedom.

Effect	Numerator DF	Denominator DF	F-value	p-value
Species	1	248	75.36	<0.0001
Percent treated grain	6	248	12.83	<0.0001
Temperature	2	248	7.86	0.0005
Species x percent treated grain	6	248	5.01	<0.0001
Species x temperature	4	248	1.49	0.2045
Percent treated grain x temperature	12	248	1.01	0.8565
Species x percent treated grain x temperature	12	248	0.35	0.4362

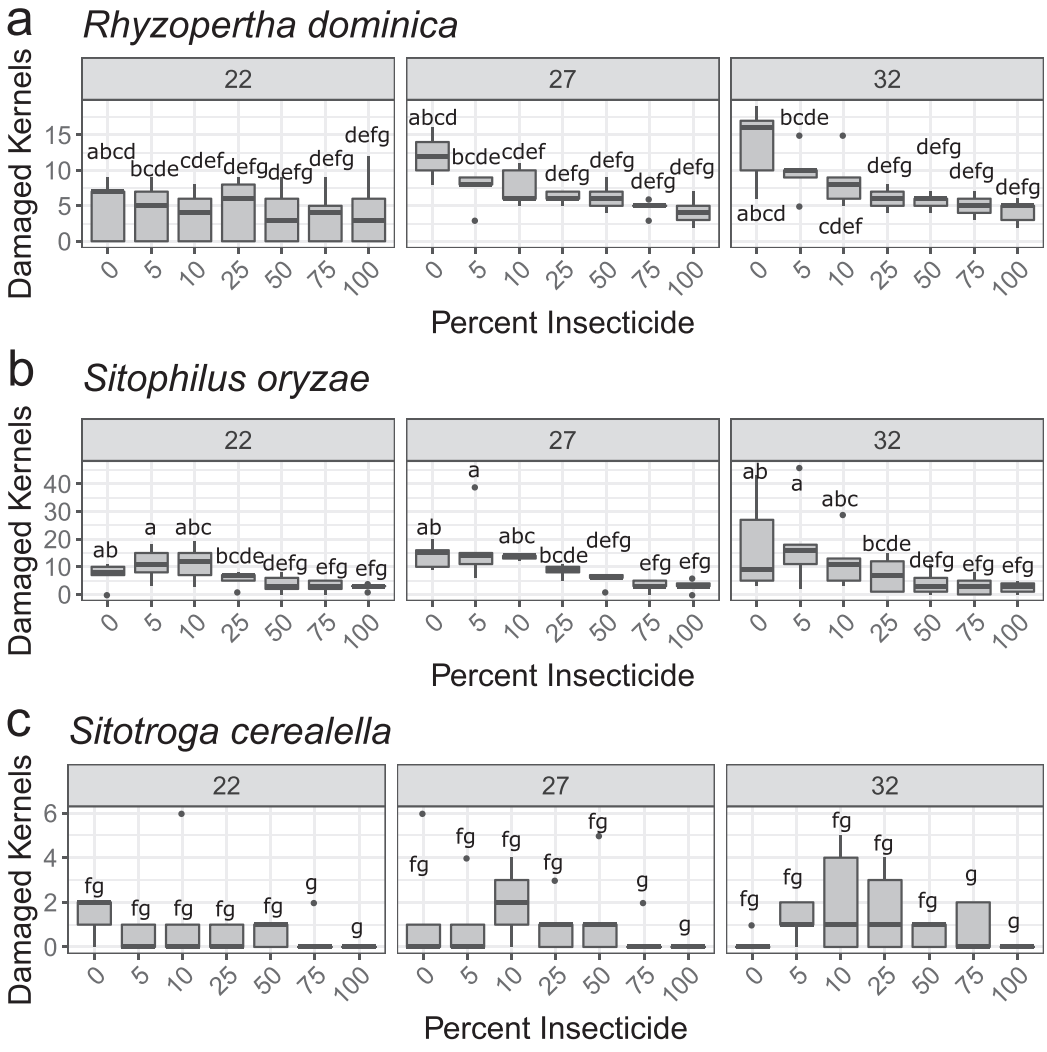

Fig. 5. Impact of temperature and Storicide II on proportion damaged kernels for (a) *Rhyzopertha dominica*, (b) *Sitophilus oryzae*, and (c) *Sitotroga cerealella*. Damage estimates were performed by taking a random subsample of 100 kernels from each jar and counting the number that were damaged. Letters indicate significant differences for species and treatment interactions at $\alpha = 0.05$. Edges of the boxes represent the IQR and the error bars represent $\pm 1.58 \times (\text{IQR})/\sqrt{(n)}$. Outliers are represented by dots.

Frass weights were not measured for *S. cerealella* because these insects typically do not produce enough frass to accurately weigh.

3.4. Principal components analysis

To investigate broader patterns among the different treatment combinations analyzed in this study and identify variabilities driving similarities between samples, principal components analysis was performed using damage counts, frass weights, total progeny counts, and number of affected adults as variables Because adult mortality and knockdown data were not collected for *S. cerealella*, this species was not included in this analysis. Overall, the *R. dominica* and *S. oryzae* control samples were separated from the majority of the Storicide II treated samples along the PC1 axis, which explained 78.9% of the variation in the data (Fig. 7a). For *R. dominica*, the separation between the Storicide II treated samples and the controls was mostly clear and distinct with the exception of the 22 °C control, which had no progeny emergence and clustered

Table 6
Type III effects for frass production. The effects of species, temperature, and percent treated grain on the mass of frass sieved from each rearing container was measured. DF = degrees of freedom.

