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Evaluation of dosimeter tubes for monitoring phosphine fumigations

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A B S T R A C T

Within integrated pest management options, fumigation of stored products is one method to help control post-harvest insect infestations in our food and agricultural products. Fumigant gas concentration monitoring is important to confirm that the treatment was adequate to achieve the desired insect control, but monitoring can be relatively expensive and labor intensive. This study evaluated how accurately dosimeter tubes could monitor phosphine fumigation treatments. The dosimeter tube is designed to continuously react with phosphine gas during the fumigation period and yields a measurement in terms of concentration * time product or CT, which can be interpreted as cumulative exposure. Two models of dosimeter tubes were evaluated (high range and low range). The reference method for these trials were wireless phosphine monitoring sensors, which recorded gas concentrations at hourly intervals during an exposure, and from this a CT product was also calculated. Model LPG-1, high-range dosimeter tube, measured within ± 25% of the phosphine monitoring sensors for CT dosages less the 70,000 ppm*hr. Model LPG-2, low-range tube, tended to significantly over-estimate phosphine CT dosage by 50%–100% of the phosphine monitoring sensor references. Secondly, bioassays of fumigant efficacy were performed using susceptible and resistant adult *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae), lesser grain borers, and *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae), red flour beetle, for estimating insect control at the varied fumigation CT treatments. For the susceptible strains, CT dosages ~5000 ppm*hr controlled both species. However, the insect control varied from 60% to 100% for resistant adults at CT dosages of ~20,000 ppm*hr. The dosimeter tubes function in these ranges of dosages where each insect species are controlled and the dosimeter tube model LPG-1 provides reasonable estimates of the fumigation dosage for a given treatment level.

1. Introduction

Phosphine fumigation of stored products is one method to help control infestations of insect pests (Phillips et al., 2012; Flinn et al., 2003). Monitoring fumigant levels during treatment is important to ensure the phosphine gas reached targeted concentrations and was held for a long enough time to provide complete control of the target pest species. The combination of phosphine concentration and holding time is referred to as the concentration*time product (CT) and can be measured in units of ppm*hr. Low CT products can lead to fumigation failures which can result in rapid population rebound and contribute to the development of insecticide resistance. Phosphine resistance in a range of stored-product insect species has become widespread throughout the world (Pimentel et al., 2010; Afful et al., 2018; Huang et al., 2019; Nayak et al., 2020).

Many fumigation monitoring technologies are available, including those designed for measuring low levels of phosphine for monitoring worker safety to those that measure the higher gas concentrations obtained during treatments. Technologies range from relatively simple gas sampling tubes to sophisticated electronic measuring systems. Because phosphine is so corrosive to metals, electronic systems have until recently used a pump to draw an air sample from the fumigation chamber or bin through a sensor system, which can be smaller, handheld systems or larger, more complex systems that can process multiple samples. These fumigation monitoring methods tend to be relatively labor and time intensive, and so, in practice fewer data points are collected during treatments, resulting in lower resolution. Newer technologies that can be placed inside a confined space during a fumigation and

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wirelessly transmit data in real time to a cloud based server offer the potential to more accurately and quickly track fumigations, while reducing the time and labor involved in collecting that information (Agrafioti et al., 2018; Glennon et al., 2018; Brabec et al., 2019). One drawback to using electronic technology is the greater cost which might be too expensive for some users such as fumigation in developing countries. Another drawback is the need for the infrastructure to receive the wireless signals and transmit them to the cloud. In some cases, postharvest agricultural structures may be composed of materials that prevent adequate transmission of signals or the fumigation location may simply lack access to basic electrical power.

Although glass tubes with material that reacts with phosphine have been widely used for years, they only provided information on the concentration at a single time and required the same time and labor as reported earlier to draw gas samples. Recently, a new technology has been developed consisting of gas-sensing tubes, or dosimeter tubes, which can be placed inside a space being fumigated and, at the end of the treatment period, provide a measure of cumulative CT that could be used to determine if the phosphine reached the appropriate concentrations. Fumigation monitoring is an essential step in determining the potential effectiveness of the fumigation. These newer dosimeter tubes react to phosphine concentrations during the entire fumigation event and the resulting tube discoloration is reported to be proportional to the CT product. While this system doesn't provide real time monitoring, it does allow greater flexibility and lower cost in terms of materials, time and labor compared to other methods and could be used to monitor fumigations conducted under transit such as in railcars or ships. Dosimeter tubes have been developed for other agricultural applications such as measuring ammonia from soils treated with chicken manure (Van Andel et al., 2017) or measuring irradiation of fruits and vegetable during phytosanitary processes (Menon et al., 2019). To our knowledge, there is no published information on these dosimeter tubes for measuring phosphine.

