Research Article

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Characterizing and predicting sublethal shifts in mobility by multiple stored product insects over time to an old and novel contact insecticide in three key stored commodities

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Abstract

BACKGROUND: There has been a push to diversify integrated pest management (IPM) programs away from exclusive fumigant use in food facilities. Residual insecticides increasingly have been included among plans. In stored products, sublethal toxicity has been neglected in favor of evaluating direct mortality. Here, we evaluated the movement of *Tribolium castaneum*, *Rhyzopertha dominica*, *Sitophilus oryzae* and *Sitophilus zeamais* in response to aged residues of an existing (Diacon IGR+® with 11.4% methoprene + 4.75% deltamethrin) and novel (Gravista® with 2.85% methoprene + 1.2% deltamethrin + 33.3% piperonyl butoxide synergist) residual insecticide.

RESULTS: Using the maximum labeled rate and two exposure times for each species, we assessed distance moved and velocity on wheat, rice and corn. Assessments were made from commodity residues aged between 0 and 12 months (at 3-month intervals). We found that after exposure, movement was reduced by 50–88% and equally by adults exposed to each insecticide formulation compared to untreated controls. After initial application, predicted distance moved increased from 4 to 14 m then 28 m in a 24 h period at 3 and 12 months post-application, respectively. Effectiveness of each insecticide at suppressing movement generally declined by 9–12 month post-application.

CONCLUSIONS: Given the quick and dramatic increases in sublethal movement after initial application, our results suggest that sanitation programs in post-harvest environments are extremely important and it may be beneficial to pair chemical control with monitoring to prevent dispersal of affected insects to new areas of a facility.

Keywords: sublethal toxicity; *Tribolium castaneum*; *Rhyzopertha dominica*; weevils; synergist; deltamethrin; behavior

1 INTRODUCTION

The post-harvest supply chain represents an essential aspect of the agricultural production system, and allows goods to move from farmers to end-use consumers around the world. This represents an enormous economic investment, with annual grain production in the United States (US) alone valued at >US$103 billion in 2017 and flour production worth a retail value of >US$40 billion in 2019.[1, 2] At each link in the supply chain, commodities are vulnerable to insect infestation, which can reduce yield by 2–50%, depending on location and access to resources.[3,4]

Typically, when an infestation is found, raw commodities in storage are most often fumigated with phosphine,[5] but food facility operators may use sulfuryl fluoride[6] with the phase-out of methyl bromide. However, all alternative compounds have drawbacks compared to methyl bromide, which had been used for many decades, but has now been mostly phased-out in the US, because of its role as an ozone-depleting compound.[7] Drawbacks with phosphine have included the development of insecticide resistance (reviewed in Nayak *et al.*[8]), corrosion of metal in buildings and equipment, and efficacy.[5] As a result, there has been a conscious move to diversify integrated pest management (IPM) programs in the post-harvest supply chain.

In de-emphasizing fumigation, increasing attention has been paid to the significant implications that poor sanitation has for most other IPM tactics available to food facilities,[9] as well as on increasing the delivery options and modes of action for chemical control techniques. The latter has resulted in the increasing adoption or expanded use of residual contact insecticides as grain protectants,[10] crack-and-crevice treatments,[11] spot treatments, aerosols (e.g. ultra-low volume formulations that are atomized[12]) and, most recently, insecticide netting.[13–16] These tactics have greatly enhanced the repertoire of chemical control alternatives at food facilities.

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One novel residual insecticide that has come to market in the past year, Gravista®™ (Central Life Sciences, Schaumberg, IL, USA), consists of an insect growth regulator (2.85% methoprene), combined with the pyrethroid, 1.2% deltamethrin and a synergist, 33.3% piperonyl butoxide.[17–18] It is an improved formulation over Diacon IGR+®™ (Central Life Sciences), which contains methoprene (11.4%) and deltamethrin (4.75%) only, but at higher concentrations. In these compounds, methoprene is meant to target immature stages, whereas deltamethrin targets adult insects. The addition of piperonyl butoxide allows less deltamethrin and methoprene to be incorporated in the production, and may also allow the product to combat resistant insect populations,[19] because the synergist is a cytochrome P450 monooxygenase inhibitor.[20] In comparing the direct mortality for these two compounds, Arthur and Morrison[17] found that both products will adequately induce death and suppress progeny production in *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae), but not as much in *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae). That study did not assess sublethal effects of brief exposures on insects. In follow-up work, Arthur *et al.*[18] found that the compounds decreased movement with increasing concentrations and found the greatest movement directly after exposure by *S. oryzae*. In addition, that study evaluated only a single commodity and did not attempt to predict changes in mobility over a common storage period of 12 months as residues aged.

Although specific formulations and modes of action may vary, many chemical control tactics collectively rely on the use of residual insecticides to provide multiple barriers to infestation by immigrating insects from the landscape and adjoining parts of a food facility. These compounds may have induced mortality or resulted in sublethal effects on insect life processes. Historically, the sublethal effects of insecticides have been neglected in post-harvest IPM[21] but these effects are essential to fully comprehend insecticide efficacy and their most appropriate use in a system. Although sublethal effects may touch on many aspects of insect behavior, reproduction and development, one of the primary factors to consider at food facilities is mobility, often using distance moved and velocity as surrogates.[13,18,22–24] It is well-known that some stored product insects may disperse long distances,[25] and may be collected far from food facilities.[26] Mobility is especially important to consider given that insects initially may be affected after exposure to an insecticide, may eventually recover, and move a significant distance before ultimately succumbing to an insecticide.[15] Thus, insects may be able to deposit eggs in raw grain or in packaged food products inside a warehouse. Furthermore, recent work in the post-harvest supply chain has documented how movement also may be used as a more impartial indicator of resistance for *T. castaneum* and *R. dominica*, with evidence of significant movement by insects even after phosphine fumigation.[24] Taken together, this makes a convincing case for considering sublethal changes in mobility after chemical application.

Although some work has been conducted on risk assessment of active ingredients in inducing sublethal effects,[21,27,28] there has been very little work attempting to quantitatively predict sublethal effects by a compound, or how these vary over time. Thus, our aims were to (i) characterize sublethal changes in movement over time after exposure to IGR+ or Gravista on different commodities (wheat, corn, rice), and (ii) develop predictions by species about how movement after exposure should change over the course of a year. Direct mortality from these compounds has been considered in previous work,[17] and was beyond the scope of this contribution.

2 MATERIALS AND METHODS

2.1 Commodities

Tests were conducted at the USDA-ARS Center for Grain and Animal Health Research (CGAHR) in Manhattan, KS, USA. The commodities used in this residual study were wheat (hard winter wheat obtained from a commercial farmer in Kansas), corn (obtained from a local Kansas farmer) and brown rice (obtained from a commercial rice milling facility in 2017). Before use, the wheat and corn had been stored at −4 °C inside a large cold storage facility at CGAHR for ≈4–5 years. The brown rice was stored in the same facility for 3 years.

2.2 Source insects

Rhyzopertha dominica and *S. oryzae* were used on wheat. Both species were cultured on the same wheat described above, and the laboratory strains had been in culture for >30 years.

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

	Max label rate (ppm)				Application rate[a] (per kg)		Mixing rate (mL product)		Amount (mL) applied to 5 kg	
	Diacon IGR+		Gravista[b]							
Commodity	Deltamethrin	Methoprene	Deltamethrin	Methoprene	Diacon IGR+[c]	Gravista[d]	Diacon IGR+[c]	Gravista[d]	Diacon IGR+	Gravista
Wheat	1.0	2.5	0.5	1.15	0.02 [e]	0.06 [e]	0.7 [e]	0.58 [e]	3.5	3.5
Corn	1.0	2.5	0.5	1.15	0.02 [f]	0.05 [f]	0.68 [f]	0.6 [f]	3.7	3.7
Rice	1.0	2.5	0.5	1.15	0.02 [g]	0.04 [g]	0.56 [g]	0.44 [g]	4.5	4.5

[a] Corresponding to the max label rates in mL product mL^{-1} H_2O.
[b] Gravista also contains 13.35 ppm of a piperonyl butoxide synergist.
[c] Mixed in 25 mL H_2O.
[d] Mixed in 10 mL H2O.
[e] Assumes 566 mL (Diacon IGR+) or 1138 mL (Gravista) of product is mixed in 5 gal (=18 920 mL) of water to cover 1000 bushels of wheat (=27 272 kg).
[f] Assumes 532 mL (Diacon IGR+) or 1062 mL (Gravista) of product is mixed in 5 gal (=18 920 mL) of water to cover 1000 bushels of corn (=25 454 kg).
[g] Assumes 424 mL (Diacon IGR+) or 854 mL (Gravista) of product is mixed in 5 gal (=18 920 mL) of water to cover 1000 bushels of rice (=20 454 kg).