Effect	Numerator DF	Denominator DF	F-value	p-value
Species	1	164	0.07	0.7964
Percent treated grain	6	164	13.98	<0.0001
Temperature	2	164	12.12	<0.0001
Species x percent treated grain	6	164	5.01	<0.0001
Species x temperature	4	164	3.79	0.0015
Percent treated grain x temperature	12	164	3.51	0.0001
Species x percent treated grain x temperature	12	164	0.94	0.5131

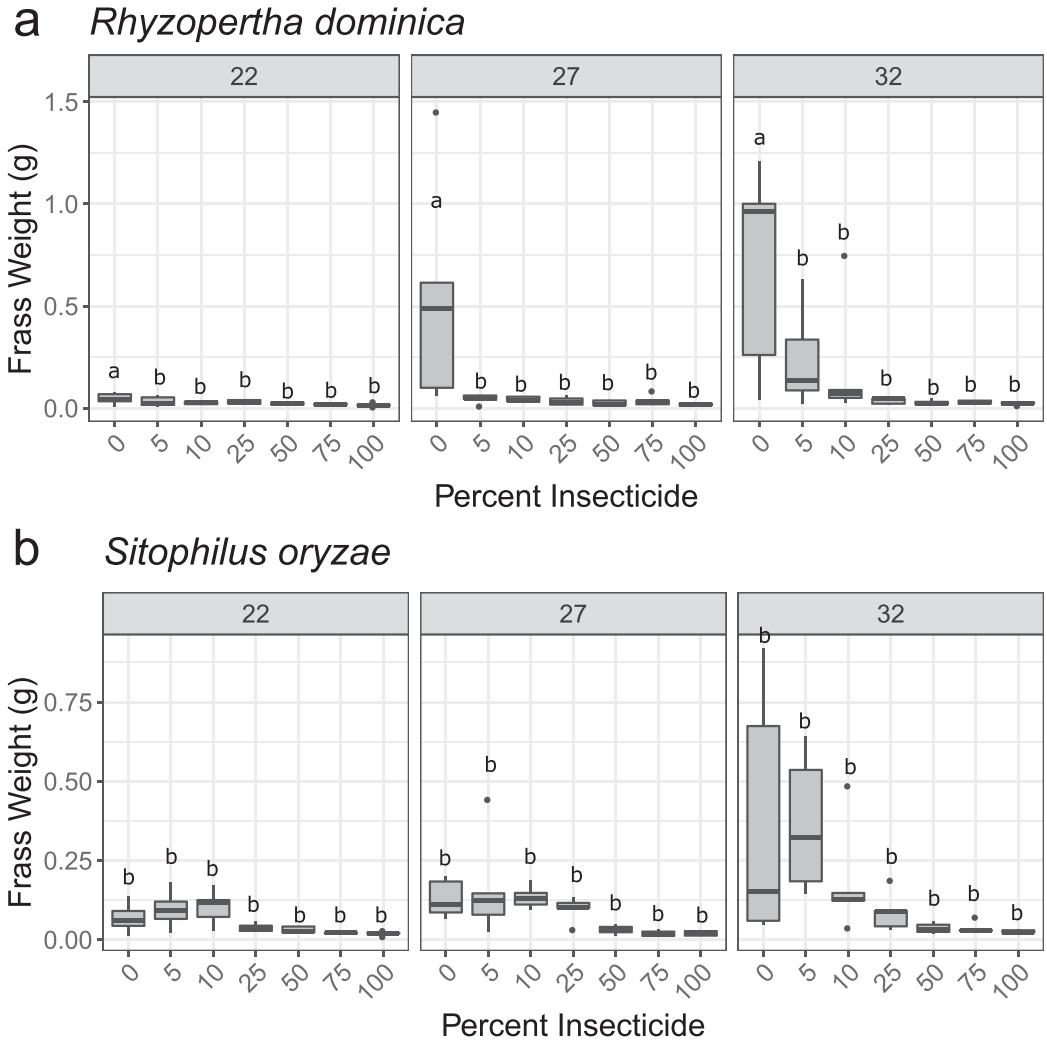

Fig. 6. Impact of temperature and Storicide II on frass weights for (a) *Rhyzopertha dominica*, (b) *Sitophilus oryzae*, and (c) *Sitotroga cerealella*. Frass produced by *Sitophilus oryzae* and *Rhyzopertha dominica* was sieved from each jar and weighed after progeny were counted. Letters indicate significant differences for species and treatment interactions at $\alpha = 0.05$. Edges of the boxes represent the IQR and the error bars represent $\pm 1.58 \times (\text{IQR})/\sqrt{(n)}$. Outliers are represented by dots.

with many of the treated samples. In contrast, the samples corresponding to the mixtures containing 5% and 10% treated brown rice for *S. oryzae* at all three temperatures were clustered with the controls, indicating that there was not much difference between the controls and the 5 and 10% mixtures (Fig. 7a). All *S. oryzae* samples from the ≥25% Storicide II treatments were clearly separated from the controls along PC1 (Fig. 7a). Among the variables analyzed, damage count explained the highest amount of the variation along this axis (29.5%), followed by progeny (25.8%), number of affected adults (24.7%), and frass weight (20.1%) (Table 7).

The PC2 axis explained approximately 11.2% of the variance and frass weight explained the majority of the variance along this axis at 73.2%. Number of affected adults (1.6%), total progeny produced (20.2%), and damage count (2.0%) explained less of the variance along this axis (Table 7). Additionally, some separation of the temperature treatments was observed along both the PC1 and PC2 axes with the 32 °C treatments appearing towards the top right

