



Exposure to a Novel Insecticide Formulation on Maize and Concrete Reduces Movement by the Stored Product Pests, *Prostephanus truncatus* (Horn) and *Sitophilus zeamais* (Motschulsky)

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Two major stored products pests of maize are the *Prostephanus truncatus* (Horn) (larger grain borer) and *Sitophilus zeamais* (Motschulsky) (maize weevil). Under climate change, *P. truncatus* may be expected to shift its distribution northward farther into the United States (US). Thus, there is a critical need to develop diversified chemical control tools in the post-harvest supply chain for these two species. In this study, we investigated the efficacy of a novel insecticide formulation, containing the insect growth regulator, S-methoprene, combined with the pyrethroid, deltamethrin and the synergist piperonyl butoxide (Central Life Science, Schaumburg, IL, US), compared to the existing commercial standard formulation without synergist, and controls at inducing direct mortality and sublethal changes in movement on treated grains as a grain protectant, and on concrete as a surface treatment. Mortality of adults was assessed visually, while movement was tracked with a network camera coupled with Ethovision software that automatically recorded velocity and distance moved by both species, after continuous exposure on treated material for 4–168 h. The novel formulation significantly induced mortality while reducing distance and velocity moved by multiple-fold compared to controls for exposed adults even after relatively brief exposure periods. In fact, the novel formulation was just as effective as the older formulation, but used only a fraction of the active ingredients, thus it may be more cost-effective. Overall, the novel insecticide formulation is a promising tool for controlling *S. zeamais* and *P. truncatus* in bulk storage and around other food facilities.

Keywords: deltamethrin, methoprene, larger grain borer, maize weevil, integrated pest management

INTRODUCTION

Cereal grains comprise 30–60% of the daily caloric intake for humans around the globe. The top three most important cereal crops are maize, rice and wheat (Awika, 2011). Maize is a major staple crop and preferred in southern and eastern Africa, Central America, and Mexico (Awika, 2011; Ranum et al., 2014). It was reported that persons in Mexico consume 267 g of maize per day (Awika, 2011).

However, 10–30% of these important cereal crops, such as maize, are lost to insect pests post-harvest as these grains are harvested, stored, processed, and marketed to consumers (Pimentel, 1991). The major stored product pests of maize are the larger grain borer, *Prostephanus truncatus* (Horn) (Coleoptera: Bostrichidae) and the maize weevil, *Sitophilus zeamais* (Motschulsky) (Coleoptera: Curculionidae). *Prostephanus truncatus* is a long-time pest of stored maize in Mexico, the US, and parts of Central and South America (reviewed in Quellhorst et al., 2021), and was first described in 1878 (Hodges, 1986). However, the insect has also become an invasive species in Africa from 1981 onwards (Golob and Hodges, 1982), where it is estimated by Boxall (2002) to cause 34–40% in losses after only 3 months of storage.

The Southern US is at the very edge of the current range of *P. truncatus*, but the species represents an emerging biosecurity threat for maize production in the country (Arthur et al., 2019). In fact, Arthur et al. (2019) documented *P. truncatus* in three Mexican states directly adjacent to the US, including Nuevo Leon, Sonora, and Tamaulipas, with the closest site located <200 km (or a 3 h drive) from Arizona. While *P. truncatus* has been documented in maize facilities in southern states like Texas (e.g., records found in the research collection at Texas A&M; pers. comm. D. Ludwick), as well as California and Arizona (Cotton and Good, 1937; Dormond, 1998), it has not yet been detected in the breadbasket of the US, such as Kansas and adjacent areas (Quellhorst et al., 2021). The distributions of agricultural insects are predicted to shift under climate change (Macfadyen et al., 2018), and this is often in a polewards direction. As a result, under a changing climate, *P. truncatus* may be expected to become increasingly problematic at higher latitudes in the US. As it does so, it will encounter endemic *S. zeamais* with increasing frequency, and both may contribute to maize damage (Quellhorst et al., 2020).

Given the threat posed by *P. truncatus* and *S. zeamais* to the biosecurity of the US and thus the world's maize supply, it is critical that we expand the integrated pest management chemical toolbox to combat them. Traditionally, stored product insect control has focused on the use of fumigants to reduce consumer complaints, such as phosphine. However, the worker safety in applying fumigants is a concern, and consumers are increasingly demanding products with fewer insecticide inputs (Batte et al., 2007). This issue coupled with a stark rise in the global resistance to phosphine (Nayak et al., 2020) has made the search for additional alternative chemical tools an important focus.

Methoprene was first registered for use after harvest in the US under the name Diacon® in the 1980s (Arthur, 2004). The first commercial formulation of deltamethrin plus methoprene used in the US was Diacon IGR+® (Central Life Sciences, Schaumburg, IL, US). Diacon IGR+ contains 0.05 kg of active ingredient [AI]/L of deltamethrin and 0.12 kg [AI]/L of the insect growth regulator (IGR) methoprene. It can be applied at two commercially labeled rates, 0.5 ppm deltamethrin + 1.25 ppm methoprene, or 1.0 ppm deltamethrin + 2.5 ppm methoprene. For species such as *P. truncatus* and *Rhyzopertha dominica* (Fabricius) (Coleoptera, Bostrichidae), methoprene alone is enough to control these insect species because the eggs are laid outside the grain kernel by mated females (Athanasio

et al., 2010a,b). Thus, there is a brief time where the larva is exposed to the insecticide. However, for species like *S. zeamais*, the addition of deltamethrin is needed to control adults because this species lays eggs inside the grain kernels, thereby protecting them from methoprene exposures (Athanasio et al., 2010a,b).

Gravista® (Central Life Sciences, Schaumburg, IL, US) is a new formulation of the Diacon IGR+ that consists of an IGR (2.85% methoprene, 0.027 kg [AI]/L), combined with the pyrethroid, 1.2% deltamethrin (0.012 kg [AI]/L) and a synergist, 33.3% piperonyl butoxide (0.32 kg [AI]/L). The main difference between the two insecticides (IGR + and Gravista) is the addition of a synergist. Synergists such as piperonyl butoxide (a cytochrome P450 detoxification enzyme inhibitor) allow for less active ingredient (deltamethrin and methoprene) to be incorporated in the product, as in this case, and may also help to control resistant insect populations.

While mortality is a common measurement for assessing the efficacy of an insecticide, it provides an incomplete picture of the full efficacy of a compound. In addition, Guedes et al. (2011) noted the importance of assessing the sublethal effects of exposure to insecticides in order to develop a comprehensive assessment of exposure to insecticides. This can be done using a variety of methodology, but one standard methodology is to use video-tracking coupled with Ethovision® software (Noldus et al., 2001, 2002; Morrison et al., 2018). Prior work has shown deltamethrin, when applied as an emulsifiable concentrate, can significantly decrease the walking ability of *S. zeamais* (Vélez et al., 2019), while no work to our knowledge has assessed sublethal effects of deltamethrin on *P. truncatus*. Further, little work has specifically been done with Diacon IGR+ or Gravista. Understanding the effects of sublethal toxicity on the behavior of insects may help to provide a better understanding of the efficacy of the insecticide in question and its ability to prevent movement and further damage after contact. Thus, our aims were to (i) characterize sublethal changes in movement over time after exposure to an existing formulation (IGR+®) and newer formulation (Gravista®) as a grain protectant on maize and residual spray on concrete surfaces, and (ii) quantify direct mortality from these compounds.

MATERIALS AND METHODS

Insects

Adult *P. truncatus* and *S. zeamais* were obtained from insect colonies kept at the United States Department of Agriculture (USDA) Center for Grain and Animal Health Research facility in Manhattan, KS. Both insects were reared on whole kernels of maize and held in incubators under constant conditions at 27 ± 0.1°C (mean ± SE), 64 ± 1% RH, and 14:10 (L:D) h photoperiod for *S. zeamais* and at 27.2 ± 0.1°C, 37 ± 1% RH, and continuous darkness for *P. truncatus* as mandated by the applicable USDA-APHIS PPQ-approved permit (P526-191021-020).

Insecticide Formulations and Spray Rates on Concrete

There were two insecticides in this study: an existing formulation (tradename: Diacon IGR+®; Central Life Sciences, Schaumburg, IL, USA), and a new formulation with

TABLE 1 | Summary of label, mixing, and application rates for insecticides used in residual assay.