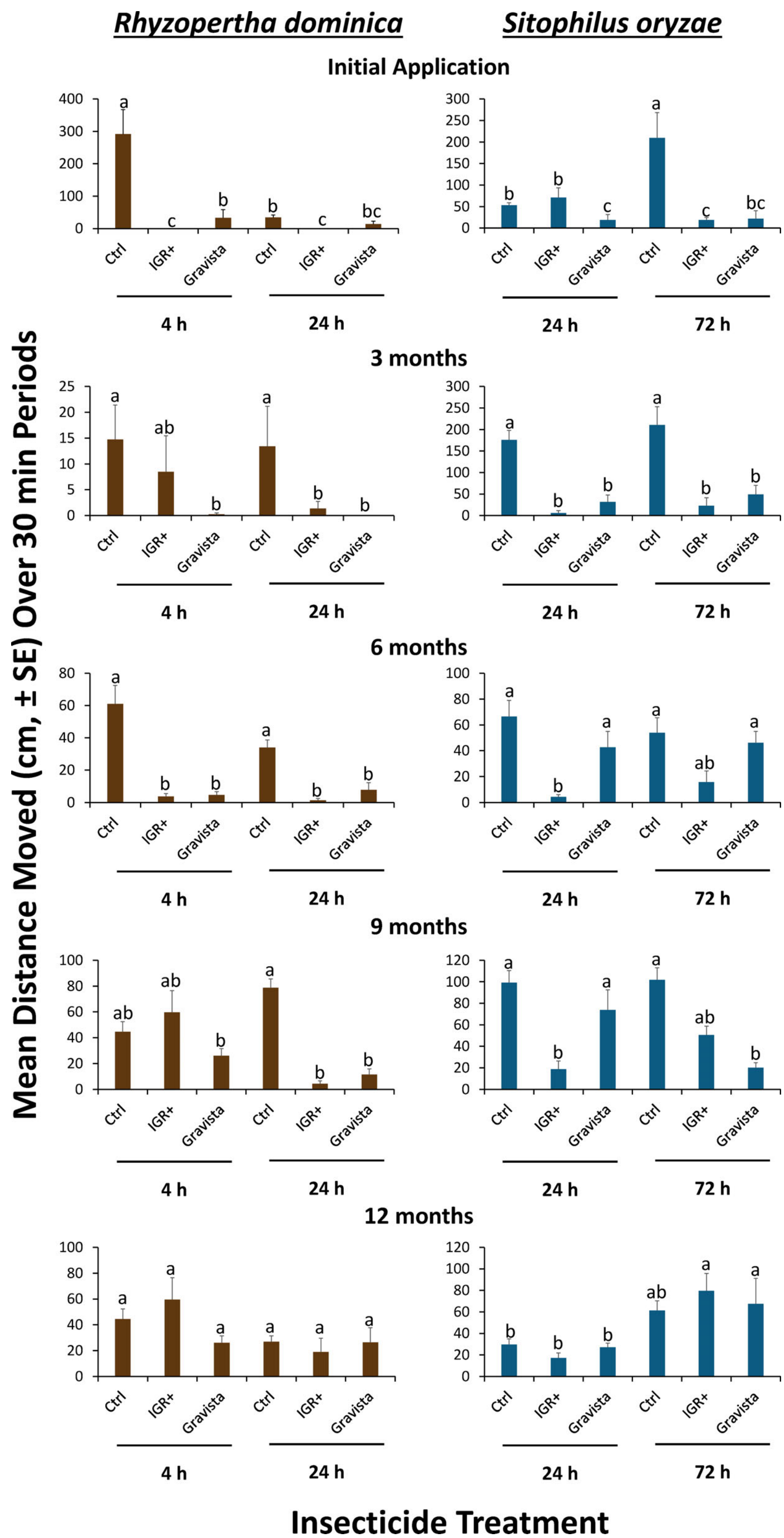

Figure 1. Mean total distance moved (cm, ± SE) over a 30-min period on Ethovision for *R. dominica* and *S. oryzae* when exposed to whole wheat directly post-application of Diacon IGR+ (methoprene + deltamethrin) or Gravista (methoprene + deltamethrin + piperonyl butoxide), or at 3, 6, 9 or 12 months post-application for 4, 24 or 72 h intervals. Bars with shared letters are not significantly different from each other (Tukey's HSD, $\alpha = 0.05$).

They were held inside a growth chamber (Percival Scientific Inc., Perry, IA, US) under constant conditions [27 °C and 60% relative humidity (RH); continuous darkness). *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae), the red flour beetle, and *S. zeamais* (Motschulsky) (Coleoptera: Curculionidae), the maize weevil, were reared on corn, whereas *R. dominica* and *T. castaneum* were reared on brown rice. The *T. castaneum* were reared on a mixture of 95% whole wheat

Table 2. General linear model results for sublethal effects on distance moved (top) and velocity (bottom) for alive and affected *R. dominica* and *S. oryzae* placed on wheat or rice in a residual efficacy experiment assessing Diacon IGR+ and Gravista at the Center for Grain and Animal Health Research (CGAHR) in Manhattan, KS

		R. dominica				*Sitophilus oryzae*			
		Wheat[a]		Rice[b]		Wheat[c]		Rice[d]	
Factor	df	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Response: log distance moved									
Residual interval	4	28.8	<0.01	13.8	<0.01	19.7	<0.01	17.8	<0.01
Exposure time	1	0.00	0.95	2.37	0.12	11.8	<0.01	0.12	0.73
Insecticide treatment	2	16.4	<0.01	25.4	<0.01	21.3	<0.01	62.3	<0.01
Residual × exposure time	4	21.5	<0.01	0.17	0.95	6.29	<0.01	1.20	0.31
Residual × insecticide	8	2.49	0.01	4.31	<0.01	7.11	<0.01	4.18	<0.01
Exposure time × insecticide	2	10.9	<0.01	1.23	0.29	8.83	<0.01	0.56	0.57
Three-way interaction	8	4.58	<0.01	1.22	0.29	4.16	<0.01	1.05	0.40
Response: log velocity									
Residual interval	4	34.5	<0.01	7.73	<0.01	16.5	<0.01	15.1	<0.01
Exposure time	1	0.14	0.71	0.04	0.84	3.43	0.06	0.49	0.48
Insecticide treatment	2	17.5	<0.01	27.1	<0.01	14.0	<0.01	75.5	<0.01
Residual × exposure time	4	20.4	<0.01	3.59	0.01	3.60	0.01	2.62	0.05
Residual × insecticide	8	4.12	<0.01	1.09	0.37	2.27	0.05	6.12	<0.01
Exposure time × insecticide	2	17.7	<0.01	1.17	0.31	6.25	0.01	2.43	0.09
Three-way interaction	8	5.62	<0.01	1.42	0.19	2.70	0.01	2.42	0.05

[a] Residual df = 298 for distance moved and velocity models in wheat for *R. dominica*.
[b] Residual df = 298 for distance moved and velocity models in rice for *R. dominica*.
[c] Residual df = 329 for distance moved and velocity models in wheat for *S. oryzae*.
[d] Residual df = 330 for distance moved and velocity models in rice for *S. oryzae*.

flour and 5% Brewer's yeast, whereas *S. zeamais* were reared on whole kernel corn, using the same variety described above. Both species were held in separate growth chambers under the same conditions listed above.

2.3 Insecticide formulations for each commodity

The insecticides used in this study included Diacon IGR+® (120 mg mL^{-1} methoprene + 50 mg mL^{-1} deltamethrin) and Gravista® (27.5 mg mL^{-1} methoprene + 12 mg mL^{-1} deltamethrin + 320 mg mL^{-1} piperonyl butoxide), which were obtained from Central Life Sciences (Schaumberg, IL, USA). In this study, a high rate of IGR+ (e.g. 2.5 ppm methoprene + 1 ppm deltamethrin) according to label specifications (see Supporting Information), a high rate of Gravista (e.g. 1.15 ppm methoprene + 0.5 ppm deltamethrin + 13.35 ppm piperonyl butoxide), and a water-treated control were compared to each other in the experiments below.

Two 5 kg lots of wheat were treated on 9 July 2018 with Diacon IGR+. Each lot was formulated by adding 0.7 mL of the compound in 25-mL volumetric flasks, and filling the remainder of the flask with distilled water (Table 1). Gravista was formulated by adding 0.58 mL compound to 10 mL water. Two 5 kg lots of corn were treated on 13 August 2018. For corn, Diacon IGR+ was formulated by mixing 0.68 mL compound with water in a 25-mL flask, and Gravista was formulated by adding 0.60 mL compound in 10 mL of water. The 5 kg of brown rice was treated on 17 September 2018. For brown rice, a total of 0.56 mL Diacon IGR+ was formulated with water in a 25-mL flask, whereas 0.44 mL Gravista was mixed with water in a 10-mL flask. There was a total of four replicate separate flask formulations mixed for each commodity and insecticide treatment, and all formulations were calculated according to equivalent use on EPA label specifications.

2.4 Residual efficacy assay

In order to evaluate how sublethal effects change after commodity residues have aged, a residual efficacy study was performed. During application of the insecticide formulations above for each replicate, the commodity was spread out on a 0.6 × 0.3 m cardboard rectangle covered with plastic. For the untreated control, a total of 3.5 mL distilled water was dispensed into the filling cap of an artists' airbrush (Badger 100 series, Badger Corporation, Franklin Park, IL, US), and equally misted over the commodity by applying to the commodity in roughly 0.8-mL increments, and mixing the commodity with a separate piece of cardboard inbetween. The procedure was repeated for each of the insecticide treatments with 3.5, 3.7 and 4.5 mL for wheat, corn and brown rice, respectively (Table 1). Separate artist's airbrushes were used for the untreated control and insecticide treatments. After treatment, the commodity was transferred to an 18.75-L plastic bucket and covered with a lid. After the four replicate formulations for each treatment and commodity were completed, all buckets with commodities were transferred to the floor of an empty 112 MT grain bin at the CGAHR to mimic field conditions.