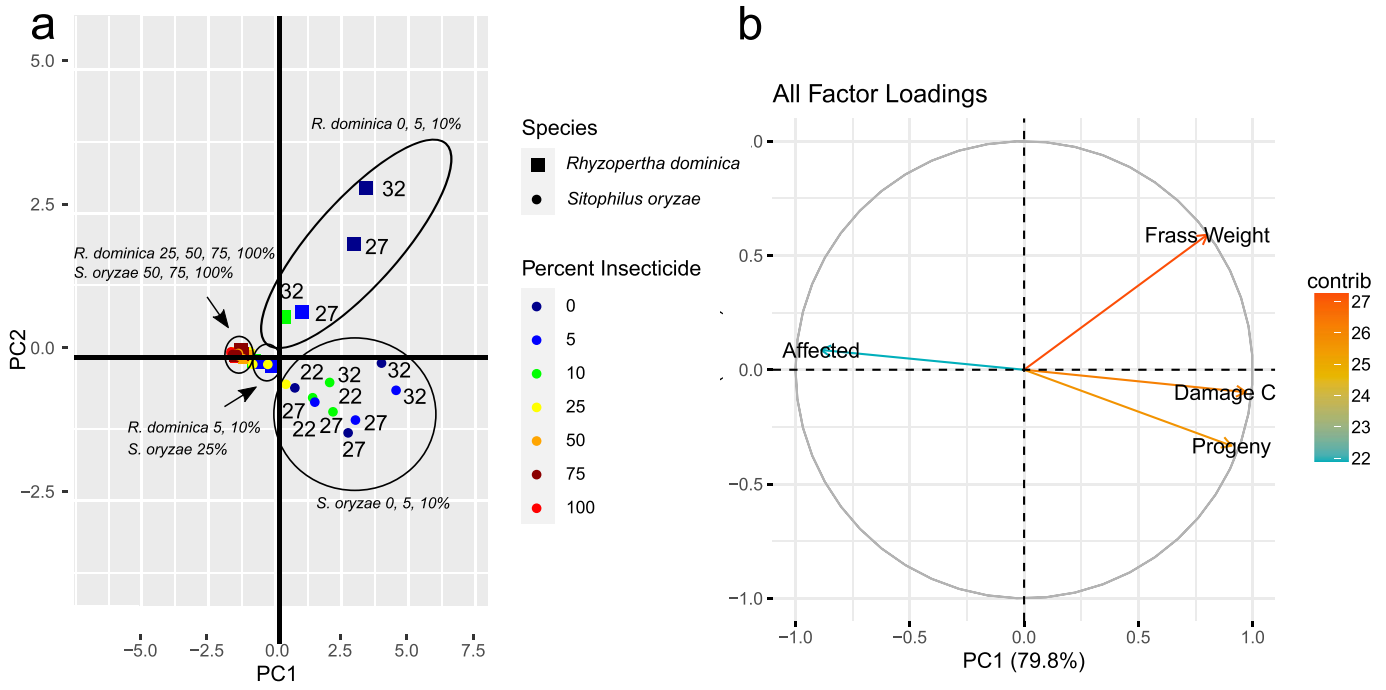

Fig. 7. Principal components analysis (PCA) of variables affected by Storicide II treatment. Panel A shows several groups of treatment combinations that had similar impacts on the variables listed above. Numbers designate the temperature treatments, shapes distinguish the two species, and shapes are color coded by percent Storicide II treatment. Temperature labels were removed on the left-hand side of the plot because all Storicide II treatments with ≥25% for *R. dominica* and ≥50% Storicide II treatments with *S. oryzae* were similar to one another. Panel B depicts the loading scores for the top four variables that explain the majority of the variation. Scores are color coded by the percent variation explained.

Table 7
Contribution of variables to principal components ordination. Principal components analysis (PCA) was performed on the *Rhzyopertha dominica* and *Sitophilus oryzae* controls and Storicide II treatments. Contribution indicates the relative contribution of each variable to the variance explained by each PCA axis and quality of representation shows the relative importance of each variable for explaining variance along each axis. PC = principal components axis.

Variable	PC Information	PC1	PC2	PC3	PC4
Damage Count	Contribution	29.48	2.02	6.50	62.00
	Quality of Representation	0.95	0.0096	0.018	0.031
Affected	Contribution	24.66	1.57	72.99	0.78
	Quality of Representation	0.78	0.0075	0.20	0.0004
Progeny	Contribution	25.76	23.18	18.06	33.00
	Quality of Representation	0.82	0.11	0.051	0.016
Frass Weight	Contribution	20.10	73.23	2.45	4.22
	Quality of Representation	0.64	0.35	0.0069	0.0021

corner of the plot and the 27 °C and 22 °C samples appearing towards the lower right corner of the plot, although a high degree of overlap was observed (Fig. 7a). No other major clustering patterns along the PC2 axis were observed; however, overlaying the variables as a biplot showed some additional factors driving similarities among groups of samples. For example, progeny and damage count were strongly associated with the control, 5%, and 10% treatments in *S. oryzae* whereas *R. dominica* overall tended to be more strongly associated with frass weight and number of affected adults (Fig. 7a and b). In addition, many of the 32 °C treatments were associated with frass weight (Fig. 7a and b), although there was a lot of variation among the different Storicide II treatments for both *R. dominica* and *S. oryzae*. Number of affected adults was associated with the majority of the treatments containing ≥25% Storicide II treated brown rice (Fig. 7a and b).

3.5. Analysis of progeny production over time

To determine how the different temperatures and treatments influenced progeny production over time, a hierarchical clustering analysis was performed to identify groups of samples that showed similarly profiles of progeny emergence. Overall, samples were assigned to 23 different clusters with five samples being assigned to their own, unique cluster (Table 8 and Fig. 8). Progeny emergence curves were especially distinct for *S. cerealella* as many progeny emergence profiles for this species did not form clusters with progeny emergence curves from the other two species. For example, the control and 5% treated grain treatments tended to produce curves with peaks in progeny counts at around 20 days after the initial appearance of progeny at 22 °C (cluster 1) whereas the control treatment produced spikes in progeny counts at approximately four to five days at 27 °C with a second peak occurring around 40 days after the initial appearance of progeny (cluster 2) (Table 8 and Fig. 8). Other clusters unique to this species were clusters 4, 5, 7, 10, 12, and 14 (Table 8 and Fig. 8). Interestingly, although 100% treated grain reduced progeny production in this species, a spike in progeny production was observed at around 35 days after the initial appearance of progeny at 22 °C (cluster 7), suggesting that progeny emergence was delayed compared to other treatment combinations (Table 8 and Fig. 8). Other clusters that were uniquely associated with one species included cluster 15 (*R. dominica* controls at 27 °C) in which spikes of progeny emergence were periodically observed throughout the 50 day observation period, and clusters 20–23, which were all associated with *S. oryzae* (Table 8 and Fig. 8).

In terms of similarities in progeny emergence among the three stored product insect species, cluster 17 had the most members with 15 different treatment combinations assigned to this cluster (Table 8 and Fig. 8). This cluster was associated with a notable lack

Table 8
Patterns of progeny emergence after exposure to Storicide II. Storicide II treatment and temperature combinations that produced similar curves in progeny emergence over time were identified using time-series analysis. The centroid of each cluster is represented in the last column with progeny on the y-axis and time in days on the x-axis.