Assay	Max label rate (ppm)				Application rate (per kg or per m ²)		Mixing rate (mL of product)		Amount (mL) applied to 5 kg maize or 93 m ² surface	
	Diacon IGR +		Gravista ^a		Diacon IGR+ ^b	Gravista ^c	Diacon IGR+	Gravista	Diacon IGR+	Gravista
	Deltamethrin	Methoprene	Deltamethrin	Methoprene						
Maize	1	2.5	0.5	1.15	0.02 ^d	0.05 ^d	0.68 ^d	0.6 ^d	3.7	3.7
Concrete	1	2.5	0.5	1.15	0.48 ^e	1.94 ^e	0.3 ^e	0.5 ^e	45	180

^aAlso contains 13.35 ppm of piperonyl butoxide synergist.

^bMixed in 25 mL H₂O.

^cMixed in 10 mL H₂O.

^dAssumes 532 mL (Diacon IGR+) or 1062 mL (Gravista) of product is mixed in 5 gal (=18 920 mL) of water to cover 1,000 bushels of corn (=25 454 kg).

^eAssumes 45 mL (Diacon IGR+) or 180 mL (Gravista) of product is mixed in 1 gal (3784 mL) of water to cover 1,000 ft² (93 m²).

synergist and less concentrated active ingredients (tradename: Gravista®). Diacon IGR+ contains 11.4% methoprene and 4.75% deltamethrin, labeled for application at 0.12 kg AI/L and 0.05 kg AI/L, respectively. Label directions for application as a residual surface treatment give a range of 28.5 mL AI/L–171 mL AI/L H₂O to cover 94 m² for both compounds. To obtain the maximum possible effect, we employed the maximum labeled rate in this study (Table 1), namely 24 mg AI/m² for deltamethrin and 57 mg AI/m² for methoprene. This corresponded to 0.3 ml of the formulation in 25 ml H₂O, sprayed at the rate of 0.3 ml per 50.3 cm² arena, using separate artist’s air brush (Badger 100 series, Badger Corporation, Franklin Park, IL, US) for each treatment. Each replicate was evenly applied to the concrete dish. The new Gravista formulation has one labeled rate, namely 684 ml formulation/L H₂O to cover 92.9 m². Scaling down, we mixed 0.5 ml of the new formulation in 10 ml H₂O. This was sprayed at the same rate as the other compound. Distilled water was used for the control arenas at 0.3 mL per arena. The arenas were allowed to dry overnight (~8 h) prior to use in experiments.

Direct Mortality on Concrete

Concrete arenas were prepared for testing by first mixing a slurry of water and Rockite cement in a large pitcher (Rockite, Cleveland, OH, US; as published prior in Arthur, 2008). Briefly, water was added until a thick paste consistency was achieved. The mixture then sat for 1 min before it was stirred vigorously, making sure any remaining clumps of dry Rockite were hydrated. Once the mixture was a loose, batter-like consistency, 8.5 ml of the slurry was poured into plastic 100 × 20 mm Petri dishes (area of the bottom: 62 cm²; hereafter, arenas). The slurry was poured to approximately 1.27 cm thick. The cement dishes were then left at room temperature to cure for 24 h. The three treatments were sprayed onto the concrete arenas included: control (distilled water), an existing insecticide (Diacon IGR+: 11.40% S-methoprene + 4.75% deltamethrin), and a novel formulation (Gravista: 2.85% S-methoprene + 1.20% deltamethrin + Synergist, 33.30% piperonyl butoxide). There were 6 exposure times: 4, 8, 24, 48, 72, 168 h. A total of 270 individuals per species (*P. truncatus* and *S. zeamais*) were collected, placed in cohorts of 15 individuals per

concrete-filled Petri dish. There were n = 3 replicate arenas per treatment combination.

After exposure, individual *P. truncatus* and *S. zeamais* were removed and placed into clean Petri dish arenas and evaluated for condition. Using a stereomicroscope (SMZ-18, Nikon Inc., Tokyo, Japan) under 60× magnification, *P. truncatus* and *S. zeamais* were classified as alive (moving normally, is able to right itself when flipped over, no twitching), affected (moving sluggishly or erratically, unable to right itself, twitching of antennae or legs may be present), or dead (completely immobile even after prodding) according to prior published definitions (Morrison et al., 2018).

Spray Rates on Treated Maize

Untreated, clean, and uninfested yellow maize was used in the experiments. Grain was sourced from a local farmer in northeast Kansas, US. The highest labeled rate for Diacon IGR+ on maize is 1 ppm deltamethrin and 2.5 ppm of methoprene, which is what we used in this study by adding 0.70 ml of the insecticide to 25 ml of H₂O. After mixing, 0.5 mL was applied to 700 g lots of maize, and this was done three separate times, one for each replicate. For Gravista, the labeled rate on maize is 0.5 ppm deltamethrin and 1.15 ppm of methoprene, which is mixed by adding 0.56 mL of insecticide to 10 mL of H₂O. After mixing, 0.5 mL was applied to 700 g lots of maize, using separate artist’s air brush (Badger 100 series, Badger Corporation, Franklin Park, IL, US; designed to spray a line 3.2–50.8 mm wide with materials of high viscosity) at a pressure of 0.70 kg/cm² for each treatment, and this was repeated three separate times, one for each replicate.

Insect Mortality on Treated Maize

To assess direct mortality on treated maize for application in bulk storage, we followed the same protocol as above for the concrete arenas with a few modifications. Specifically, the experiment was conducted with three bioassay replicates (n = 3); each with 18 combinations of three treatments (IGR+, Gravista, and H₂O Control) and six exposure times (4, 8, 24, 48, 72, 168 h) of each treatment. For each bioassay replicate, 700 g of maize were treated with each insecticide or H₂O (e.g., control) as described above. Each of 18 vials (120-mL volume) was filled with 100 g of treated maize and 30 adult insects of either species, and the

insects were maintained at $27 \pm 0.02^\circ\text{C}$. Only 15 of the 30 insects in each vial were randomly selected and evaluated for direct toxicity at each of six exposure times. Extra insects were provided in these experiments to account for a subset of individuals that may bore completely into the kernel (e.g., especially *P. truncatus*), which would prevent them from being classified and counted. To recover the insects, the grain was sieved (No. 10, 2.0 x 2.0 mm mesh, W.S Tyler, Mentor, OH, US), and shaken to maximize the number of insects falling into the collection pan.

Insect Mobility on Concrete Arenas and in Treated Maize

To assess insecticide effect on insect mobility in both of the bioassays above, a random subset individuals classified as either alive or affected were tracked with a network camera (Basler AG, Ahrensburg, Germany) coupled with Ethovision XT (v. 14.0, Noldus Software, Leesburg, VA, US). Individuals of each species were placed individually into arenas consisting of 90 mm Petri dishes lined with 85 mm Whatman #1 filter (GE Healthcare, Chicago, IL, USA). The camera was placed 80 cm above the arenas, and trials lasted 30 min. At the end of the trial, the total distance moved (cm) and the average instantaneous velocity (i.e., speed with a direction, cm/s) was recorded. To account for cursor bounce, an input filter was applied that discarded the accumulated distance if it was >5 cm per s. Each trial was manually checked for irregularities, and any with suspicious accumulations of distance were re-run. There were a total of $n = 15$ replicate individuals per treatment combination, totaling to 234 h of video for 468 individuals per species and assay.

Statistical Analysis

A generalized linear model (GLM) based on a quasibinomial to account for overdispersion in the dataset (Aho, 2017) was used to assess differences in percentage of mortality, with a separate model used for each species and assay. Fixed, explanatory variables included insecticide (Diacon IGR+, Gravista, and control), exposure time (0, 4, 8, 24, 48, 72, 168 h), and their interaction. Likelihood ratio tests were conducted to calculate the χ^2 -value and determine significance of each variable in a model with (alternative hypothesis) or without the variable (null hypothesis) in question. Upon a significant result from the GLM, Tukey HSD was used for multiple comparisons and implemented with the glht function from the multcomp package (Hothorn et al., 2008). Unless otherwise specified, $\alpha = 0.05$, and R Statistical Software was used (R Core Team, 2019).

To analyze the data from the insecticide effect on insect mobility, a special case of a GLM was used based on a Gaussian distribution (e.g., general linear model) with distance moved and velocity as the response variables to account for the fact that each response was continuous, unbounded, and normally distributed (Bolker et al., 2009). A single model was created for each assay and species. The model used insecticide, exposure time, and their interaction as fixed, explanatory variables. Upon a significant result from the general linear model, Tukey HSD was used for multiple comparisons.