On the day after placement of commodities in the bin, the wheat was sampled as follows. An equal mixture from the four replicates for each insecticide treatment totaling 80 g of a given commodity was placed in two 120-mL plastic vials. A total of 50 one-week-old, mixed-sex adult insects of the appropriate species for each commodity were exposed in each vial. Based on

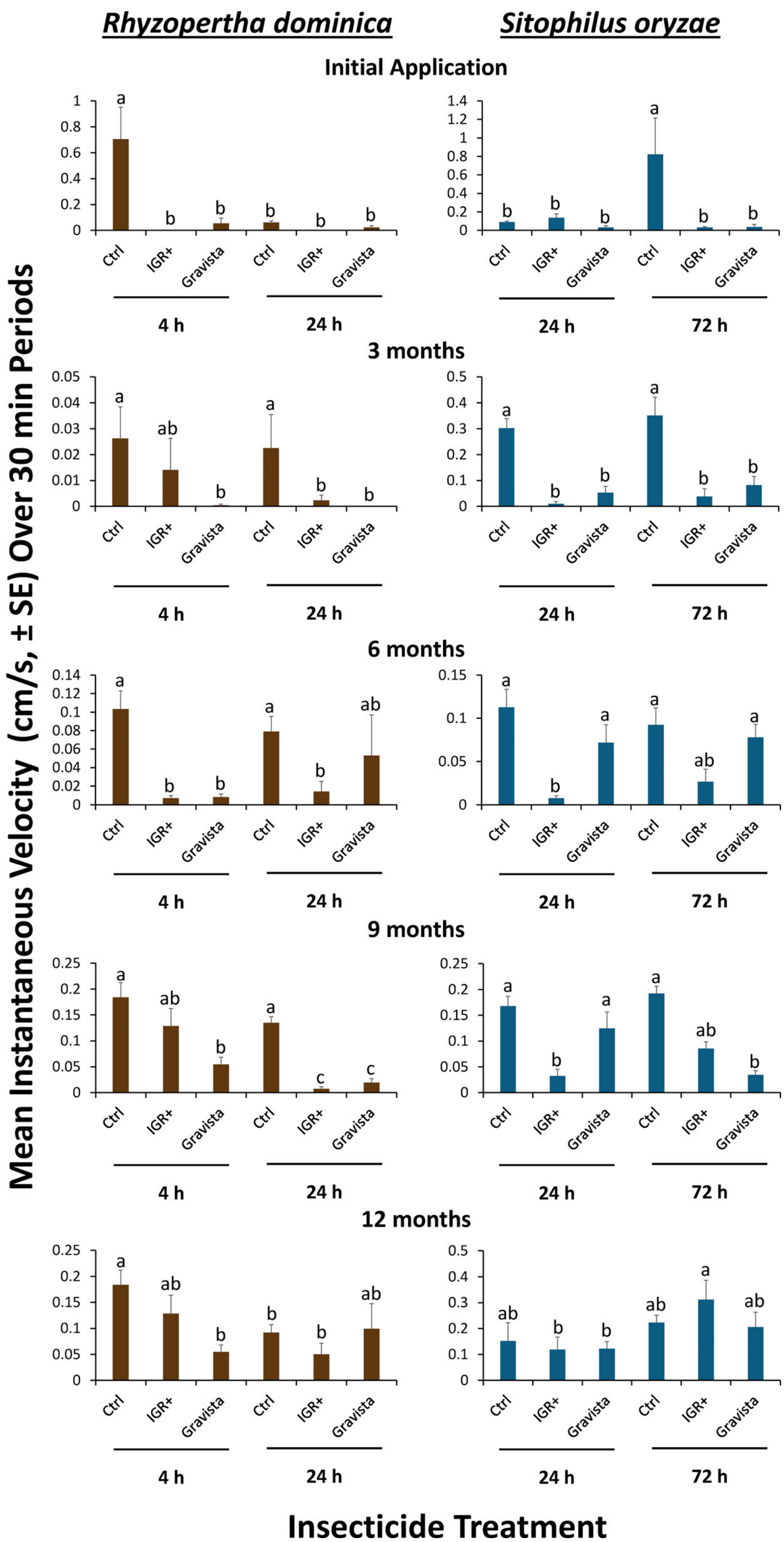

Figure 2. Mean instantaneous velocity (cm s^{-1}, ± SE) over a 30-min period on Ethovision for *R. dominica* (brown) and *S. oryzae* (blue) when exposed to whole wheat directly after application of Diacon IGR+ (methoprene + deltamethrin) or Gravista (methoprene + deltamethrin + piperonyl butoxide), or at 3, 6, 9 or 12 months post-application for 4, 24 or 72 h intervals. Bars with shared letters are not significantly different from each other (Tukey's HSD, $\alpha = 0.05$).

differential susceptibility of each species,[29] cohorts of *R. dominica* and *T. castaneum* were exposed for 4 or 24 h on the commodity, whereas *S. oryzae* was exposed for 24 or 72 h. During the holding periods, all vials were placed inside an environmental chamber set at 27 °C and 60% RH as above. At each time period, one vial was sieved, and adults were placed on a petri dish (62 cm^2) lined

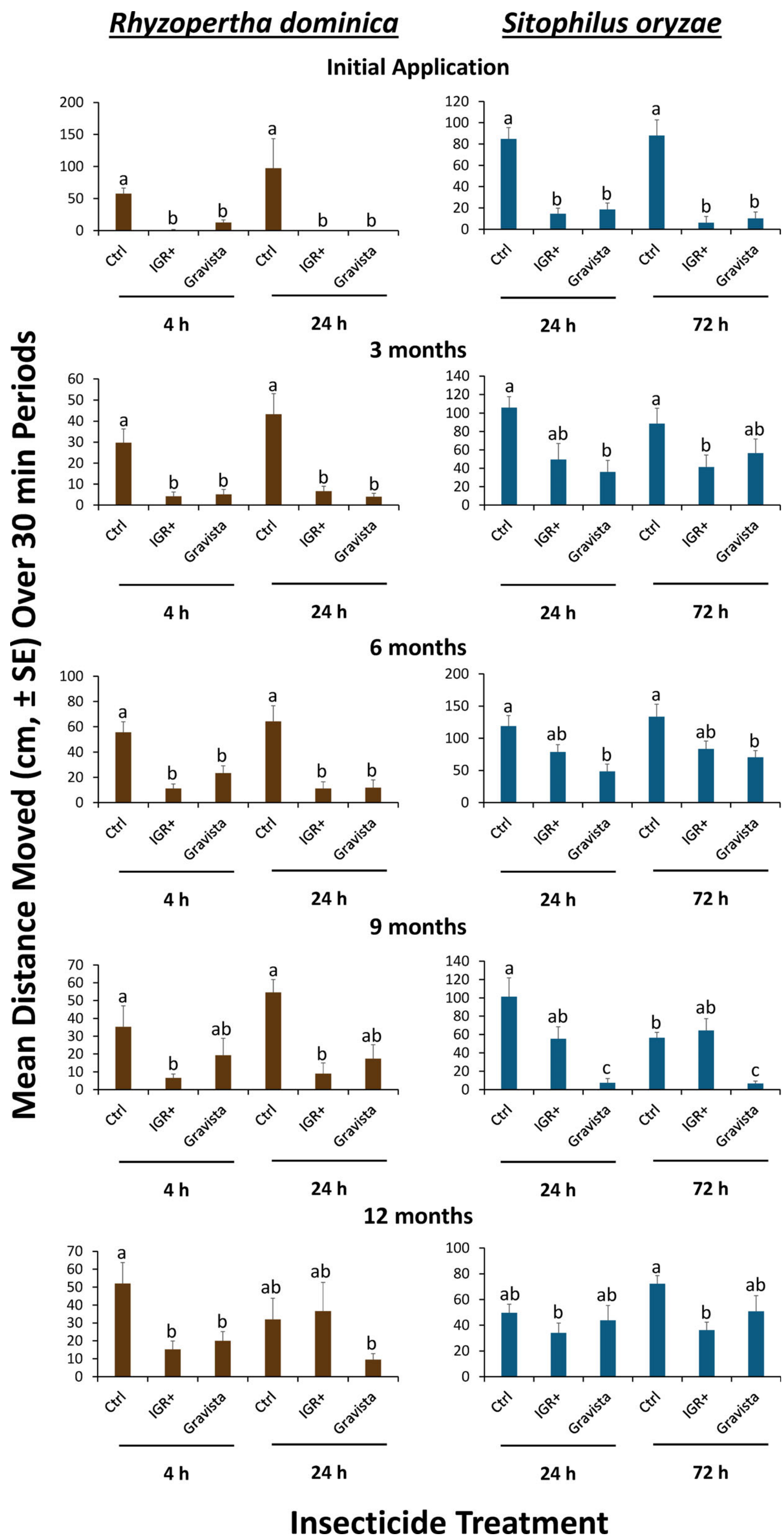

Figure 3. Mean total distance moved (cm, ± SE) over a 30-min period on Ethovision for *R. dominica* (brown) and *S. oryzae* (blue) when exposed to brown rice directly after application of Diacon IGR+ (methoprene + deltamethrin) or Gravista (methoprene + deltamethrin + piperonyl butoxide), or at 3, 6, 9 or 12 months post-application for 4, 24 or 72 h intervals. Bars with shared letters are not significantly different from each other (Tukey's HSD, $\alpha = 0.05$).

with filter paper under a dissecting stereomicroscope (SMZ18, Nikon Inc., Minato City, Japan) for assessment into three categories: alive, affected or dead (e.g. Morrison *et al.*[13]). Only adults rated as alive or affected were used in the movement assay below. Species were exposed to commodities after they had aged 0, 3, 6, 9 or 12 months after initial treatment of grain.