Cluster	Size	*Sitotroga cerealella* Members	*Rhzyopertha dominica* Members	*Sitophilus oryzae* Members
1	2	22 °C at 0, 5%	None	None
2	1	27 °C at 0%	None	None
3	3	32 °C at 0%	22 °C at 0% 32 °C at 50%	None
4	2	22 °C at 10, 25%	None	None
5	1	27 °C at 10%	None	None
6	3	32 °C at 5, 10, 75%	None	None
7	1	22 °C at 100%	None	None
8	3	27 °C at 100%	27 °C at 10%	22 °C at 75%
9	3	32 °C at 25, 100%	None	27 °C at 50%
10	1	27 °C at 25%	None	None
11	3	27 °C at 5%	None	27 °C at 5, 25%
12	2	22 °C at 50, 75%	None	None
13	5	27 °C at 50%	32 °C at 5, 10%	32 °C at 25, 75%
14	2	27 °C at 75% 32 °C at 50%	None	None
15	1	None	27 °C at 0%	None
16	2	None	32 °C at 0%	32 °C at 50%
17	15	None	22 °C at 5, 10, 25, 50, 75, 100% 27 °C at 50, 75, 100%	22 °C at 100% 27 °C at 75, 100% 32 °C at 100%
18	2	None	27 °C at 25% 32 °C at 100%	None
19	2	None	27 °C at 5%	22 °C at 50%
20	2	None	None	22 °C at 0, 5%
21	2	None	None	27 °C at 0, 10%
22	3	None	None	22 °C at 10, 25% 32 °C at 0%
23	2	None	None	32 °C at 5, 10%

of progeny emergence over the 50-day time course and included all *R. dominica* samples at 22 °C and all *R. dominica* samples at both 27 and 32 °C that were reared on brown rice mixtures containing ≥50% treated grain (Table 8). All *S. oryzae* samples exposed to 100% treated brown rice were also assigned to this cluster along with the 75% treatment at 27 °C (Table 8 and Fig. 8). Cluster 13 also had high shared membership among the three stored product species (five different treatment combinations) and was characterized by two spikes in progeny emergence at 20 days after the initial appearance of progeny followed by another spike at 25 days (Table 8 and Fig. 8). Samples that were assigned to this cluster included *S. cerealella* on 50% treated brown rice at 27 °C, *R. dominica* on 5% and 10% treated brown rice at 32 °C, and *S. oryzae* on 25% and 75% treated brown rice at 32 °C (Table 8). Other clusters that were represented by at least two different species included clusters 3, 8, 9, 11, 16, and 19 (Table 8 and Fig. 8). Although the patterns of progeny patterns among all three species were diverse, one consistent trend that emerged is that treatments containing lower percentage of Storicide II treated grain for *R. domincia* tended to form clusters with treatments containing higher percentages of Storicide II treated grain for the two other species, again reflecting the sensitivity of this insect to the grain protectant.

When clustering analysis was performed for each species independently, some additional trends emerged. Five different patterns of progeny emergence were observed for *S. oryzae* (Table 9). At 22 °C in *S. oryzae*, progeny emergence was more gradual and occurred over a longer period of time with a maximum burst of progeny observed at approximately 18 days after the initial appearance of progeny in the control and 5% Storicide II treatment (cluster 1) (Table 9 and Figure S1). Although a more gradual emergence of progeny was also observed in the other Storicide II treatments at this temperature, the spike in progeny emergence at 18 days was reduced and progeny emerged more asynchronously over time in the 10 and 25% treatments (cluster 3) (Table 9 and Figure S1). The same trends were observed in the 50 and 75% treatments at 22 °C, although lower numbers of progeny emergence were observed before 20 days unlike the treatments containing lower percentages of Storicide II treated brown rice (cluster 5) (Table 9 and Figure S1). No progeny emergence was observed at 100% treated brown rice at 22 °C (cluster 4) (Table 9 and Figure S1). In contrast, higher bursts of progeny emergence over a much shorter time interval were observed at the 27 and 32 °C treatments in the controls and the treatments containing lower percentages of Storicide II (cluster 2) (Table 9 and Figure S1). For example, at 27 °C, *S. oryzae* exhibited a spike in progeny emergence at ~10 days after the first appearance of progeny and this spike in progeny was observed at treatments containing up to 50% Storicide II treated brown rice; however, progeny production was completely suppressed at 75 and 100% at this temperature (cluster 4) (Table 9 and Figure S1). Thus, temperature did not have as drastic of an impact on progeny production curves at 27 °C compared to 22 °C in this species (Table 9 and Figure S1). At 32 °C, a spike in progeny emergence was observed at 10 days after the initial detection or progeny, but more progeny emerged at earlier timepoints compared to the 22 °C and 27 °C treatments (Table 9 and Figure S1). This trend was observed in the controls and in treatments containing up to 75% treated brown rice, but no progeny were observed at the 100% treatment at 32 °C (Table 9 and Figure S1).

For *R. dominica*, nine predominant patterns were apparent and very little progeny production was observed at 22 °C and in many of the Storicide II treatments at both 27 and 32 °C (Table 9 and Figure S2). However, some notable differences were observed between the 27 and 32 °C controls and the treatments containing 5–10% treated brown rice (Table 9 and Figure S2). At 27 °C, progeny emergence was spread out over an approximately 40 day interval in the controls whereas several spikes in progeny emergence were noted between 10 and 20 days after the initial appearance of progeny in the 32 °C controls and the treatments containing both 5