TABLE 2 | Summary of statistical GLM model results for mortality of *S. zeamais* and *P. truncatus* in the concrete assay and maize assay.

Factor	Concrete assay			Maize assay		
	df	χ^2	P	df	χ^2	P
<i>S. zeamais</i>						
Alive						
Insecticide	2	582	0.0001	2	2421	0.0001
Exposure	5	6.95	0.22	5	1.99	0.85
Interaction	10	85.9	0.0001	10	61.1	0.0001
Affected						
Insecticide	2	2509	0.0001	2	509	0.0001
Exposure	5	58.6	0.0001	5	25.7	0.001
Interaction	10	19.9	0.05	10	1.01	0.99
Dead						
Insecticide	2	20.3	0.0001	2	19.2	0.0001
Exposure	5	84.0	0.0001	5	104	0.0001
Interaction	10	11.8	0.30	10	4.22	0.94
<i>P. truncatus</i>						
Affected						
Insecticide	2	8,37,709	0.0001	2	1,13,944	0.0001
Exposure	5	15.0	0.05	5	15.0	0.05
Interaction	10	0.00	1.00	10	0.00	1.00
Dead						
Insecticide	2	2,573	0.0001	2	529	0.0001
Exposure	5	103	0.0001	5	42.7	0.0001
Interaction	10	2.35	0.99	10	2.75	0.99
Dead						
Insecticide	2	30.9	0.0001	2	30.5	0.0001
Exposure	5	60.6	0.0001	5	87	0.0001
Interaction	10	5.11	0.88	10	4.79	0.90

RESULTS

Mortality on Concrete

On concrete, the insecticide had a significant overall effect on mortality (percent of individuals affected and dead) compared to the control for both *S. zeamais* and *P. truncatus* (Table 2, Figure 1). However, mortality was not significantly different for the two insecticide formulations, IGR+ and Gravista. The exposure time also significantly affected the condition of adults of both *S. zeamais* and *P. truncatus*, with more adults dead at 72 and 168 h than at other periods (Figure 2). *Prostephanus truncatus* was slightly more susceptible to both insecticide formulations with no “alive” individuals (all affected or dead) compared to some *S. zeamais* individuals remaining alive. Mortality reached 33–37% for insecticide-exposed *S. zeamais*, while it was 40–47% for insecticide-exposed *P. truncatus* by 168 h. Nonetheless, the majority of individuals were simply still affected. There was no significant interaction between exposure time and insecticide among the health conditions and species for the concrete assay, except for the percent of affected adult *S. zeamais*, where there were initially more alive adults after treatment with Gravista (Figure 2).

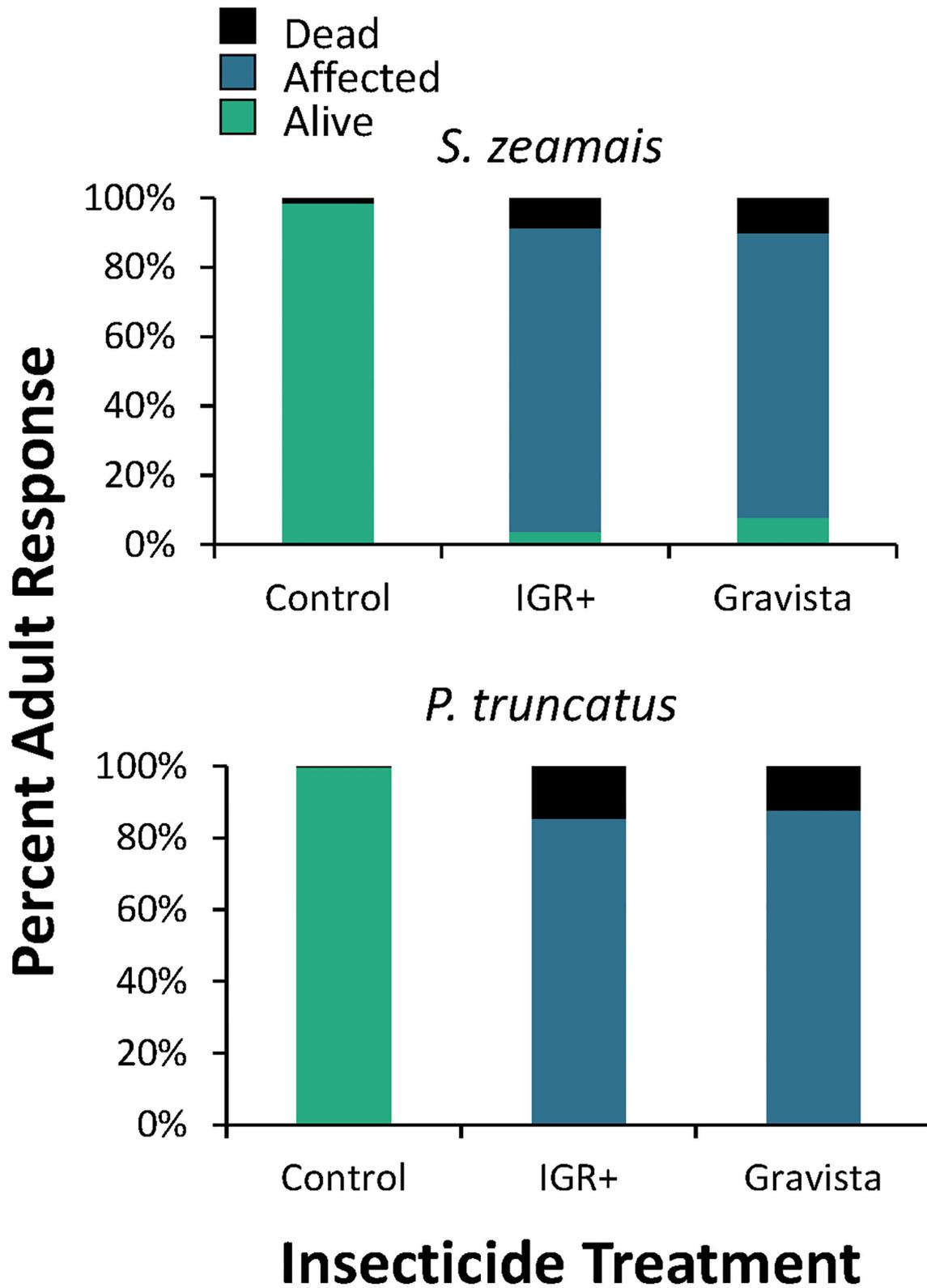
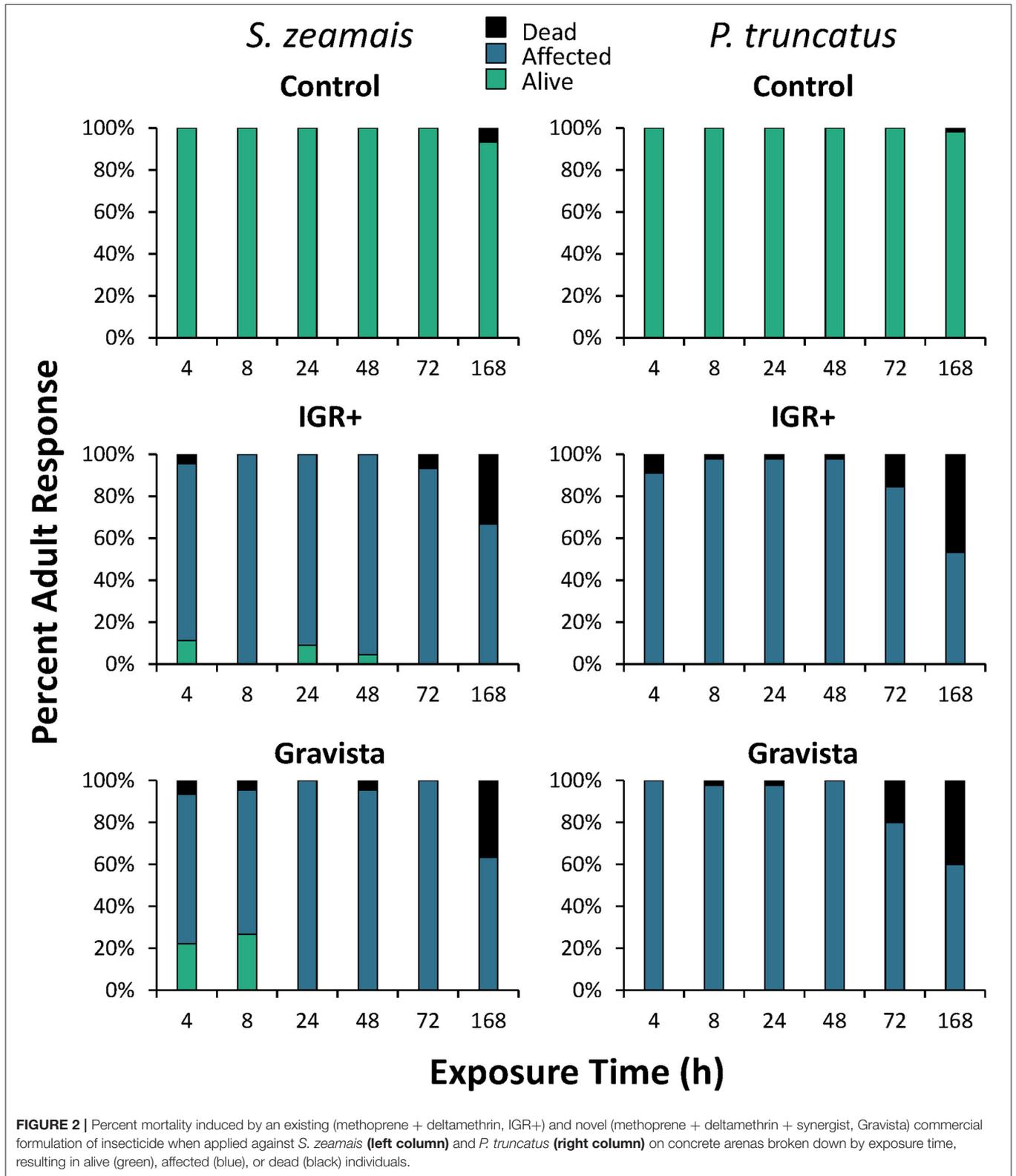