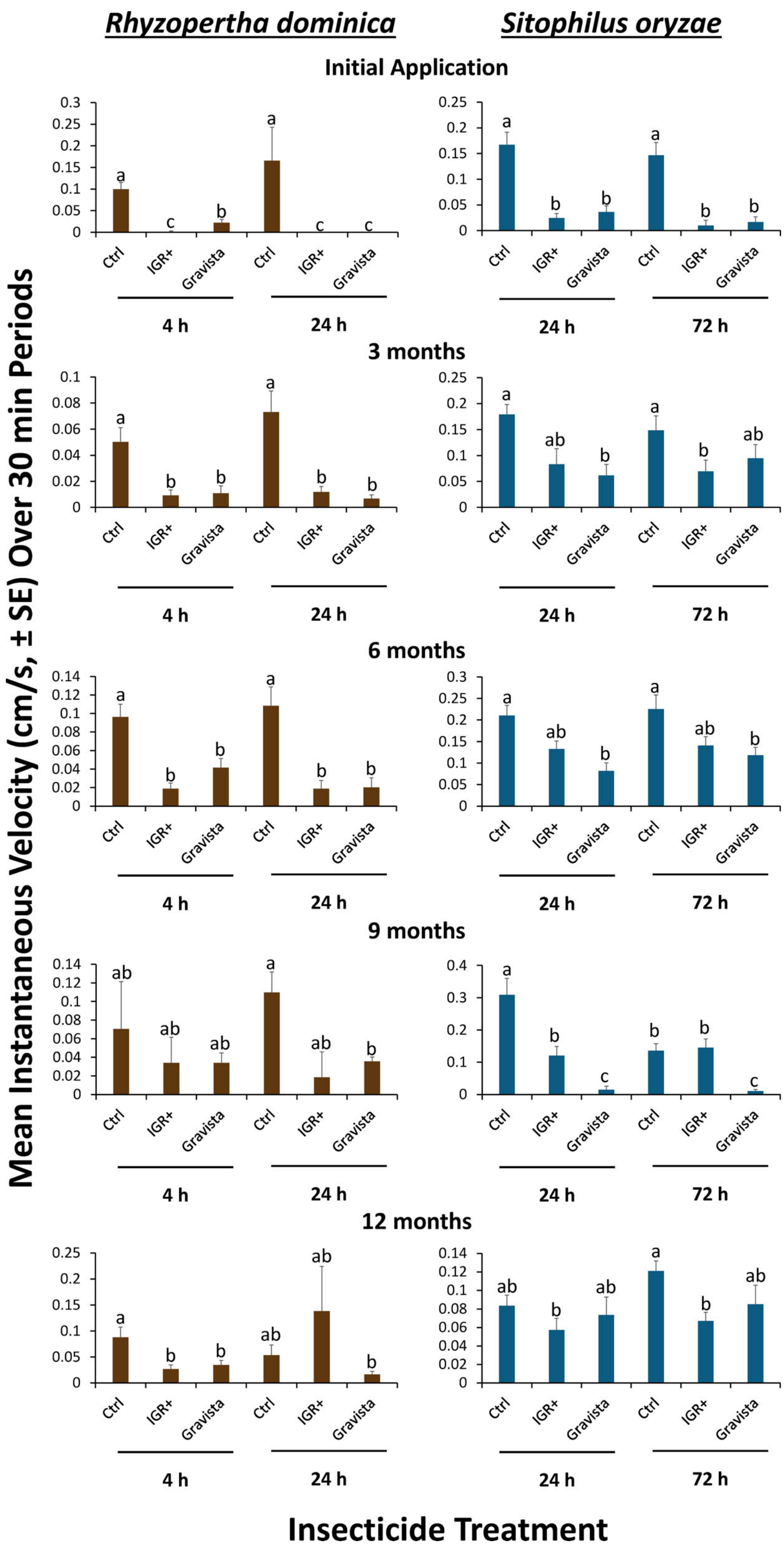

Figure 4. Mean instantaneous velocity (cm s^{-1}, ± SE) over a 30-min period on Ethovision for *R. dominica* (brown) and *S. oryzae* (blue) when exposed to brown rice directly after application of Diacon IGR+ (methoprene + deltamethrin) or Gravista (methoprene + deltamethrin + piperonyl butoxide), or at 3, 6, 9 or 12 months post-application for 4, 24 or 72 h intervals. Bars with shared letters are not significantly different from each other (Tukey's HSD, $\alpha = 0.05$).

2.5 Movement measurements

In order to impartially observe movement,[23,24,28] video tracking of insects was used. Cohorts of six insects were tracked for 30 min simultaneously in individual, filter paper-lined petri dishes (85 mm D), consisting of two replicate alive or affected individuals from each insecticide treatment (Diacon IGR+, Gravista or

Table 3. Predicted distance moved (cm) by four species of stored product beetles in a 24-h period after exposure to commodities treated with residual insecticides at different time points over a year at CGAHR in Manhattan, KS

				Total predicted distance moved in 24 h after exposure (cm)					Fold increase in mobility compared to initial treatment			
Species	Insecticide	Fitted equation	R^2	0 month	3 months	6 months	9 months	12 months	3 months	6 months	9 months	12 months
T. castaneum	Diacon IGR+	$y = 2.2 + 12.2(x^{0.50})$	0.58	86	1123	1526	1862	2136	13	18	22	25
T. castaneum	Gravista	$y = 27.9–26.9e^{(-x)}$	0.87	48	1277	1330	1440	1440	27	28	28	28
S. oryzae	Diacon IGR+	$y = 26.2 + 6.0(x^{0.42})$	0.59	655	1714	1872	1982	2088	3	3	3	3
S. oryzae	Gravista	$y = 43.7–26.2e^{(-x)}$	0.68	22	1963	2006	2011	2011	89	91	91	91
S. zeamais[a]	Diacon IGR+	—	—	—	—	—	—	—	—	—	—	—
S. zeamais	Gravista	$y = 0.3 + 24.7(x^{0.30})$	0.81	1277	1795	1927	2035	2136	1	2	2	2
R. dominica	Diacon IGR+	$y = 3.2 + 0.2(x^{5.67})$	0.30	152	147	180	418	1493	1	1	3	10
R. dominica	Gravista	$y = 5.7 + 0.13^{(-x/-24)}$	0.59	278	283	346	945	1238	1	1	3	4

[a] Equation could not be reliably derived because of high variability in dataset.

untreated control) described above. The petri dishes were backlit using an artist's LED lightbox (42 cm wide × 30 cm long, LPB3, Litup, Shenzhen, China) to optimize tracking of insects. A network video camera (GigE, Basler AG, Ahrenburg, Germany) affixed 80 cm above the insects livestreamed the video to an adjacent computer and was directly captured by Ethovision v14.0 (Noldus Inc., Leesburg, VA, USA) software, which recorded the total distance moved (cm) and instantaneous velocity (cm s^{-1}) for each individual. An individual was never used more than once. There was a total of $n = 12$ replicate individuals per combination of insecticide treatment, commodity, exposure time and residual time.

2.6 Statistical analysis

In order to determine differences in movement, distance moved and velocity by insects were used as response variables in a generalized linear mixed model (GLMM) framework. The model was based on an underlying Gaussian distribution using an identity link function. Fixed, explanatory variables included insecticide treatment (Diacon IGR+, Gravista, Ctrl), commodity (wheat, corn, rice), exposure time (2 or 24 h, or 24 or 72 h), and residual time (0, 3, 6, 9 or 12 months) in a full factorial design. Date of run was included as a random, blocking variable. Inspection of residuals confirmed that assumptions of normality and homogenous variances were fulfilled. Separate models were run for each species and commodity. Levene's test for homoscedasticity of responses using treatments nested in residual interval revealed no deviations. Thus, comparison of insecticide treatments was confined to within a residual time for each species and commodity. Upon a significant overall model result, Tukey's honestly significant difference (HSD) was used for multiple comparisons using the *ghlt* function in the *multcomp* package implemented in R software.[30] For this and all other tests, $\alpha = 0.05$.

In order to develop predictions about changes in mobility with aging residues on commodities, nonlinear regression was implemented using Table Curve 2D software (Systat Software, San Jose, CA, US) to determine the best-fit line. In particular, residual time was used to predict distance moved or velocity for each combination of species and insecticide. Equations for each line was used to develop predictions of total distance moved by insects over a 24-h period directly post-application, or 3, 6, 9 or 12 months later.

3 RESULTS

3.1 Characterizing and predicting sublethal effects on movement by *R. dominica*

In wheat, the residual interval significantly affected the sublethal movement of *R. dominica* (Fig. 1; Table 2), with a 3–524-fold greater distance moved 3–12 months later compared to movement of individuals exposed to wheat initially after application. Each insecticide performed likewise, reducing the distance moved by *R. dominica* ≈79–84% compared to control individuals (Table 2). By contrast the exposure time did not significantly affect the distance moved by insecticide-treated *R. dominica* (Table 2). Every secondary and three-way interaction among the variables was significant. Generally, the distance moved by *R. dominica* progressively increased over time with mobility of insecticide-exposed individuals becoming equal to controls by 9–12 months post-application (Fig. 1).

Likewise, in wheat the velocity of *R. dominica* was significantly affected by the residual interval (Table 2; Fig. 2), with instantaneous velocity of insecticide-exposed individuals rising by