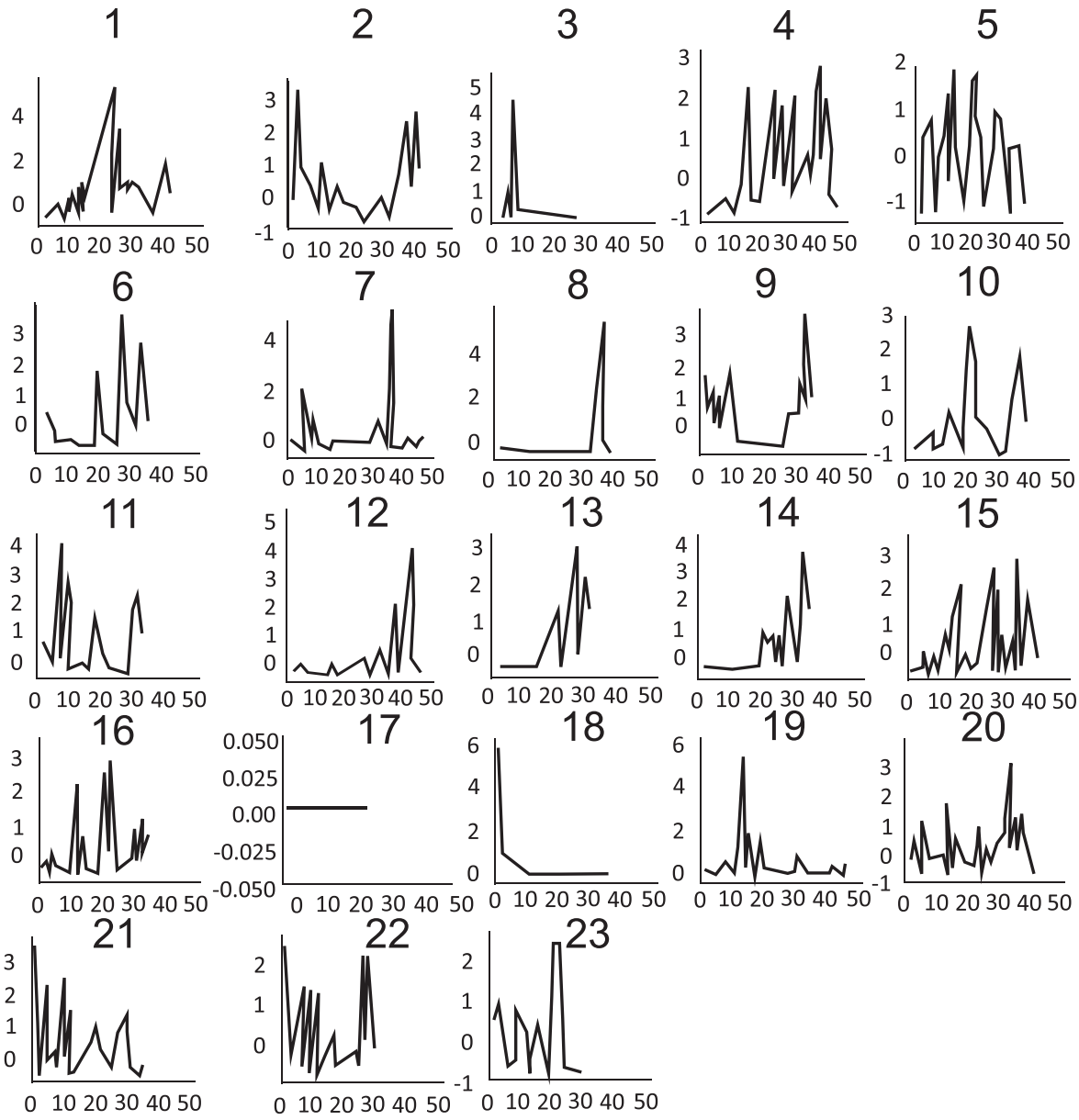

Fig. 8. Progeny emergence curves of insects exposed to Storicide II-treated and control brown rice. Time-series clustering analysis was collectively performed on progeny emergence data from *Rhyzopertha dominica*, *Sitotroga cerealella*, and *Sitophilus oryzae*, which was collected over a 50-day time period to determine the effect of Storicide II and temperature on progeny emergence over time. Twenty-three unique time-series curves were identified for the three species. Information regarding cluster membership is presented in Table 8.

and 10% treated brown rice (Table 9 and Figure S2). Progeny emergence was completely suppressed in treatments containing ≥25% treated brown rice at 32 °C (Table 9 and Figure S2). In addition, progeny emergence was significantly delayed at 27 °C in the 10% treatment as early emergence of progeny was significantly reduced followed by a spike in emergence at ~30 days after the first detection of offspring (Table 9 and Figure S2). In contrast to both the control and the 10% treatment at 27 °C, progeny emergence largely occurred in a single early burst in the 5% treatment at 27 °C and some of the later bursts that were observed in the control treatment at this temperature were suppressed (Table 9 and Figure S2).

Finally, for *S. cerealella*, six different patterns of progeny emergence were apparent and temperature had a notable impact on progeny emergence in both the controls and in the different Storicide II treatments (Table 9 and Figure S3). At 22 °C, progeny emergence was spread over an approximately 50-day interval with a major peak in emergence around 20 days after the initial appearance of progeny followed by a smaller peak at 25 days (cluster 1) (Table 9 and Figure S3). Although the emergence profile for the 5% treatment was similar to the control, shifts in the emergence profiles at 22 °C (cluster 1) were apparent at 10 and 25% treated brown rice, in which progeny emergence became more erratic, and in the treatments containing ≥50% treated brown rice where the spike in progeny emergence had shifted to 25 days after the first appearance of progeny (cluster 4) (Table 9 and Figure S3). At 27 °C, progeny emergence was spread over an approximately 40-day interval with multiple peaks of progeny emergence observed in this span (cluster 6) (Table 9 and Figure S3). The emergence profiles for the controls and 5, 50, and 75% treated brown rice at 27 °C were

Table 9
Clustering analysis for progeny emergence performed independently for each species. Time series analysis was performed on each species independently to identify temperature and Storicide II treatment combinations that yielded similar profiles of progeny emergence over time. The centroid of each cluster is represented in the last column with progeny on the y-axis and time in days on the x-axis.

Species	Cluster	Size	Members and Shape
Sitotroga cerealella	1	2	22 °C at 0, 5%
	2	5	27 °C at 0, 5, 50, 75%
	3	8	27 °C at 100%
			32 °C at 0, 5, 10, 25, 50, 75%, 100%
	4	2	22 °C at 10, 25%
	5	1	27 °C at 10%
	6	3	22 °C at 50, 75 and 100%
Rhyzopertha dominica	1	2	22 °C at 0%
			32 °C at 50%
	2	1	27 °C at 0%
	3	1	32 °C at 0%
	4	11	22 °C at 5, 10, 25, 50, 75, 100%
			27 °C at 100, 50, 75%
			32 °C at 25, 75%
	5	1	27 °C at 10%
	6	1	32 °C at 10%
	7	2	27 °C at 25%
			32 °C at 100%
	8	1	27 °C at 5%
	9	1	32 °C at 5%
Sitophilus oryzae	1	2	22 °C at 0, 5%
	2	11	27 °C at 0 5, 10, 25, 50%
			32 °C at 0, 5, 10, 25, 50, 75%
	3	2	22 °C at 10, 25%
	4	4	22 °C at 100%
			27 °C at 75, 100%
			32 °C at 100%
	5	2	22 °C at 50, 75%

identical (cluster 2) whereas the peaks of progeny emergence in the 10% treated were broader in comparison (cluster 5) (Table 9 and Figure S3). In the 100% treatment in this temperature, progeny emergence was compressed to a 30 day interval with a spike an emergence occurring at approximately 10 days after the initial appearance of progeny (cluster 3) (Table 9 and Figure S3). Interestingly, the 32 °C control treatment and all of the 32 °C treatments containing up to 75% treated brown rice were also assigned to cluster 3 (Table 9 and Figure S3).