FIGURE 1 | Overall percent mortality induced by an existing (methoprene + deltamethrin, IGR+) and novel (methoprene + deltamethrin + synergist, Gravista) commercial formulation of insecticide when applied against *S. zeamais* (top panel) and *P. truncatus* (bottom panel) on concrete arenas after exposure for 4–168 h, resulting in alive (green), affected (blue), or dead (black) individuals.



Mobility Effects on Concrete

On concrete, the distance moved by *S. zeamais* was significantly affected by exposure to both insecticide formulations (IGR+ and Gravista; **Table 3**). IGR+-exposed *S. zeamais* showed a nearly 5-fold decrease in movement compared to the control, while Gravista-exposed *S. zeamais* exhibited a nearly 7-fold decrease

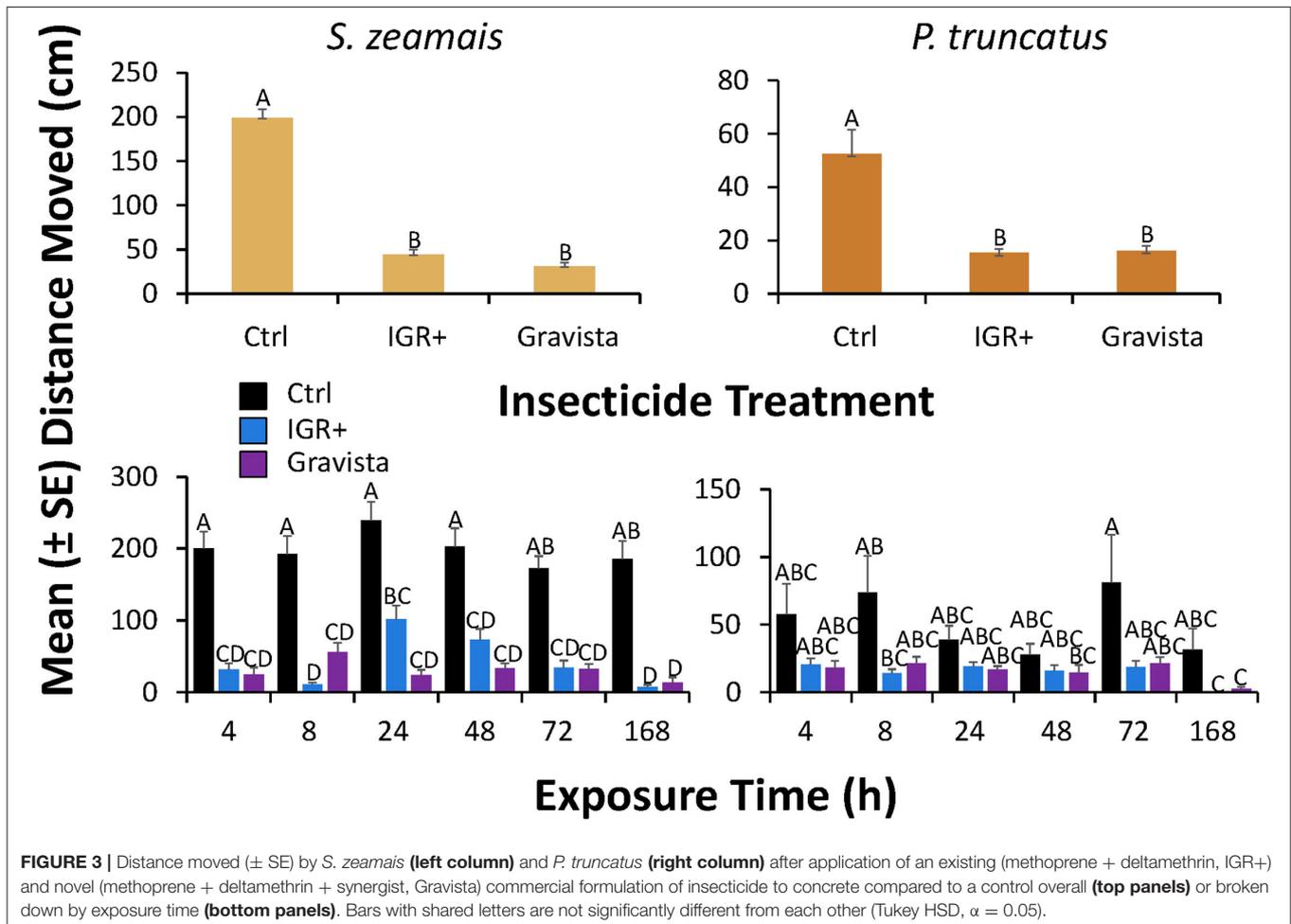
(**Figure 3**). Exposure time significantly affected the distance moved by *S. zeamais*, with adults moving 1.8-fold less 168 h after exposure compared to 24 h after exposure. The interaction between insecticide and exposure time was not significant for *S. zeamais*.

Moreover, on concrete, the distance moved by *P. truncatus* was also significantly affected by exposure to both insecticide formulations (**Table 3**). IGR+-exposed *P. truncatus* saw a nearly 4-fold decrease in distance moved compared to the control. Gravista-exposed *P. truncatus* saw a 3-fold decrease in movement compared to the control (**Figure 3**). *Sitophilus zeamais* moved an average of 200 cm compared to 53 cm by *P. truncatus*. Thus, a greater decrease in movement can be seen for both insecticide formulations for *S. zeamais*. Exposure time significantly affected the distance moved by *P. truncatus*, with adults moving 3.6-fold farther 72 h after exposure than 168 h after exposure. The interaction between insecticide and exposure time was significant (**Table 3**), with less movement in control individuals at 24 and 48 h compared to 4, 8, and 72 h (**Figure 3**).

On concrete, the velocity by *S. zeamais* was significantly affected by exposure to both insecticide formulations (IGR+ and Gravista) compared to controls (**Table 4**). IGR+-exposed *S. zeamais* exhibited a nearly 5-fold decrease in velocity compared

TABLE 3 | Summary of statistical model results for GLM of distance moved by *S. zeamais* and *P. truncatus* in the after exposure in the concrete assay and maize assay.