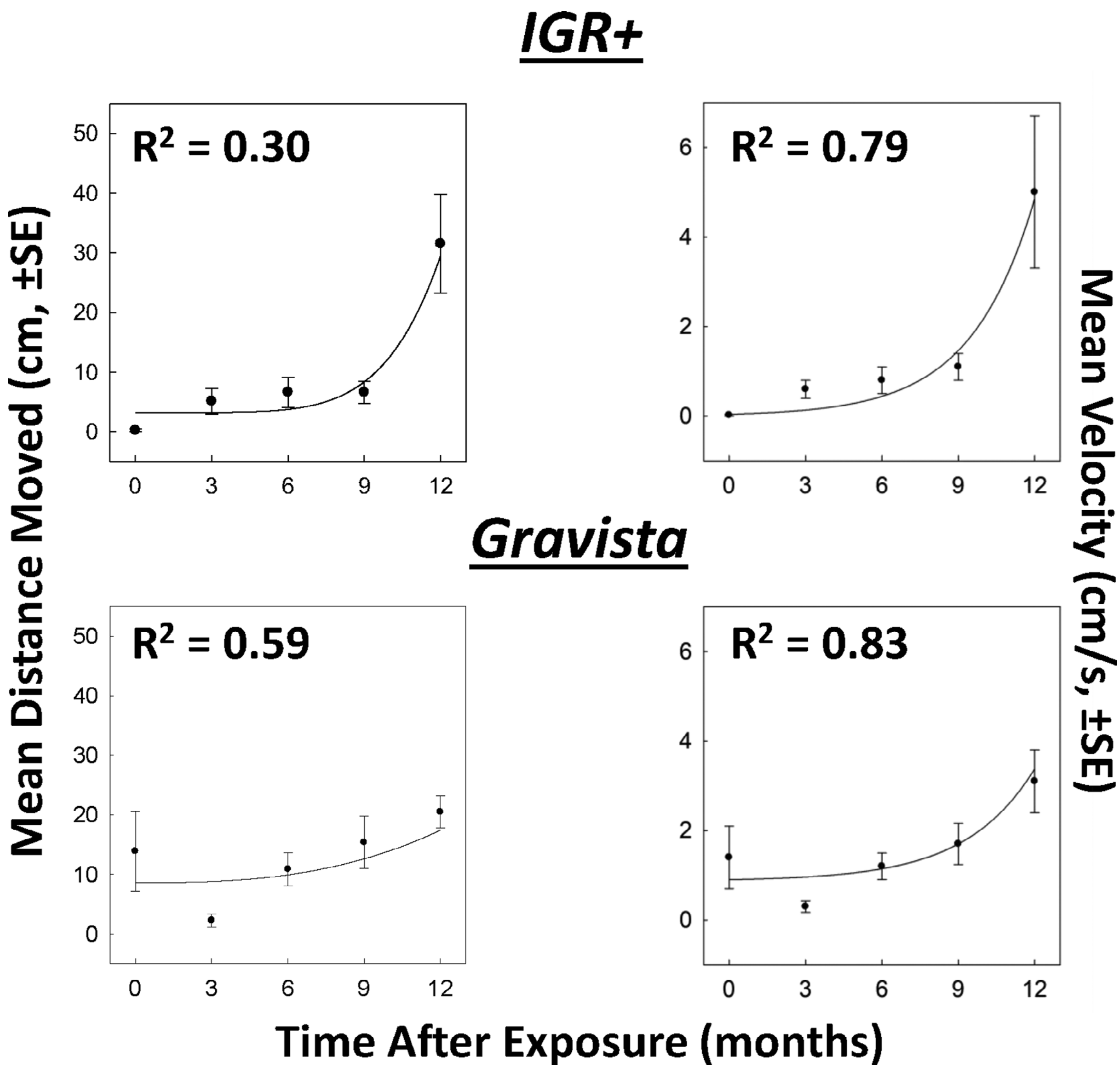

Figure 5. Nonlinear regression of mean distance moved (cm, ± SE; left-hand panels) and mean instantaneous velocity (cm s^{-1}, ± SE; right-hand panels) against time on commodities (wheat and rice) by *R. dominica* adults exposed to aged Diacon IGR+ (top panels) or Gravista (bottom panels) residues. Fitted lines are shown in solid black with corresponding goodness-of-fit (R^2); graphs without did not have models that were significant.

3–209-fold 3–12 months after the initial application. Treatment also had a significant influence on the velocity of *R. dominica* (Table 2), but both insecticides performed equally well, reducing movement by 77–84%. Again, exposure time did not significantly affect velocity (Table 2). All the two- and three-way interactions among the variables were significant (Table 2). Depending on specific combination of treatments, the velocity moved by exposed *R. dominica* on the treated wheat gradually increased over time and was no better than the controls at 9 months after a 4 h exposure or 12 months at both exposure levels (Fig. 2).

In rice, the distance moved by *R. dominica* also was significantly affected by the residual interval (Table 2; Fig. 3), with movement individuals increased by 13–62-fold after 3–12 months compared to initial application. The treatment significantly affected the distance moved (Table 2), which was reduced by 75–81% for insecticide-exposed individuals compared to controls. Similar to wheat, the exposure time did not significantly affect the distance moved by *R. dominica*. None of the interactions among the variables were significant except for the two-way interaction between residual interval and insecticide treatment (Table 2). In particular, Gravista was slightly less effective after 9 months, whereas IGR+ was less effective after 12 months. Both compounds provided control, as evidenced by the distance moved by *R. dominica* between 0 and 6 months post-application (Fig. 3).

Similar to distance moved in rice, the velocity of movement in rice by *R. dominica* significantly increased by 14–113-fold at 3–12 months compared to velocity of individuals exposed on the treated wheat after initial application (Table 2; Fig. 4). Likewise, the two insecticides significantly reduced *R. dominica* velocity by 70–74% compared to controls (Table 2). Although the exposure time did not significantly affect the velocity of *R. dominica* in rice, there was a significant exposure time by residual interval interaction on velocity, which was the only significant interaction (Table 2; Fig. 4).

Out of all the species, *R. dominica* initially showed the slowest recovery after exposure to either Diacon IGR+ or Gravista (Table 3; Fig. 5). After 3–12 months from application, *R. dominica* moved a predicted average of 631 cm in a 24 h period compared to a

Table 5. Predicted mean velocity (cm s^{-1}) by four species of stored product beetles in a 24-h period after exposure to commodities treated with residual insecticides at different time points over a year

Species	Insecticide	Fitted equation	R^2	Mean predicted velocity in 24 h after exposure (cm min^{-1})					Fold increase in mobility compared to initial treatment			
				0 month	3 months	6 months	9 months	12 months	3 months	6 months	9 months	12 months
T. castaneum	Diacon IGR+	$y = 1.1 + 0.02(x^{2.51})$	0.95	1.10	1.42	2.90	6.07	11.3	1	3	6	10
T. castaneum	Gravista	$y = 3.3–3.2e^{(-x)}$	0.99	0.10	3.14	3.29	3.30	3.30	31	33	33	33
S. oryzae	Diacon IGR+	$y = 2.8 + 0.05(x^{1.84})$	0.98	2.80	3.18	4.15	5.65	7.64	1	1	2	3
S. oryzae	Gravista	$y = 1.9 + 1.2(x^{0.49})$	0.58	1.90	3.96	4.79	5.42	5.95	2	3	3	3
S. zeamais[a]	Diacon IGR+	—	—	—	—	—	—	—	—	—	—	—
S. zeamais[b]	Gravista	$y = 4.6–4.6e^{-x}$	0.93	0	4.37	4.59	4.60	4.60	—	—	—	—
R. dominica	Diacon IGR+	$y = 0.04^{(-x/-2.5)}$	0.79	0.04	0.13	0.44	1.44	4.86	3	11	36	122
R. dominica	Gravista	$y = 0.9 + 0.03^{(-x/-2.71)}$	0.83	0.90	0.96	1.14	1.70	3.38	1	1	2	4

[a] Equation could not be reliably derived because of high variability in dataset.
[b] Values cannot be divided by zero, thus fold-increase in mobility cannot be calculated.

predicted 215 cm after the initial application of either compound, representing an almost three-fold increase in distance moved. The predicted velocity after initial application of insecticides was 0.47 cm min^{-1}, which rose to 1.76 cm min^{-1} in the ensuing intervals (e.g. a four-fold increase; Table 5).

3.2 Characterizing and predicting sublethal effects on movement by *S. oryzae*

In wheat, the distance moved by *S. oryzae* was significantly affected by the residual interval, treatment, and exposure time (Table 2; Fig. 1). In addition, all the two- and three-way interactions significantly affected the distance moved by adults (Table 2). Over succeeding months from the initial application, the distance moved by adults after exposure to the Gravista treatment increased by 1.7–2.3-fold. Adult *S. oryzae* exhibited a 62–72% reduction in movement on treated wheat compared to movement on untreated wheat (Fig. 1). After 24 h of exposure, insecticide-exposed individuals showed a 53–74% reduction in movement, whereas adults showed a 68–71% reduction in distance moved after 72 h. However, factoring in the interactions, there were inconsistent effects on the distance moved by *S. oryzae* in wheat, depending on residual interval, compound and exposure time (Fig. 1).

The velocity of *S. oryzae* in wheat was significantly affected by the residual interval, with insecticide-exposed individuals moving 2–5-fold faster in the 3–12 months following application (Table 2; Fig. 2). Treatment had a significant effect on the velocity of adults (Table 2), with insecticide-exposed individuals moving 66–68% slower. Exposure time did not significantly affect the velocity of adults, but all of the two- and three-way interactions significantly affected the velocity of *S. oryzae* (Table 2). Overall, there were inconsistent effects on the velocity of *S. oryzae* depending on residual time, compound and exposure time (Fig. 2).

In rice, the distance moved by adult *S. oryzae* was affected by the residual interval (Table 2); in particular, IGR+- and Gravista-exposed adults moved 3–7-fold and 3–4-fold more in later intervals compared to the movement of individuals exposed at the initial application (Fig. 3). The treatment also affected the distance moved by adults (Table 2), with a 50–61% reduction in movement for insecticide-exposed adults, depending on compound (Fig. 3). The exposure time did not affect the distance moved, but the residual-by-insecticide treatment interaction did. In particular, Gravista appeared more effective for a longer residual time compared to IGR+ (Fig. 3).

The velocity of adult *S. oryzae* in rice also was significantly affected by the residual interval (Table 2; Fig. 4). IGR+- and Gravista-exposed adults moved 3–7-fold and 0.5–4-fold faster in later residual months compared to the initial application. Additionally, the treatment significantly affected the velocity, with IGR+ reducing velocity by 52% and Gravista by 65%. Exposure time did not significantly affect the velocity of adults, but all of the interactions among the variables were significant with the exception of exposure time-by-insecticide (Table 2). In short, except at initial application IGR+ was not successful at reducing movement compared to the controls unless *S. oryzae* was exposed for a longer time (Fig. 4). By 12 months post-application, both insecticides had mostly lost effectiveness at reducing movement compared to controls.

The predicted distance moved by *S. oryzae* in a 24-h period increased from 339 cm directly post-application to 1956 cm 3–12 months post-application, corresponding to an almost