3.6. Risk thresholds with mortality and damage

Mortality was used to estimate the percent treated brown rice required to achieve LD_{50} and LD_{90} for *R. dominica* and *S. oryzae* at 22, 27, and 32 °C. Overall, LD_{50} and LD_{90} values tended to be slightly higher for *R. dominica* adults compared to *S. oryzae*, especially at 22 °C (Fig. 9). Additionally, significantly higher percentages of treated brown rice were estimated for LD_{50} and LD_{90} at 22 °C compared to 27 °C and 32 °C for *R. dominica* (Fig. 9). Although higher percentages of treated brown rice were also required to achieve LD_{50} and LD_{90} for *S. oryzae* at 22 °C compared to 27 °C and 32 °C, the magnitude of the differences between the LDs at 22 °C and the other two temperatures was much less pronounced than the differences predicted between 22 °C and the other two temperatures in *R. dominica* (Fig. 9). Overall, this finding suggests that the mode of action is not as strong at lower temperatures and the impact of temperature on mode of action is more pronounced in *R. dominica* than in *S. oryzae*.

Additionally, we modeled the impact of insecticide treatment on kernel damage. When all species and temperatures were analyzed in a single model, the predicted percentage of damaged kernels for the controls containing no insecticide was 8% while only 2% damaged kernels were predicted in the 100% insecticide treatments and 4% damaged kernels were predicted in the 50% treatment (Fig. 10). Overall, the observed percentages of damaged kernels were quite low for both *R. dominica* and *S. cerealella*, so the probabilities of the damage estimates based on the percentage of insecticide treated brown rice in a sample are low for these two species and the confidence intervals for these estimates are wide (Fig. 10). For example, a 1% kernel damage estimate was predicted for treatments containing 9.6–65.4% insecticide treated brown rice at 27 °C while at 22 °C, 1% of the kernels were estimated to be damaged in treatments containing 0–28% insecticide-treated brown rice (Fig. 10). When all temperatures were combined, a 1% kernel damage estimate was predicted for the 5.6–34.5% insecticide treated brown rice (Fig. 10). For *R. dominica*, 10–11% kernel damage was estimated in the controls at 32 °C while 4% kernel damage was predicted for the 100% insecticide treatments at this temperature (Fig. 10). At 27 °C, the damage estimate dropped to 9% in the controls but like 32 °C, 4% damage was predicted at the mixtures containing 100% insecticide treated brown rice (Fig. 10). The highest estimated kernel damage was predicted in *S. oryzae* with a prediction of 2% damage at in the 100% insecticide treatment and 17% damage predicted for the controls at 32 °C. Damage estimates dropped to 10% damage for the controls at 22 °C, but remained at 2% damage for the 100% treatment (Fig. 10). Five percent damage was predicted for mixtures containing 50% treated brown rice in this species (Fig. 10). Notably, no treatments were predicted to completely prevent damage.

4. Discussion

Grain protectants can be used alone or in conjunction with other IPM approaches to reduce damage from insect feeding during storage (Arthur, 1996). Although many formulations with efficacies against a broad range of stored product insects are available (Arthur, 2019a; Daglish et al., 2018; Kavallieratos et al., 2017; Kljajić and Perić, 2009), environmental factors can influence efficacy of these products, including temperature and humidity (Arthur et al., 1992; Athanassiou et al., 2008; Kavallieratos et al., 2017; Singano et al., 2020; Strong and Sbur, 1960). In addition, grain is often mixed with untreated grain as it moves through the supply chain, which can reduce the efficacy of the protectant when it is used alone or in conjunction with cold temperatures or other IPM tactics (Arthur, 1992a). The efficacy of these protectants can also differ among species with different life histories (Mubayiwa et al., 2018). For example, external feeders are exposed to the protectant as both larvae and adults and immediate impacts to both life stages are expected. In contrast, internal feeders that spend the majority of their larval stages feeding inside grain kernels and are not exposed to the insecticide until after adult emergence. Formulation and mode of action can also impact the efficacy of grain protectants on different life stages, sex and insect species (Arthur, 1992b, 2012; Arthur et al., 2020; Banken and Stark, 1997; Yao et al., 2019) Resistance to insecticides, fumigants, and grain protectants has become more common in stored product insects (Arthur, 1996; Boyer et al., 2012; Daglish and Nayak, 2018; Ding et al., 2002) and thus, many insecticides and protectants contain multiple ingredients with different modes of action or with synergists (Arthur et al., 2020; Bomzan et al., 2018; Hewlett, 1951; Panini et al., 2016). Regardless of whether or not a population is resistant to a particular formulation or active ingredient, some life stages are more tolerant than others due to lower rates of feeding or different feeding preferences (Guedes et al., 2009; Zalucki and Furlong, 2017) and different insect species may have slight differences in amino acid sequence or three-dimension structure of protein/receptor binding site that may make it more difficult for the insecticide to bind or interact with its target proteins (Baek et al., 2005; Moores et al.,