Factor	Concrete assay			Maize assay		
	df	F	P	df	F	P
<i>S. zeamais</i>						
Insecticide	2	23.5	0.0001	2	103	0.0001
Exposure	5	3.71	0.01	5	14.8	0.0001
Interaction	10	1.47	0.15	10	10.3	0.0001
<i>P. truncatus</i>						
Insecticide	2	50.2	0.0001	2	219	0.0001
Exposure	5	13.8	0.0001	5	11.9	0.0001
Interaction	10	3.36	0.0001	10	2.32	0.01



to the control, while Gravista-exposed *S. zeamais* showed a nearly 7-fold decrease (Figure 4). Furthermore, exposure time significantly altered the velocity by *S. zeamais*, with adults moving 1.8-fold slower 168 h after exposure compared to 72 h afterwards. The interaction between insecticide and exposure time had a significant effect on the velocity (Table 4), with *S.*

zeamais moving 1.4–14-fold faster 24 h after exposure to IGR+ compared to 4 and 168 h (Figure 4).

Similarly, the velocity by *P. truncatus* was significantly affected by exposure to IGR+ and Gravista compared to a control (Table 4). IGR+- and Gravista-exposed *P. truncatus* showed a 2-fold decrease in velocity compared to the control. Again, it should be noted that, *S. zeamais* is a much more mobile species compared to *P. truncatus*, with control *S. zeamais* velocity on average 0.11 cm/s compared to 0.03 cm/s by *P. truncatus*. Thus, the greater decrease in velocity can be seen for both insecticide formulations for *S. zeamais*. By contrast, exposure time did not significantly affect the distance moved by *P. truncatus*. There was no significant interaction between the insecticide and exposure time on velocity.

TABLE 4 | Summary of statistical model results for GLM of velocity by *S. zeamais* and *P. truncatus* in the after exposure in the concrete assay and maize assay.

Factor	Concrete assay			Maize assay		
	df	F	P	df	F	P
<i>S. zeamais</i>						
Insecticide	2	21.8	0.0001	2	78.8	0.0001
Exposure	5	4.91	0.001	5	29.1	0.01
Interaction	10	3.75	0.001	10	6.07	0.15
<i>P. truncatus</i>						
Insecticide	2	2.97	0.05	2	2.90	0.05
Exposure	5	0.811	0.54	5	0.07	0.99
Interaction	10	2.08	0.05	10	2.42	0.01

Mortality on Maize

The overall mortality on maize was significantly different after exposure to both IGR+ and Gravista compared to the control for both *S. zeamais* and *P. truncatus* (Table 2, Figure 5). However, mortality (percent dead and percent affected) was not significantly different for the two insecticide formulations, IGR+ and Gravista. Exposure time significantly affected mortality by both species also (Figure 6), but there was no significant

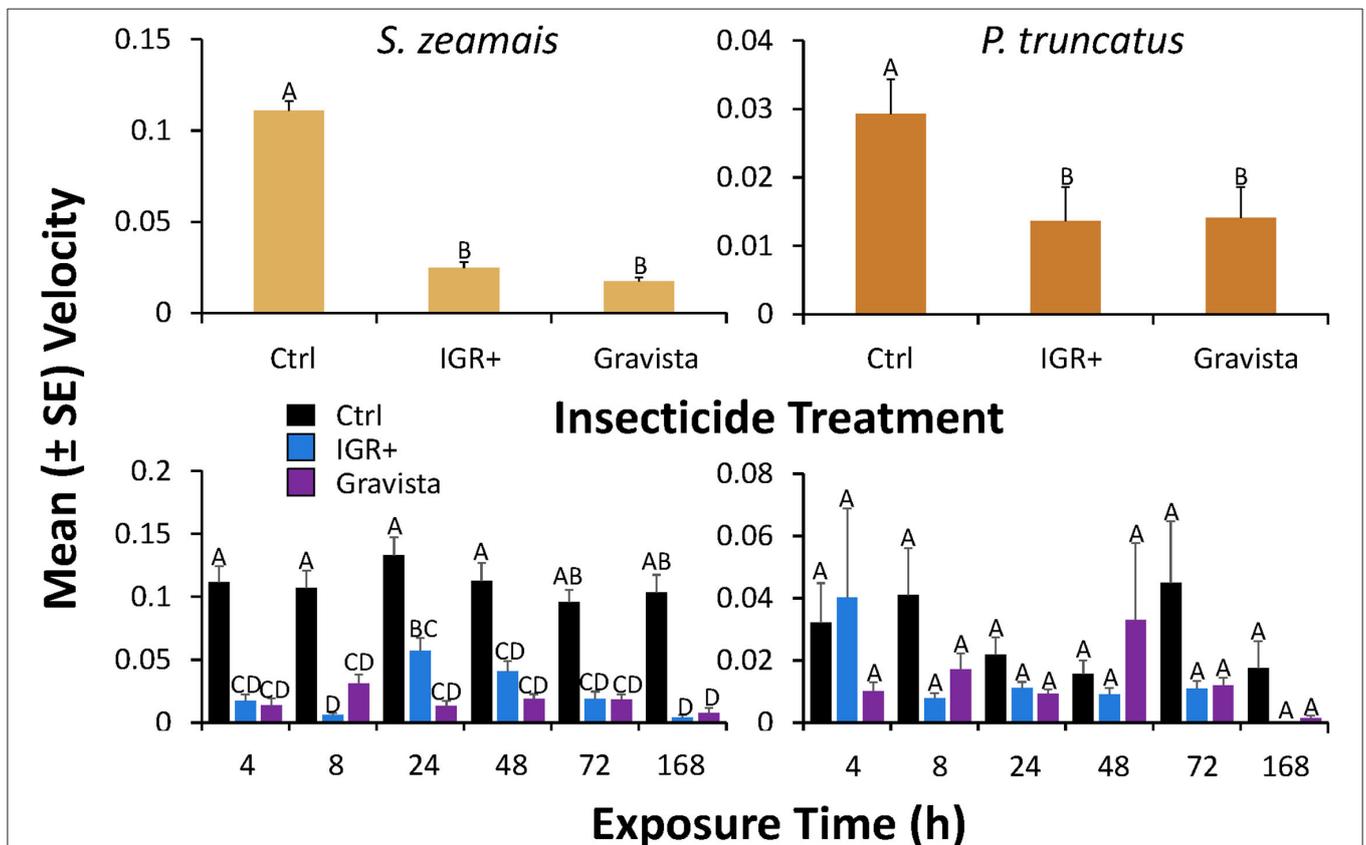


FIGURE 4 | Velocity (± SE) by *S. zeamais* (left column) and *P. truncatus* (right column) after application of an existing (methoprene + deltamethrin, IGR+) and novel (methoprene + deltamethrin + synergist, Gravista) commercial formulation of insecticide to concrete compared to a control overall (top panels) and broken down by exposure time (bottom panels). Bars with shared letters are not significantly different from each other (Tukey HSD, $\alpha = 0.05$).