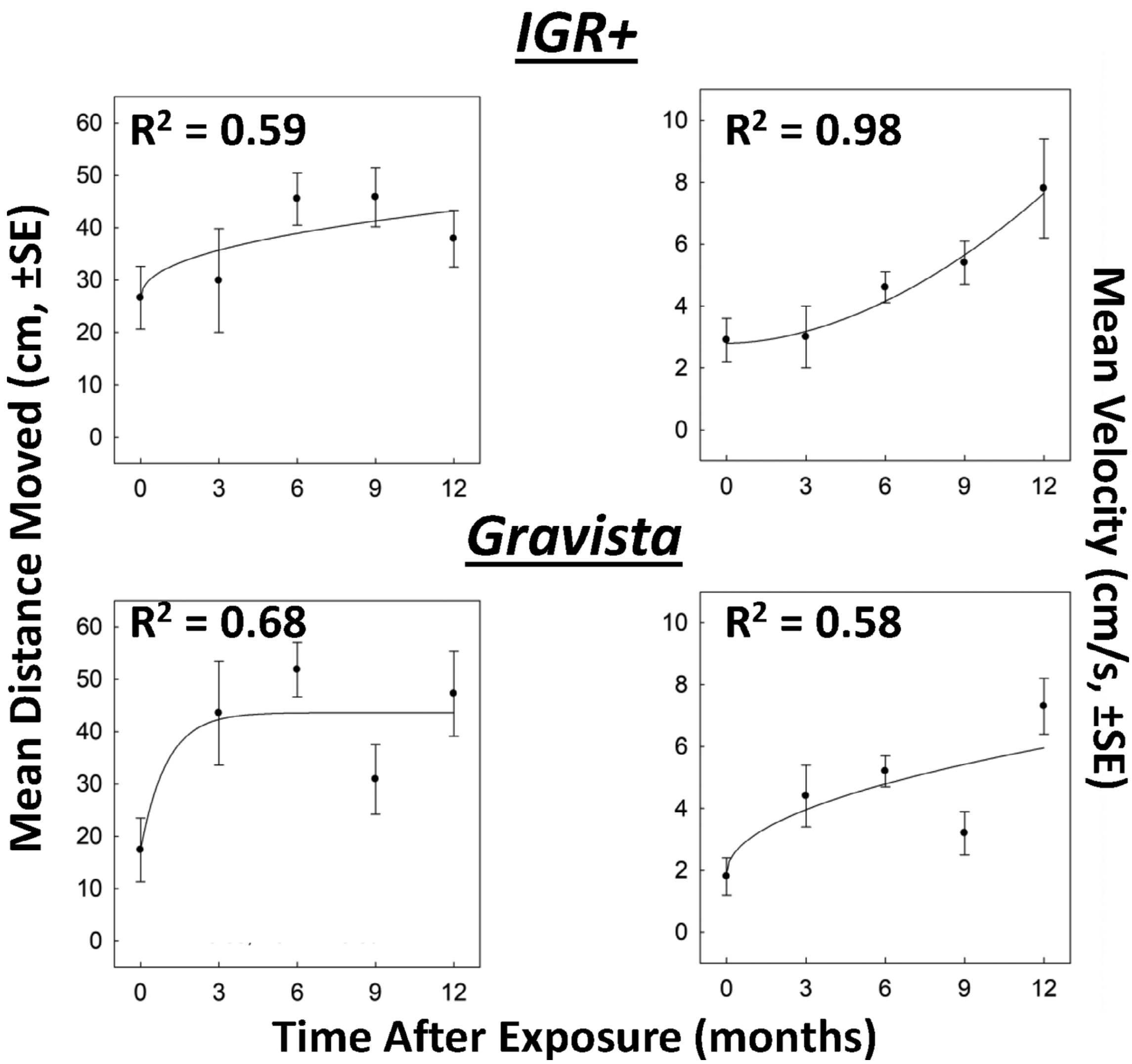

Figure 6. Nonlinear regression of mean distance moved (cm, ± SE; left-hand panels) and mean instantaneous velocity (cm s^{-1}, ± SE; right-hand panels) against time on commodities (wheat and rice) by *S. oryzae* adults exposed to aged Diacon IGR+ (top panels) or Gravista (bottom panels) residues. Fitted lines are shown in solid black with corresponding goodness-of-fit (R^2); graphs without did not have models that were significant.

six-fold increase in movement (Table 3; Fig. 6). Likewise, the predicted velocity for *S. oryzae* after initial insecticide application was 2.35 cm min^{-1}, which increased by two-fold to 5.09 cm min^{-1} 3–12 months post-application (Table 5).

3.3 Characterizing and predicting sublethal effects on movement by *T. castaneum*

In corn, the distance moved by *T. castaneum* was significantly affected by the residual interval, with insecticide-exposed individuals moving 6–31-fold more after the initial application up to 12 months (Table 4; Fig. 7). In addition, the treatment also significantly impacted the distance moved by adults (Table 4), with movement for insecticide-exposed beetles reduced by 85–88% compared to untreated controls (Fig. 7). There was no significant effect of exposure time on distance moved by *T. castaneum*, nor were any of the interactions significant.

Likewise, velocity of movement by *T. castaneum* in corn was significantly affected by residual interval (Table 4; Fig. 8). Insecticide-exposed adults moved 6–73-fold faster 3–12 months post-application compared to initial treatment. Likewise, treatment significantly affected the velocity, with the two insecticides decreasing movement by 76–88%. In addition, exposure time did not significantly affect velocity, nor were any of the interactions significant except the two-way interaction between residual interval and insecticide treatment (Table 4; Fig. 8).

Among the species tested, *T. castaneum*'s predicted movement increased most significantly in the ensuing period post-application (Fig. 9). When *T. castaneum* were exposed on the treated wheat after initial application, the predicted distance moved was 67 cm over a 24 h period, but after 3–12 months, the predicted distance moved increased to 1517 cm, an increase of 23-fold (Table 3). Likewise, after initial application of insecticides, predicted velocity of *T. castaneum* was 0.6 cm min^{-1}, which increased by seven-fold to 4.34 cm min^{-1} 3–12 months post-application (Table 5).

3.4 Characterizing and predicting sublethal effects on movement by *S. zeamais*

In corn, the distance moved by *S. zeamais* was significantly affected by the residual time post-application (Table 4; Fig. 7);

Table 4. General linear model results for sublethal effects on distance moved (top) and velocity (bottom) for alive and affected *T. castaneum* and *S. zeamais* placed on corn in a residual efficacy experiment assessing Diacon IGR+ and Gravista at CGAHR in Manhattan, KS

		Corn			
		T. castaneum[a]		*S. zeamais*[a]	
Factor	df	*F*	*P*	*F*	*P*
Response: log distance moved					
Residual interval	4	5.45	<0.01	4.35	0.01
Exposure time	1	0.02	0.88	2.98	0.06
Insecticide treatment	2	42.3	<0.01	122	<0.01
Residual × exposure time	4	1.35	0.25	6.02	<0.01
Residual × insecticide	8	1.45	0.18	9.32	<0.01
Exposure time × insecticide	2	0.19	0.83	3.09	0.05
Three-way interaction	8	0.74	0.66	3.17	0.01
Response: log velocity					
Residual interval	4	8.86	<0.01	4.67	0.01
Exposure time	1	9.74	0.01	3.96	0.05
Insecticide treatment	2	30.9	<0.01	130	<0.01
Residual × exposure time	4	0.98	0.42	6.35	<0.01
Residual × insecticide	8	2.61	0.01	8.76	<0.01
Exposure time × insecticide	2	2.49	0.08	4.12	0.01
Three-way interaction	8	1.80	0.08	3.12	0.01

[a] Residual df = 330 for distance moved and velocity models in corn for *R. dominica* and *S. oryzae*.

exposed adults moved 41–95-fold more in the 3–12 months after the exposures at initial application. Insecticide-exposed adult *S. zeamais* experienced a 63–66% reduction in distance moved compared to controls (Table 4). Exposure time did not significantly affect the distance moved by *S. zeamais*, but every pairwise and three-way interaction among the variables was significant. Generally, the efficacy of both insecticides in reducing movement of *S. zeamais* adults was inconsistent by 9–12 months post-application.

The velocity moved by *S. zeamais* adults in corn also was significantly affected by the residual time, with beetles moving 34–96-fold faster by 3–12 months (Table 4; Fig. 8). Likewise, insecticide-exposed adult *S. zeamais* showed a 63–66% reduction in velocity compared to controls (Table 4). However, exposure time did not affect the velocity of adults (Table 4). Every two- and three-way interaction was significant among the variables (Table 4).

Among the species tested, *S. zeamais* movement was predicted to be the one that was least affected initially, and thus who would have had the lowest fold-change in the ensuing period (Fig. 10). After initial application of insecticides, the predicted distance moved by *S. zeamais* was 1277 cm over 24 h, which increased marginally by 1.5-fold to 1973 cm 3–12 months post-application (Table 3). Although the initial application of Gravista brought velocity to a halt, it increased to 4.54 cm min^{-1} after 3–12 months (Table 5).

4 DISCUSSION

As prior work has noted, the sublethal effects of insecticides on stored product insects historically has been neglected but is essential for a complete evaluation of compound efficacy.[21] For the first time, we have comprehensively assessed the sublethal effects on mobility after exposure to an existing (e.g. IGR+) and novel (Gravista) formulation labeled as a grain protectant or as residual insecticide applied to surfaces. This has included evaluating the distance moved and velocity of four insects on three common stored commodities. Overall, application of both compounds provided excellent control of movement by stored product insects directly post-application, but 3–12 months post-application, each species exhibited multiple-fold increases in mobility to varying degrees. Suppression of movement by the compounds was most inconsistent for *S. oryzae*. Both insecticides performed equally on other susceptible strains of stored product insects.[17–] However, for insecticide resistant strains, Gravista is expected to perform better even if it has less of the active ingredients deltamethrin and methoprene, because it contains a synergist, which suppresses P450 activity.[20] To confirm this hypothesis, future work should evaluate deltamethrin-susceptible compared to deltamethrin-resistant stored product insect strains.

Shorter exposure times often were found to be statistically equivalent to longer exposure times in affecting mobility of most species on most commodities tested here. Depending on the residual interval since initial treatment, this may mean that insects are not moving much at all initially, or that the insects become very mobile as the residues age, regardless of whether exposure is 4 h or (≤) 72 h. Similar results were found for exposure of *T. castaneum* and *R. dominica* to long-lasting insecticide-incorporate netting with 0.4% deltamethrin; adults exposed to netting for 1, 5 or 10 min experienced equal decreases in mobility after exposure.[13] This suggests that sublethal reductions in movement may be quickly induced, but that the effectiveness of aged residues on movement may decline with residual time.