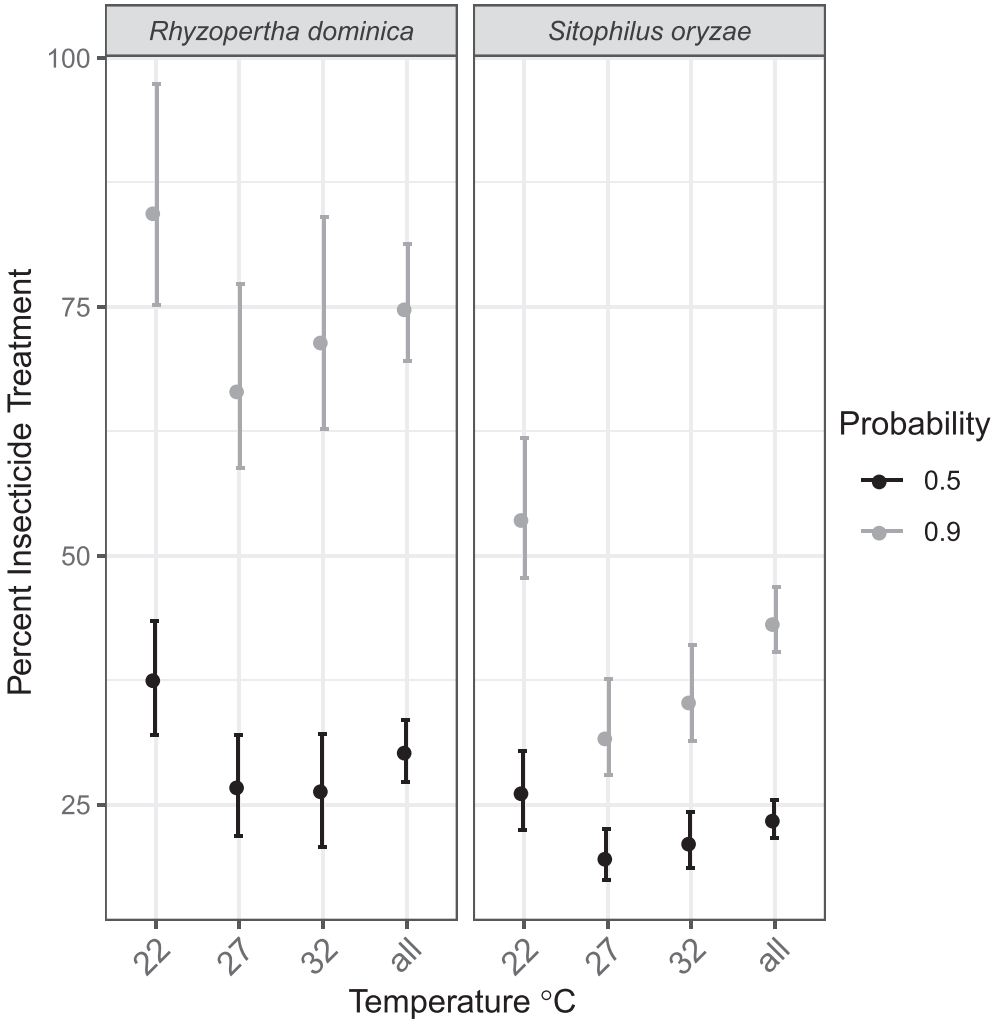

Fig. 9. Estimates of percent Storicide II-treated brown rice to achieve LD_{50} and LD_{90}. Black represents the estimated percentage of treated brown rice needed to achieve 50% mortality and gray represents the estimated percentage required for 90% mortality. Error bars represent 95% confidence intervals. "All" represents LD_{50} and LD_{90} when data from all temperatures were pooled together. LD_{50} and LD_{90} estimates for each temperature are also presented separately to show how temperature impacts these values. LD values for *Sitotroga cerealella* were not calculated because adult mortality data were not collected for this species.

1994). Collectively, these factors make it challenging to identify grain protectants that will adequately reduce damage caused by multiple stored product insects; however, combining insecticide treatment with other pest management tactics, such as cold storage, can provide additional protection from feeding damage. For this study, we investigated the efficacy of Storicide II against three different species of stored product insects at three different temperatures. Because grain is often mixed with untreated grain during processing, we also studied the impact of mixing in untreated grain on efficacy.

Overall, *R. dominica* was consistently more susceptible to lower amounts of Storicide II-treated grain in comparison to *S. oryzae* and *S. cerealella* as indicated by higher adult mortality and lower progeny emergence. Although this susceptibility is partially explained by differences in lifestyle as only *R. dominica* is exposed to the protectant during the larval stage, adult mortality was also significantly higher in *R. dominica* compared to *S. oryzae* even though the adults of both insect species are exposed to the protectant. These findings are consistent with previous studies that also demonstrated that *R. dominica* was more susceptible than *S. oryzae* to pyrethroid grain protectants (Arthur, 1992b). Currently, the underlying mechanisms for this differential susceptibility are not known; however, several factors could be responsible for driving these responses. Both chlorpyrifos-methyl and deltamethrin are contact insecticides that are absorbed through the cuticle and thus, differences in cuticular thickness (Balabanidou et al., 2018) or the presence of waxy, lipids, or sebaceous compounds on the cuticle that reduce the solubility of the insecticide or its ability to penetrate the exoskeleton could be responsible (Motoyama et al., 1992; Patil and Guthrie, 1979). Additionally, the differences in the ability to detoxify the active ingredients may also explain the differential impacts of Storicide II on these two species. Copy numbers of genes coding for detoxification enzymes vary tremendously among insect species (McKenna et al., 2016; Rane et al., 2016) and copy number variations in genes coding for detoxification enzymes have also been linked to tolerance to insecticides previously (Bass and Field, 2011; Puinean et al., 2010). In addition, differences in amino acid sequences of target proteins between these two species may also explain our results. This protectant contains two active ingredients that target different parts of the central nervous system, chlorpyrifos-methyl, which targets acetylcholinesterase, and deltamethrin, which targets sodium ion channels. Differences in the three dimensional structures and/or amino acid sequences of acetylcholinesterase or sodium ion

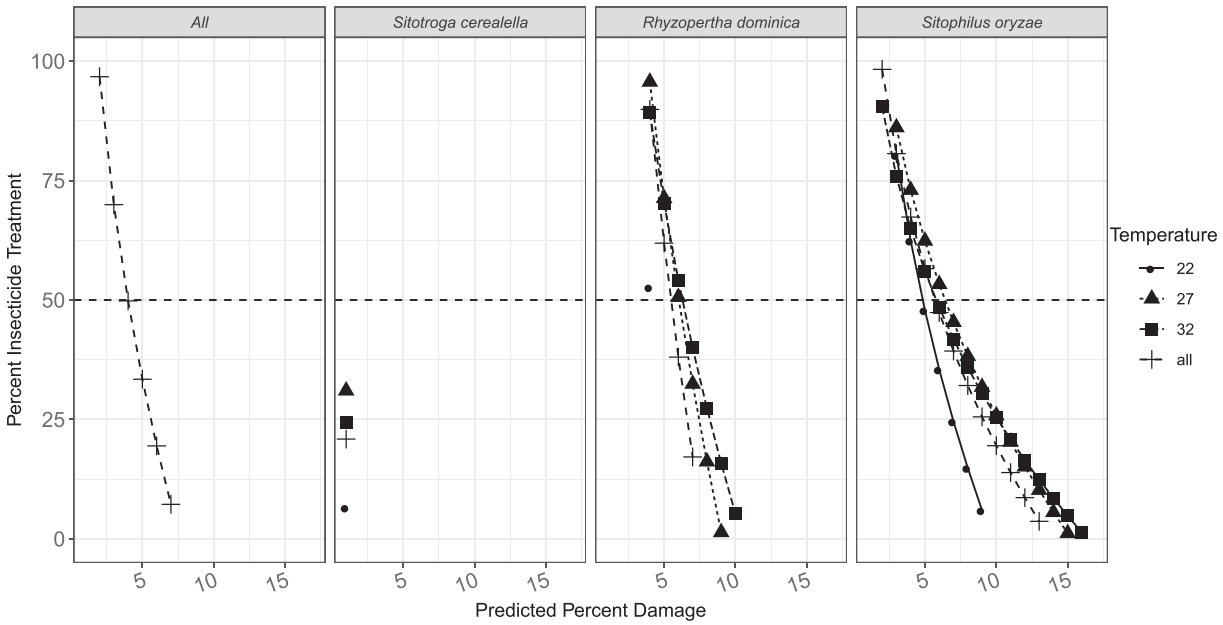

Fig. 10. Estimates of percentage kernel damage for percentage of Storicide II treated brown rice. Numbers in each box represent the percent damage estimate for each percent Storicide II treatment. Error bars represent the upper and lower bounds of the 95% confidence interval.