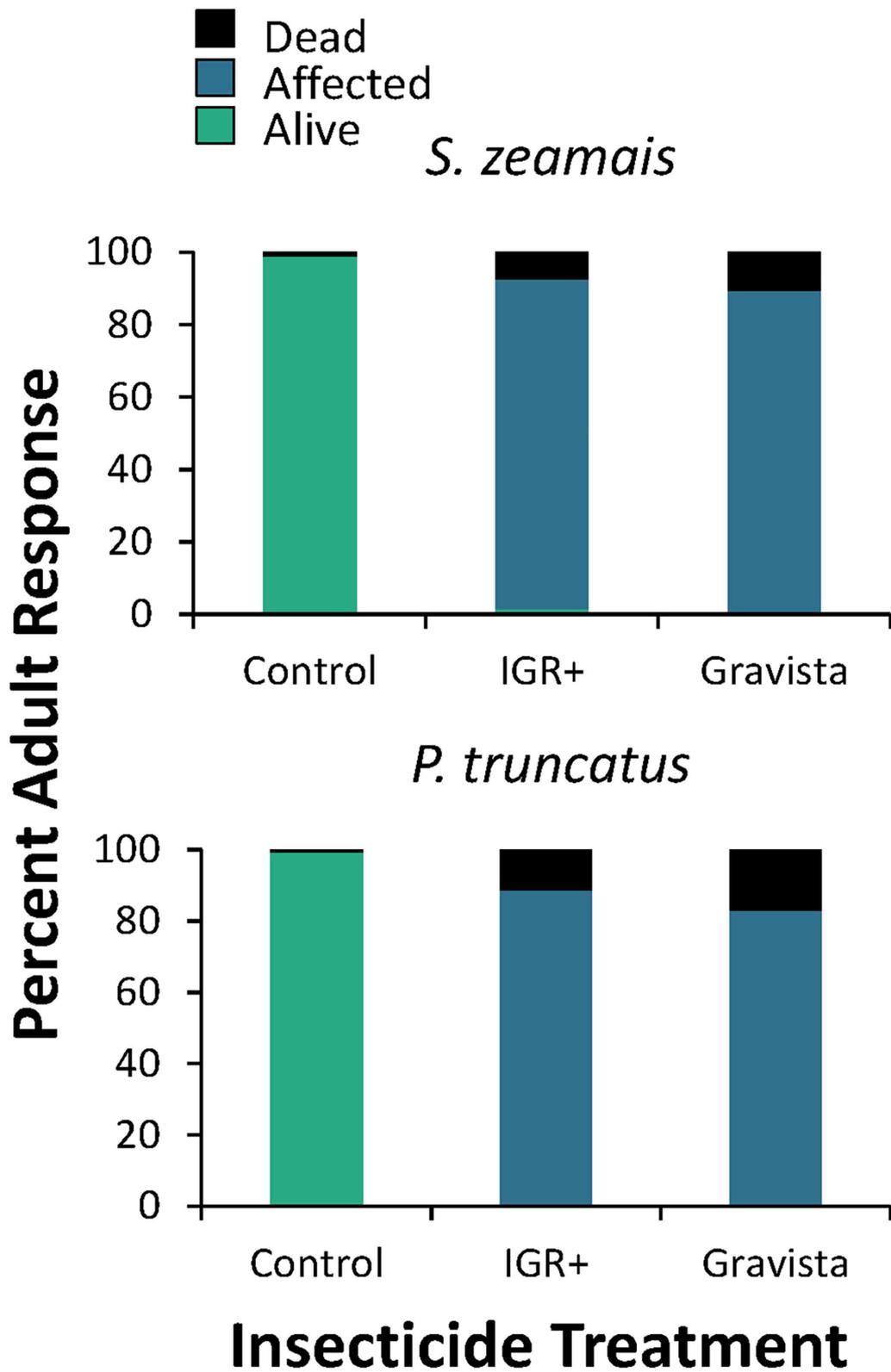
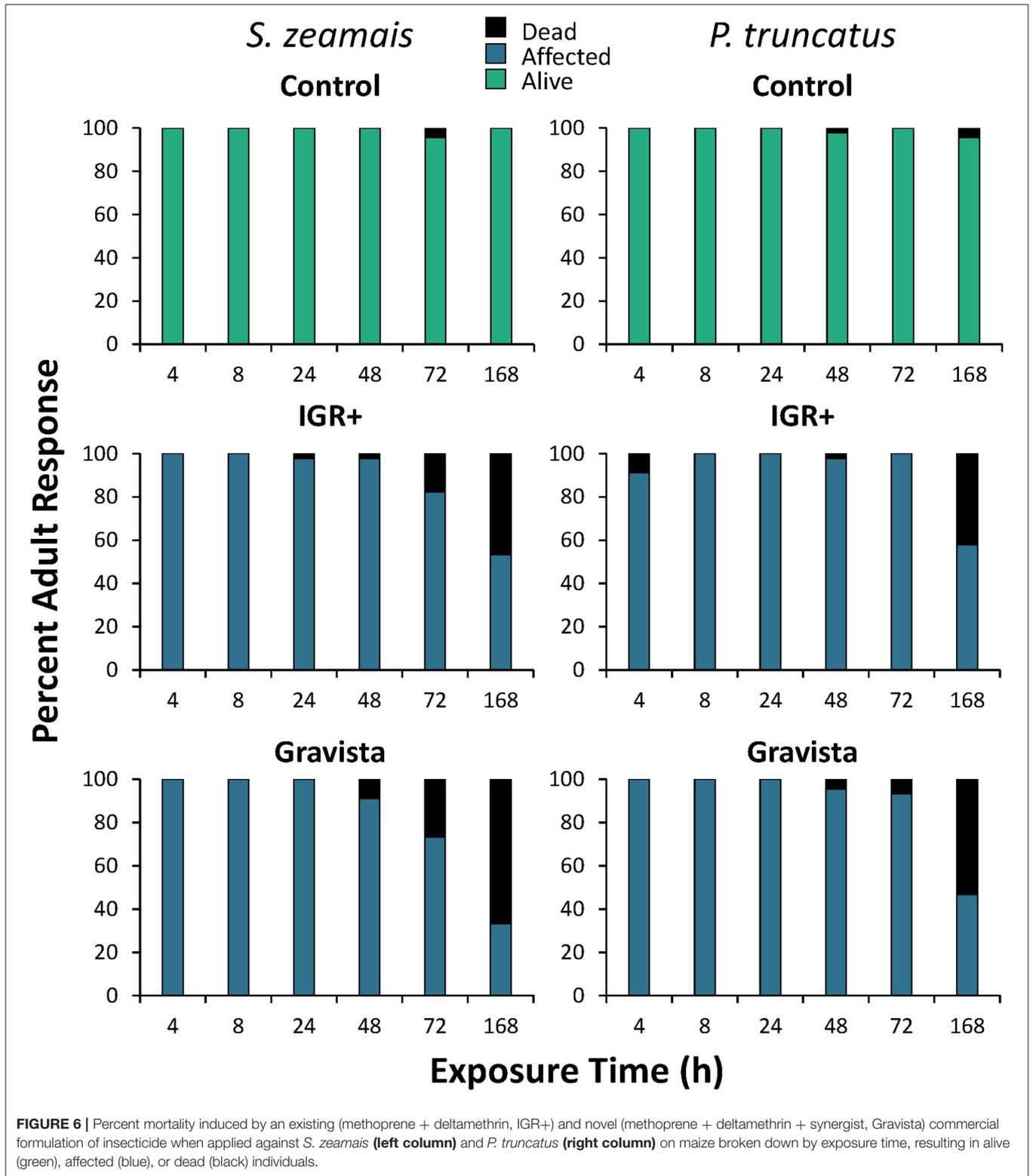
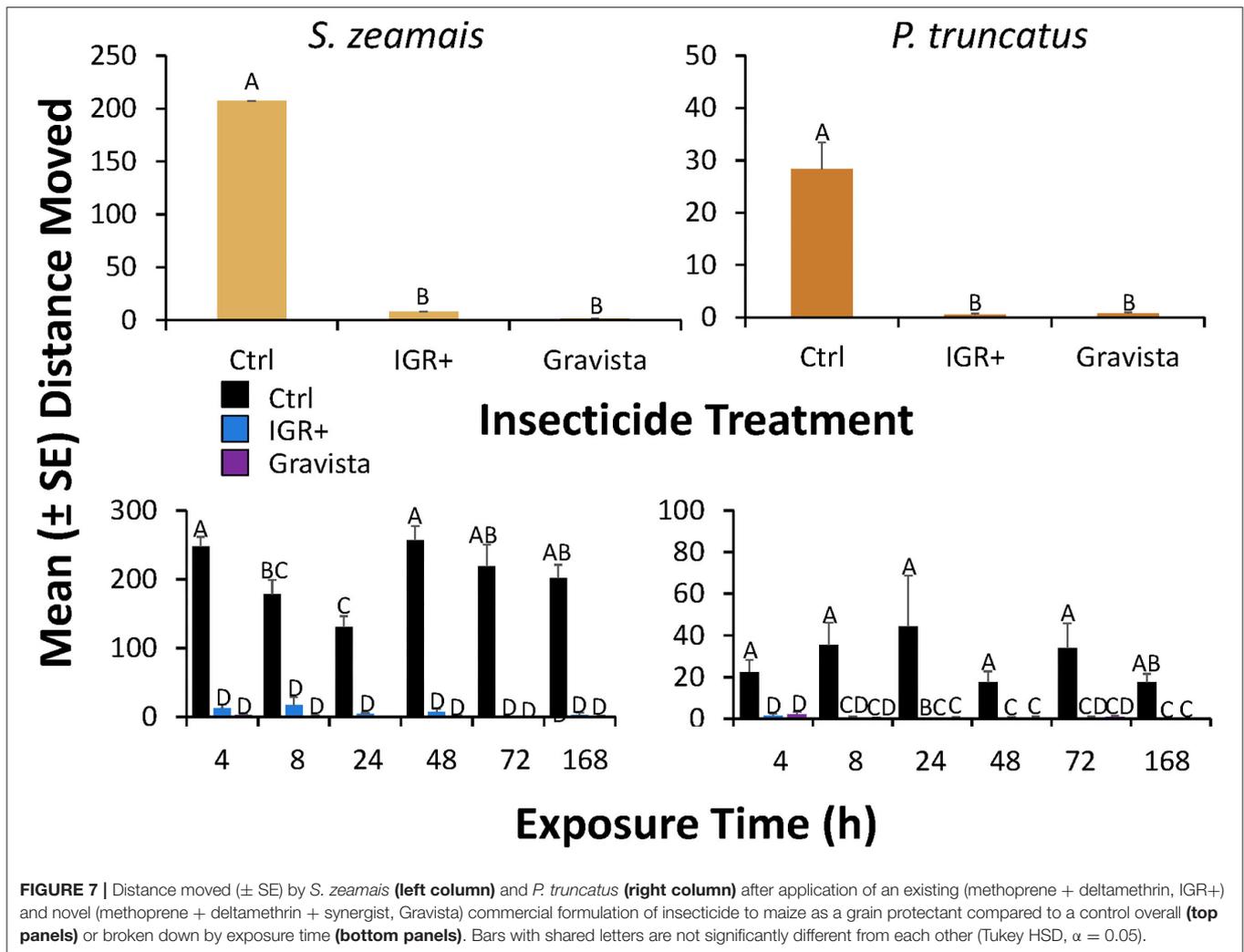