Although there was excellent control of mobility by stored product species after initial application, there was noticeably more movement after 3 months, with differences between the control and insecticide-exposed individuals disappearing by

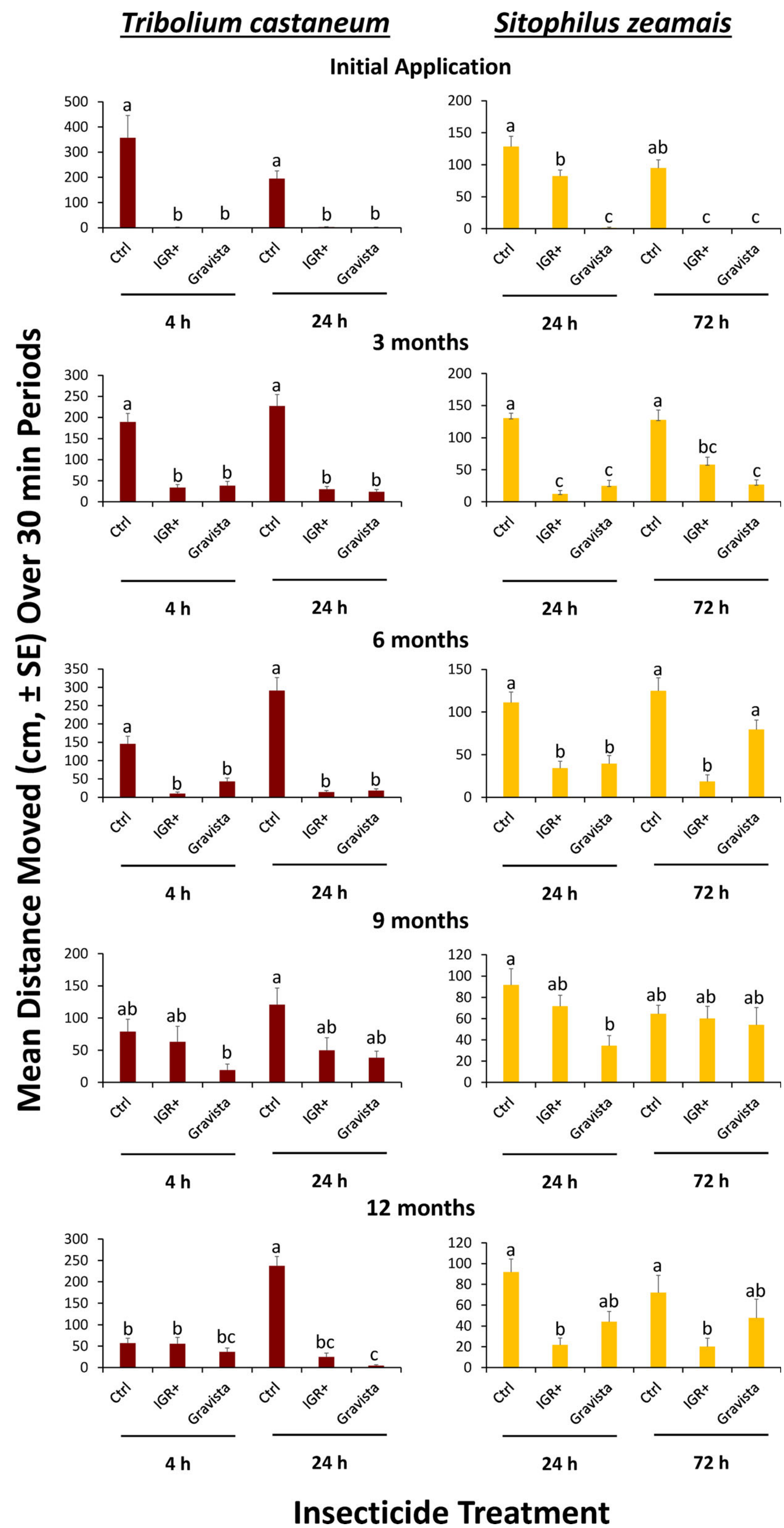

Figure 7. Mean total distance moved (cm, ± SE) over a 30-min period on Ethovision for *T. castaneum* (red) and *S. zeamais* (yellow) when exposed to corn directly after application of Diacon IGR+ (methoprene + deltamethrin) or Gravista (methoprene + deltamethrin + piperonyl butoxide), or at 3, 6, 9 or 12 months post-application for 4, 24 or 72 h intervals. Bars with shared letters are not significantly different from each other (Tukey's HSD, $\alpha = 0.05$).

9–12 months, depending on species. This may be a reasonable length of efficacy given typical storage durations for grain in the US Great Plains region. Prior work has considered the direct toxicity of deltamethrin and β-cyfluthrin, and found that the former did not decrease in efficacy over a 3.5-month period, whereas the latter did against *Sitophilus* spp.[31] In the companion study to

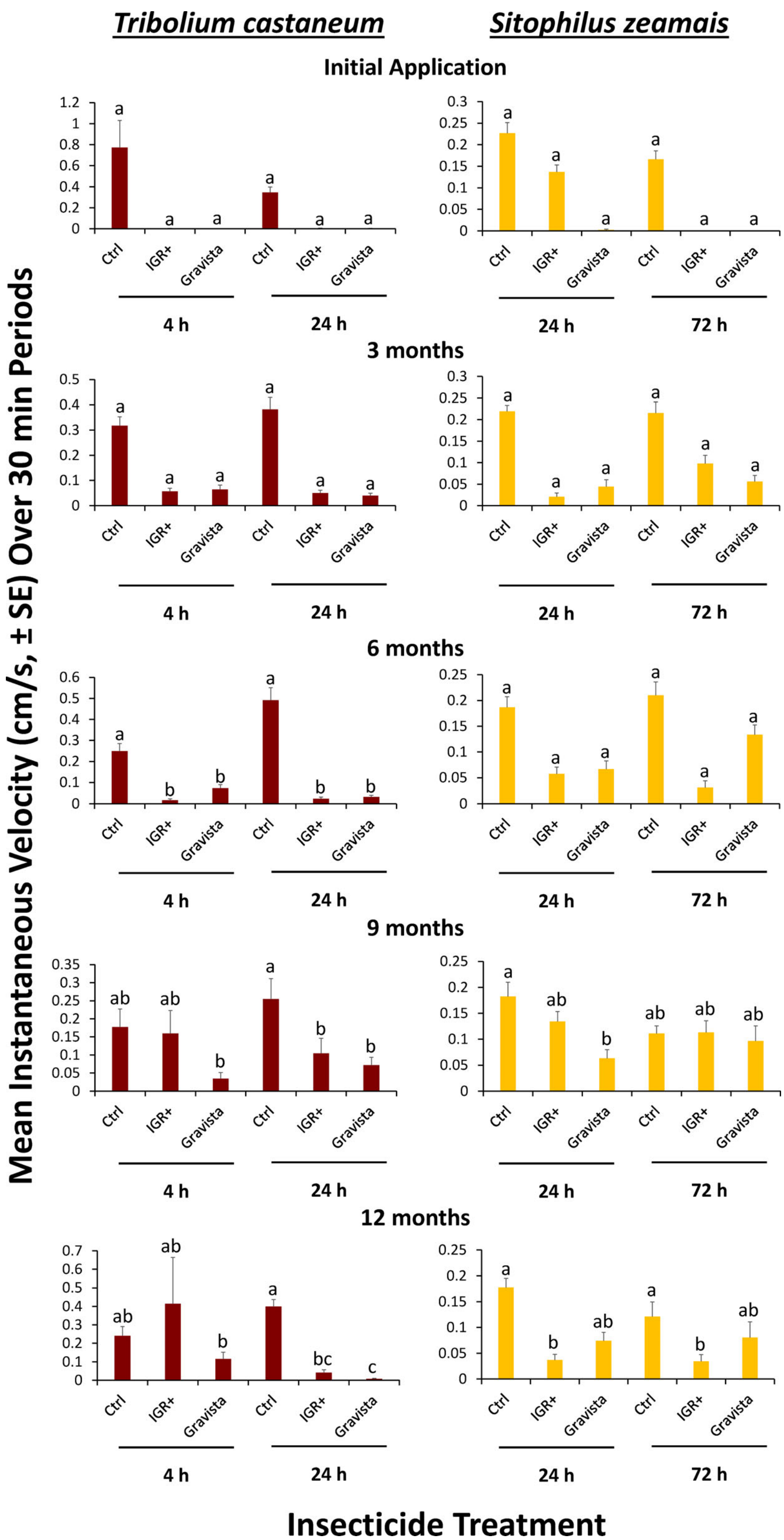

Figure 8. Mean instantaneous velocity (cm s^{-1}, ± SE) over a 30-min period on Ethovision for *T. castaneum* (red) and *S. zeamais* (yellow) when exposed to corn directly after application of Diacon IGR+ (methoprene + deltamethrin) or Gravista (methoprene + deltamethrin + piperonyl butoxide), or at 3, 6, 9 or 12 months post-application for 4, 24 or 72 h intervals. Bars with shared letters are not significantly different from each other (Tukey's HSD, $\alpha = 0.05$).

this one looking at the same treatments, but evaluating direct lethality, both Gravista and IGR+ were found to kill adults and prevent progeny production by *R. dominica* and *T. castaneum*, but documented less consistent results with *S. zeamais* and especially *S. oryzae*.[17] We found similar results, with much more movement by *Sitophilus* spp. overall after exposure. By 9–12 months, there