Fumigation monitoring is vital for knowing whether the targeted CT was achieved, and the treatment was adequate to control the target insects. Given the levels of phosphine resistance that are present, it is also important that the target CT is sufficient to provide control of the specific target species or strains of those species present at a location. The objectives of this study were to (1) evaluate the accuracy of the CT estimate from dosimeter tubes in comparison with CT estimates calculated from data obtained using wireless phosphine monitoring devices; and (2) relate CT values to efficacy against susceptible and resistant strains of two common stored product insect species, *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae), the lesser grain borer, and *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae), the red flour beetle.

2. Materials and methods

2.1. Fumigation tests

Experiments were carried out at the USDA-ARS-Center for Grain and Animal Health Research (CGAHR) in Manhattan, Kansas during the summer and fall of 2019. The fumigation treatments were done in 55-gallon barrels which were half filled with ~80 kg of hard red winter wheat. The barrels were covered with lids which contained rubber gaskets and a turn-bucket ring to hold the lid tightly to the top of the barrel. The lids were modified with inlet and outlet airlines and valves which were used to flush the barrel with fresh air and exhaust the phosphine gas after each treatment period. The inlet plastic lines were 6.4 mm I.D. and 9.5 mm O.D. lines while the exhaust lines were tygon tubing with 19 mm I.D, and 25 mm O.D. (Supplemental Fig.).

There were five targeted levels of phosphine initial concentrations: 0, 70, 210, 700, and 1400 ppm. These concentrations of phosphine were achieved using two different types of phosphine generating pellets. The first pellet had a base of magnesium phosphide (Mg_3P_2) and was part of the Detia-Degesh Phosphine Tolerance test kit (Degesch America, Weyers Cave, VA) and these Mg_3P_2 pellets are not the same as those for commercial fumigations. For the target of 70 ppm in the barrel, a single Mg_3P_2 pellet was used. For the target of 210 ppm, three Mg_3P_2 pellets were used. The second type of pellet had a base of aluminum phosphide (Al P) (Phos-Toxin, Industrial Fumigant Company, Lenexa, KS). The Phos-Toxin pellets weighed 0.6 g each. The testing was performed in 208 L barrels which were half full of grain. A single pellet was found to generate peak phosphine concentrations of ~700 ppm target concentration within the barrel while two pellets were found to generate ~1400 ppm peak concentration. The pellets were added to the top 8 cm of the grain. The 0 dose or control barrel did not have any phosphine pellets added.

Three holding times or exposure periods were evaluated (24, 48, and 96 h). Holding time was the blocking factor per trial. Experimental observations were collected from nine trials, which represented the three holding times and three replications. For each trial, five barrels were used with one barrel at each target concentration (0, 70, 210, 700, or 1400 ppm). Each barrel received dosimeter tubes, WiFi gas monitoring sensors, and insect bioassays (details on each is provided below). The WiFi sensors were set on top of the grain while the dosimeter tubes and insect bioassays were inserted into the top 10 cm of the grain. All measuring methods were placed within 25 cm of each other.

This experiment was conducted in the summer months of July and August of 2019 at USDA-ARS facilities in Manhattan, Kansas. The average temperatures were 29 °C inside the barrels and ranged ± 4 °C over the course of the diurnal cycle. The experiment was repeated during Autumn under cooler ambient temperatures (average 15 °C, and ranged from 13 to 18 °C), but only two target phosphine concentrations (200 and 700 ppm) and a single 48-h exposure period were used. In the Autumn experiment there were three replicates. Six barrels were used with three barrels at ~200 ppm (3 Mg_3P_2 pellets per barrel) and with three barrels at ~700 ppm (1 Al P pellet per barrel). Control barrels were not used in the fall experiment based on results of previous experiment, rather phosphine measurements were collected near the barrels inside the bin. The barrels were tight and low leakage occurred. Any leakage that occurred would dissipate in the larger volume of the bin which was well ventilated.