FIGURE 5 | Overall percent mortality induced by an existing (methoprene + deltamethrin, IGR+) and novel (methoprene + deltamethrin + synergist, Gravista) commercial formulation of insecticide when applied against *S. zeamais* (top panel) and *P. truncatus* (bottom panel) as a grain protectant on maize after exposure for 4–168 h, resulting in alive (green), affected (blue), or dead (black) individuals.



interaction between insecticide and exposure time on the percent of affected and percent of dead adults for either species (Table 2). As exposure time increased, so too did the number of dead

adults, which reached 47–67% and 42–53% for insecticide-exposed *S. zeamais* and *P. truncatus* by 168 h, respectively. *Prostephanus truncatus* was slightly more susceptible to both



insecticide formulations with no “alive” individuals (all affected or dead) compared to the *S. zeamais*. *Sitophilus zeamais* was more susceptible on maize compared to exposure on concrete with <1% left “alive” compared to nearly 8% “alive” on concrete.

Mobility Effects on Maize

On maize, the distance moved by *S. zeamais* was significantly affected by exposure to IGR+ and Gravista compared to the control (Table 3). IGR+-exposed and Gravista-exposed *S. zeamais* showed a 26-fold and 148-fold decrease, respectively, in distance moved compared to the control (Figure 7). Exposure time significantly affected the distance moved by *S. zeamais*, with adults 4 h after exposure moving 2-fold more than those 24 h afterwards. There was a significant interaction between insecticide and exposure time on the distance moved by *S. zeamais*, with control adults moving the least 24 h after exposure to the insecticide treatments and the most 4 and 48 h afterwards (Figure 7).

In addition, on maize, the distance moved by *P. truncatus* was significantly affected by exposure to both insecticide

formulations (Table 4). IGR+-exposed *P. truncatus* saw a 49-fold decrease in movement compared to the control, while Gravista-exposed *S. zeamais* exhibited a 39-fold decrease in movement compared to the control. Exposure time did not significantly affected the distance moved by *P. truncatus*, with adults moving 2.5-fold more after 24 h exposure compared to 168 h afterwards. There was a significant interaction between insecticide and exposure time on distance moved by *P. truncatus*, with movement less at later exposure times for the control adults (Figure 7). It should be noted that both *S. zeamais* and *P. truncatus* were more susceptible to both insecticide formulations when exposed on maize vs. concrete, as both species saw nearly zero movement after exposure compared to the controls. When exposed on concrete, *S. zeamais* moved on average as much as 44 cm after exposure and *P. truncatus* 16 cm after exposure.

Similarly, on maize, the velocity by *S. zeamais* was significantly affected by exposure to both insecticide formulations (Table 4). IGR+-exposed and Gravista-exposed *S. zeamais* saw a 30-fold and 150-fold decrease, respectively, in velocity compared to the control (Figure 8). Exposure time significantly affected the

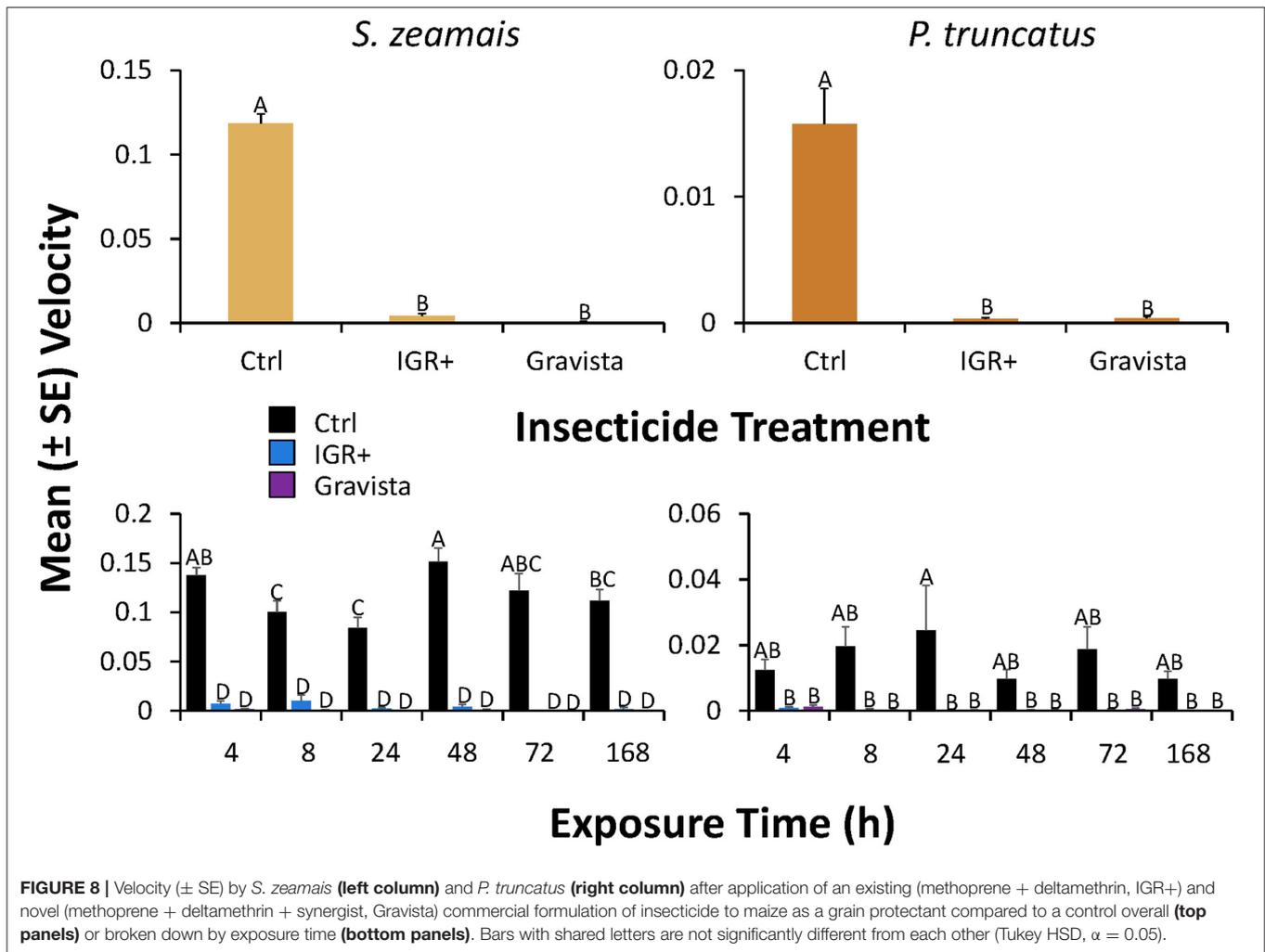


FIGURE 8 | Velocity (± SE) by *S. zeamais* (left column) and *P. truncatus* (right column) after application of an existing (methoprene + deltamethrin, IGR+) and novel (methoprene + deltamethrin + synergist, Gravista) commercial formulation of insecticide to maize as a grain protectant compared to a control overall (top panels) or broken down by exposure time (bottom panels). Bars with shared letters are not significantly different from each other (Tukey HSD, $\alpha = 0.05$).

velocity by *S. zeamais*, with adults moving 1.8-fold more 48 h after exposure compared to 24 h afterwards. The interaction between insecticide and exposure time did not have a significant effect on velocity moved by *S. zeamais*.