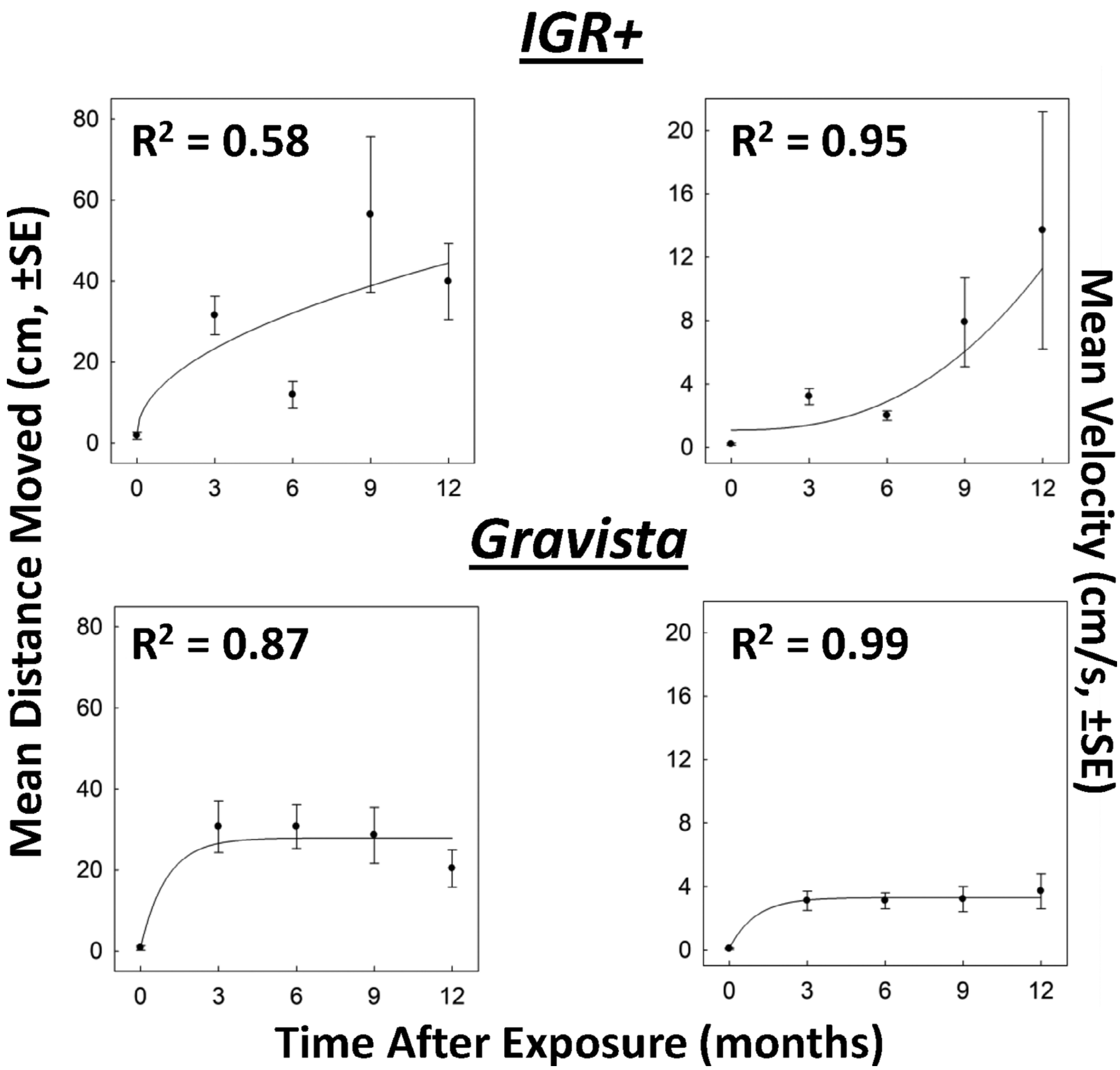

Figure 9. Nonlinear regression of mean distance moved (cm, ± SE; left-hand panels) and mean instantaneous velocity (cm s^{-1}, ± SE; right-hand panels) against time on commodities (corn) by *T. castaneum* adults exposed to aged Diacon IGR+ (top panels) or Gravista (bottom panels) residues. Fitted lines are shown in solid black with corresponding goodness of fit (R^2); graphs without did not have models that were significant.

was a loss of some efficacy for direct mortality,[17] but this loss of effectiveness appears even greater for the movement assessed in this study. A study evaluating Gravista for different sublethal effects on mobility by concentration of product found decreasing distance moved and velocity as concentration increased.[18] In this experiment, we used the maximum labeled rate, and thus our results represent a best-case estimate of the insecticide on mobility.

The predicted movement of each species is expected to dramatically increase with time. For example, after initial application, insects are expected to move only 4 m in the first 24 h after exposure, whereas by 3 months post-application, this increases by 3.5-fold to 14 m, and by 12 months another two-fold increase to 28 m. These distances are not trivial on the scale of a large grain bin or a food milling and processing facility. In fact, they represent the difference between insects not escaping the area of application and succumbing, or dispersing to a new food patch or bin in a facility and successfully reproducing. The residues were aged on the floor of an empty grain bin at the CGAHR, but even warmer climates may expect an even more precipitous increase in movement post-application. For instance, Arthur[32] found that there were an increased number of degree-hours above 32.2 °C in nonclimate-controlled bins during summer, which led to decreased efficacy of deltamethrin applied to concrete exposure arenas for control of *T. castaneum* compared to efficacy of exposures on arenas in autumn or held in climate-controlled chambers. In the last couple decades, there has been increasing awareness that behavioral data should be taken into account when assessing risk from insecticides, but others have noted that such an approach will require a larger foundational set of data to predict longer-term consequences on species.[29] Although there has been work carried out on examining the mechanisms of sublethal exposure in stored product entomology,[33] and characterizing sublethal changes after exposure,[23] few attempts have been made to predict sublethal changes before exposure. More recently, some have predicted that climate change may increase sublethal impacts of chemicals on a variety of species.[34] Our values here provide testable hypotheses for future work. This predicted sublethal movement should be taken into

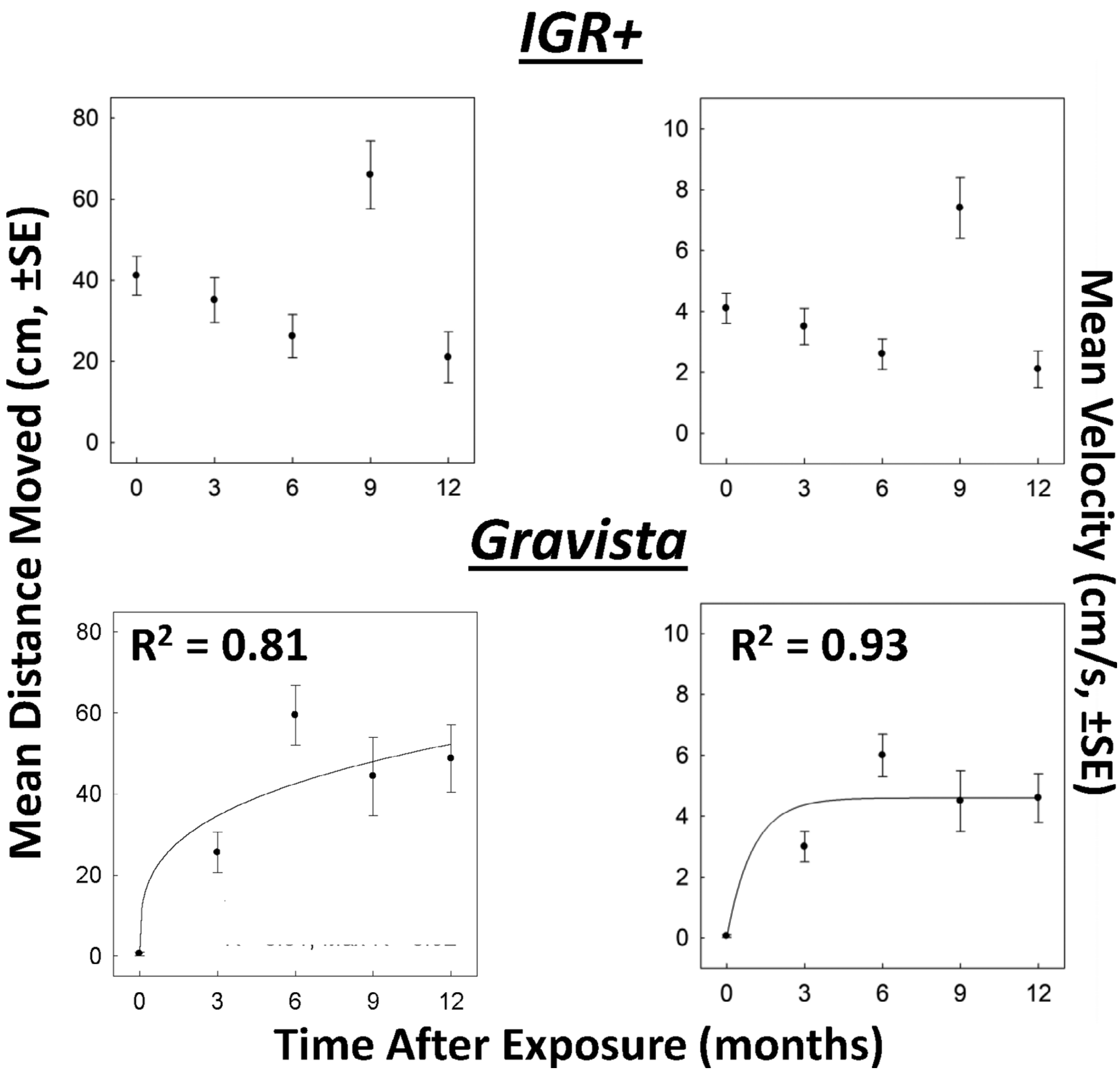

Figure 10. Nonlinear regression of mean distance moved (cm, ± SE; left-hand panels) and mean instantaneous velocity (cm s^{-1}, ± SE; right-hand panels) against time on commodities (corn) by *S. zeamais* adults exposed to aged Diacon IGR+ (top panels) or Gravista (bottom panels) residues. Fitted lines are shown in solid black with corresponding goodness-of-fit (R^2); graphs without did not have models that were significant.

account when considering application for management of insects in milling and processing facilities.

Given the abundant capacity of stored products insects for movement after even prolonged exposure to residual insecticides, the current work emphasizes two important messages. First, proper sanitation protocols are imperative; their conspicuous absence at a facility will present increased opportunities for treated insects to escape residual insecticides, feed, recover and likely infest new parts of a facility. Prior work also has found that decreased sanitation impaired a range of IPM tactics, including chemical control.[9] Second, it appears important to combine killing agents with effective, broad-spectrum attractants to effectively retain stored product insects and prevent subsequent dispersal to safety or new parts of a food facility. Prior work has shown that the behavior of stored product insects may be amenable to behavioral manipulation,[35–39] including combining a killing agent with attractants in a potential attract-and-kill approach to intercepting insects outside a facility.[16] Correcting the historical bias against evaluating sublethal effects by insecticides on stored product insects in the future for other compounds may deliver additional insights and produce a more comprehensive evaluation of insecticide use in the post-harvest supply chain.

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CONFLICT OF INTEREST

The authors acknowledge that they received partial funding from Central Life Sciences for this research, and declare that they have no other conflict of interests.

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