channels between these two species may impact the ability of these insecticides to interact with their targets (Jackson et al., 2013). Lastly, these differences in susceptibility could also be linked to differences in feeding rates (Gatton et al., 2013; Panini et al., 2016). Even though both ingredients primarily function as contact insecticides, they can both cause impacts on the nervous system if the insecticides are ingested. Adults of both species actively feed on the exterior surfaces of the grain, but the feeding rate or the amount of food consumed could differ between adults of two species, leading one to ingest a higher amount of insecticide than the other.

Sitotroga cerealella were least impacted by the insecticide in comparison to the other two insects when progeny emergence was considered. The efficacy of this insecticide quickly tapered off when 25% or more untreated grain was added to the mixture at 27 °C and when 50% or more untreated grain at 32 °C. There was a significant interaction between temperature and Storicide II treatment on progeny emergence in this species as lower progeny emergence was observed in mixtures containing 25% or more treated grain at 22 °C, indicating that cooling or chilling could be used in conjunction with a grain protectant to reduce progeny emergence during storage. However, these effects were no longer observed when ≤10% treated grain was added to the mixture. The lower efficacy on progeny emergence in this species compared to *S. oryzae* and *R. dominica* and the reduced efficacy of partial insecticide treatments to bulk grain against this species are consistent with previous studies (Arthur, 2019a, 2019b). These observations are probably primarily due to lifestyle. For example, Adult *S. cerealella* do not feed after they emerge from the kernel and are very short-lived (Borzoui and Naseri, 2016). Therefore, they probably do not interact with the exterior surfaces of the grain or come into direct contact with the insecticide as frequently as the other two species. In addition, it is possible that the larvae of *S. cerealella* either may be able to bore into a rice kernel faster than neonate *R. dominica* larvae, or have greater natural tolerance to insecticides, as seen with studies involving rough rice and brown rice treated with the insect growth regulator methoprene (Arthur, 2016). Despite its lower efficacy on this species, Storicide II did reduce progeny emergence relative to the controls, especially when it was stored at cooler temperatures, suggesting that storing the grain at cooler temperatures in conjunction with the grain protectant could reduce damage from *S. cerealealla* (Liu et al., 2016). The same effect was also observed from the other two species. In addition, although total progeny production was not reduced in treatments containing 5–25% treated grain at 27 and 32 °C, the timeline for progeny emergence was significantly altered relative to control treatments. In the control treatments, progeny emergence was largely synchronized and large numbers of progeny tended to emerge around the same time; however, progeny emergence was asynchronous in treatments containing as little as 10 and 25% treated rice. Since this species is short-lived, spreading progeny emergence over longer time could reduce mating and subsequent progeny production into the next generation. Thus, while immediate impacts on progeny production were not necessarily observed when grain was mixed at 10 or 25%, these treatments could have longer term impacts on population levels of *S. cerealella* as has been observed in other insects (Mahmoodi et al., 2020). Similar effects are also likely for *R. dominica* and *S. oryzae* as shifts in progeny emergence curves were also observed for some of the mixtures containing lower percentages of Storicide II-treated grain.

Interestingly, even though adult mortality was higher for *R. dominica* compared to *S. oryzae*, the LD_{50} and LD_{90} values were slightly higher for this species, especially at 22 °C, indicating that the mode of action is not as efficacious. Although temperature also had impacts progeny emergence when it was used either alone or in combination with on *S. oryzae* and *S. cerealella*, the effect of temperature was strongly pronounced in *R. dominica*. Although *R. dominica* can complete development at temperatures as low as 20 °C, but developmental time is prolonged and mortality is often high at low temperatures (Andreadis and Athanassiou, 2017). Thus, for this insect, temperature likely has a more pronounced negative impact on life history, which reduces LD values when reared at low temperatures. In addition, higher LD_{50} and LD_{90} values were also observed for *R. dominica* at 27 and 32 °C even though more mortality was observed in comparison to *S. oryzae*. The reason for the higher LD estimates is likely due to differences in variation. While average mortality was higher in *R. dominica*, more variation in

mortality was observed at almost all treatment combinations tested. In contrast, treatments containing ≥50% treated brown rice had mortalities that were consistently close to 100% for *S. oryzae*. This finding suggests that variation in efficacy among replicates is also an important factor that should be included when assessing an insecticide or a grain protectant.

Overall, Storicide II differentially impacted the life history of three internal feeders when insects were exposed to 100% treated grain regardless of temperature. Immediate impacts to adult mortality were observed to *S. oryzae* and *R. dominica* and impacts to progeny production were observed in all three species, including *S. cerealella*. Efficacy gradually declined as the ratio of untreated grain to treated grain increased, but holding the grain at a lower temperature of 22 °C increased efficacy of the protectant when larger amounts of untreated grain were added to the mixture. The effect of temperature was most pronounced on *R. dominica* as treatments containing as little as 10% treated grain were still successful in reducing progeny emergence in comparison to the control treatment at this temperature. Although adding in ≥50% untreated grain to the mixtures reduced efficacy in the other two insect species, Storicide II treatment impacted the timing and duration of progeny emergence, which could have longer term impacts on population levels beyond those observed in this current study. These impacts on progeny emergence were more pronounced at lower temperatures. Thus, lowering storage temperature is a tactic that managers can implement to improve the efficacy of grain protectants when treated and untreated grain are mixed together.

CRediT authorship contribution statement

Erin D. Scully: Conceptualization, Data curation, Formal analysis, Writing - original draft. **Alison R. Gerken:** Conceptualization, Data curation, Formal analysis, Writing - original draft. **Adriane Fifield:** Data curation. **Valerie Nguyen:** Data curation. **Nicholas Van Pelt:** Data curation. **Frank H. Arthur:** Conceptualization, Data curation, Writing - original draft.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

Funding was provided by USDA-ARS CRIS project number 3020-43000-033-00D. Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture. USDA is an equal opportunity employer and provider.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jspr.2020.101758.

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