Finally, the velocity by *P. truncatus* on maize was significantly affected by exposure to both insecticide formulations (IGR+ and Gravista) (Table 4). IGR+-exposed *P. truncatus* showed a nearly 67-fold decrease in movement compared to the control, while Gravista-exposed *P. truncatus* exhibited a 50-fold decrease (Figure 8). Exposure time did not significantly affect the distance moved by *P. truncatus*. Baseline mobility was greater for *Sitophilus zeamais*, with *S. zeamais* control individuals moving an average of 0.12 cm/s compared to 0.02 cm/s by *P. truncatus*.

DISCUSSION

We examined both mortality and sublethal effects by the novel insecticide (Gravista) compared to IGR+ for two stored products pests. Up to this point, no one has evaluated the baseline mobility or sublethal effects of insecticides on the invasive

P. truncatus. Importantly, we found that both compounds significantly reduced the movement of *P. truncatus* and *S. zeamais* compared to the controls, even after brief 4-h exposures. This is the first reported experiment using Ethovision coupled with video-tracking to capture sublethal reduction in movement (e.g., distance moved and velocity) related to an insecticide for *P. truncatus*. Baseline movement of *P. truncatus* decreased from 28–52 cm in 30 min in the controls to 0.56–16 cm, on average, while *S. zeamais* distance moved decreased from 199–207 cm to 7–44 cm in the same period. The reduction in movement by *S. zeamais* (5–27-fold) is in alignment with prior results on sublethal exposure of the species to deltamethrin (Morrison et al., 2021). *Prostephanus truncatus* movement was reduced by 3–50-fold compared to control individuals. The use of IGR+ and Gravista has the potential to manage both *P. truncatus* and *S. zeamais* and prevent their further movement. As Guedes et al., 2011 pointed out, understanding the sublethal effects of insecticide exposure allows for a more comprehensive picture of efficacy compared to solely focusing on direct lethality. Our study adds to a growing body of literature with different species of stored products insects that document sublethal exposure to

insecticides (Morrison et al., 2018, 2019, 2021; Arthur et al., 2020; Wilkins et al., 2020).

Both IGR+ and Gravista provided effective control of both *P. truncatus* and *S. zeamais*, but the latter does it with 4-fold less active ingredient because of the addition of a synergist. Both insecticides caused direct mortality in the two pests, though markedly greater in *P. truncatus* compared to *S. zeamais*. It was clear *P. truncatus* was the more susceptible species, with a mortality of 40–47% by 168 h on concrete, whereas *S. zeamais* mortality reached 33–37%. Interestingly, mortality was higher when insects were exposed on grain instead of concrete. Mortality reached 47–67% and 42–53% for insecticide-exposed *S. zeamais* and *P. truncatus* by 168 h on maize. By contrast, immediately after treatment with Gravista in a residual study, *S. oryzae* mortality was 91% after 168 h (Arthur and Morrison, 2020). The differences in the mortality for the two weevils could be the result of the recent range expansion of *S. zeamais* (Corrêa et al., 2017), and its greater resistance to insecticides more generally (Ribeiro et al., 2003). Regardless, both IGR+ and Gravista can be considered effective at inducing mortality in both species even over the brief exposure times evaluated in this study.

In our study, we evaluated the relative efficacy of IGR+ and Gravista as a residual treatment on concrete or as a grain protectant on maize. We found efficacy was greater when insects were placed on maize than on concrete. There have been many studies that examine how the efficacy of insecticides varies on different substrates. These studies often compared concrete to varnished wood, metal, tile or laminated paper (Williams et al., 1983; White and Leesch, 1996; Arthur, 1997, 2008; Hagstrum and Subramanyam, 2006; Jenson et al., 2009; Wijayaratne et al., 2012). Generally, insecticides are found to have the greatest efficacy on non-porous substrates such as metal or tile, followed by porous substrates like concrete or wood. In addition, residual efficacy of insecticides was found to be greatest when applied on metal surfaces (Jankov et al., 2013). Concrete generally absorbs much of the formulation applied to it. In a few cases, depending on the insecticide and the insect species, efficacy was actually greater on concrete compared to other substrates. For example, when insecticidal pyrrole chlorfenapyr was applied to concrete, vinyl tile, and plywood surfaces, control of two *Tribolium* species was greater on concrete compared to vinyl tile and plywood (Arthur, 2008). As Chadwick (1985) noted, substrate type and its porosity may impact the absorption of the insecticide into the substrate and subsequent degradation of the insecticide. It would appear that at least in this study, IGR+ and Gravista were more effective on maize kernels that had been sprayed with insecticide compared with concrete, which had likely absorbed more of the insecticide below the surface, sequestering it from contact with the insects. It is possible that only the legs come into contact with the residual insecticides when insects move on the treated but flat concrete surface whereas the rest of the body can also come into contact with the residual insecticides when insects move around the treated maize kernels. However, both IGR+ and Gravista seem useful both as a residual surface treatment and as a grain protectant despite their varying efficacy in those forms. Both residual treatments

around a food facilities and grain protectants for bulk storage situations have an role to play in comprehensive integrated pest management programs (Arthur and Subramanyam, 2012), with both of the compounds assessed in this study showing promise for incorporation.

Another aspect that may affect efficacy is the development of resistance to deltamethrin by one or both species. The use of deltamethrin in the US is relatively new, and to date, there has been no documented instance of resistance. However, there has been documented deltamethrin resistance in a variety of other countries, including Australia (Daglish and Nayak, 2018) and Brazil (Guedes et al., 2009). Incomplete dosing is known to induce resistance, but this is not likely to occur with the lower amounts of active ingredients in Gravista, because there is a much higher amount of synergist, which often acts to break resistance to pyrethroids in a variety of systems (Forrester et al., 1993).

Currently, *S. zeamais* is one of the most important pests of stored maize grains in the US and elsewhere. Despite the significance of the *S. zeamais* as a pest of maize grains globally, there is relatively little information compiled about the life history and biology of the insect pest. This could be due to the fact that *S. zeamais* is nearly indistinguishable from *S. oryzae* and was often referred to as “*Sitophilus* species” in the literature. *Sitophilus zeamais* is less selective when it comes to the commodities it attacks, feeding on commodities as diverse as apples, rice, wheat, and maize (Longstaff, 1981). The damage caused on maize by *S. zeamais* has been estimated by Boxall (2002) to be 10–20% after only 3 months in storage.

While *S. zeamais* is already present in the US, *P. truncatus* may invade under shifting distributions from climate change. Both are important and pernicious pests of maize (Quellhorst et al., 2021). Thus, the use of IGR+ and Gravista as crack-and-crevice treatments in or around food facilities in the US where grains are typically kept and grain protectant treatments of kernels stored for extended periods in bulk storage may be especially beneficial. Most food facilities lose some maize in both quantity and quality over the time if stored for extended periods. Both compounds appear like feasible options. Gravista, a formulation with much less active ingredient, may be an option to delay resistance development or overcome existing resistance problems for food facilities, because of the synergist.

While we have assessed both direct and sublethal effects of two compounds on two economically important species in stored products using two application methods, in the future it will be important to (1) determine whether exposure to these compounds affects dispersal to novel food patches, (2) evaluate changes in flight capacity after exposure to insecticides (e.g., Morrison et al., 2017), and (3) elucidate molecular mechanisms of stress after exposure to these compounds. While prior work has documented that *S. zeamais* and *P. truncatus* prefer different ends of the temperature spectrum (Quellhorst et al., 2020), it is unknown how temperature mediates response especially to the new formulation with synergist (e.g., Gravista). Follow-up studies on these topics will help more fully characterize important new compounds coming onto the market for stored

product protection, while providing information on potential new chemical tools for stakeholders.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

HQ, FA, and WM conceived of the experiments and methodology. AB, FA, and HQ set up the experiments and collected the data. HQ and WM wrote a first draft of the MS and jointly performed the statistical analysis. FA, HQ, WM, and KZ obtained funding for the research. FA, KZ, and WM provided supervision and facilities. All authors reviewed and edited drafts of the manuscript. All authors contributed to the article and approved the submitted version.

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