2.2. WiFi phosphine monitoring sensors

Wireless phosphine monitoring devices (Centaur Analytics Inc., Ventura, CA) were used in each barrel to provide the baseline data on phosphine concentrations over time (Fig. 1) and simultaneously recorded temperature data. The devices were programed to record both measurements every hour. Each monitoring device transmitted the sensor data to a WiFi interface that uploaded the data to a cloud-based server. The hourly data were processed and a total CT dosage was computed as a summation of the hourly concentrations over the trial exposure period of either 24, 48, or 96 h.

2.3. Dosimeter tubes

Two models of phosphine dosimeter tubes (Uniphos Envirotronic, Sugar Land, TX, USA) were tested; LPG-1 tubes (high range max. 200,000 ppm*hr) and LPG-2 tubes (low range max. 20,000 ppm*hr). Barrels with low phosphine CT levels contained both low and high range tubes. However, for high phosphine CT

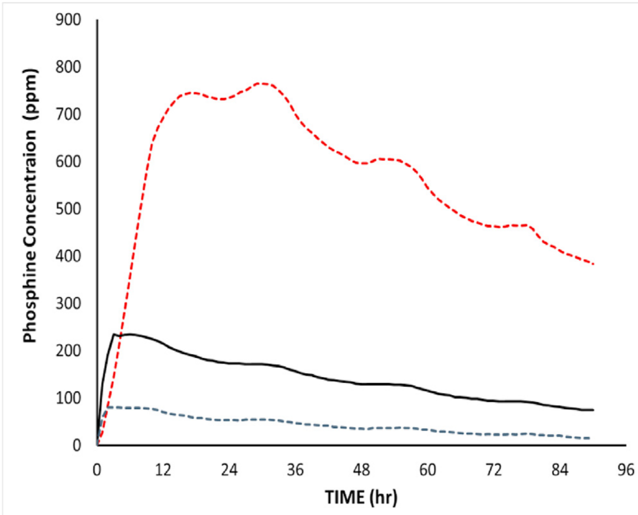

Fig. 1. Example of the data collected by Centaur phosphine monitoring devices showing hourly phosphine concentration with either 1 Mg_3P_2 (low, black dashed line), 3 Mg_3P_2 (medium, solid black line) or 1 Al P pellet (high, red dashed line) treatments. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

barrels, only the high range tubes were used, because the phosphine CT exceeded the designed maximum range of the low-range tubes. Each glass dosimeter tube was scored around the circumference near one end by the manufacturer. Thus, the glass end could be carefully snapped off, which exposed the dosimeter to the surrounding air or phosphine gas. Tubes were secured by rubber grommets inside vented aluminum caps (Fig. 2) and then fitted securely into the PVC body (CF0973-dosimeter tube holder, Uniphos Envirotronic, Sugar Land, TX), which was added to a barrel.

2.4. Insect bioassays

Two insect species, *T. castaneum and R. dominica*, and a phosphine susceptible and resistant strain for each species, were evaluated. For *T. castaneum*, four-to eight-week-old adults of susceptible (laboratory strain maintained in culture for over 30 years at the USDA-ARS in Manhattan, KS) and phosphine-resistant (field-collected strain from Pachecho, Brazil in 1988) strains were used. For *R. dominica*, four to eight week old adults of susceptible (field strain collected outside a mill in Kansas in 2012)

Fig. 2. Protective PVC tube, red rubber grommets, and aluminum caps for holding dosimeter glass tubes while deployed in commodities and three dosimeter tubes which were exposed during fumigation trials. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

and phosphine-resistant (field strain collected in Enid, Oklahoma, USA in 2010) strains were used. *Rhyzopertha dominica* was reared on organic wheat and *T. castaneum* was reared on a mixture of 95% unbleached, organic flour and 5% brewer's yeast. Susceptible colonies were maintained at 27.5 °C, 65% RH, and 14:10 (L:D) h photoperiod, while resistant colonies were reared at 25 °C, 65% RH, and 16:8 (L:D).

All beetles used in experiments were collected from colonies on the same day as testing and held in individual plastic containers (9 × 9 cm, Maryland Plastics, Federalsburg, MD, USA) prior to addition to bioassay tubes. The bioassay tubes were plastic, 10 cm long, 1.2 cm I.D., with ends sealed with corks. Each tube had ten ~0.5 mm holes, five on a side, which allowed for gas penetration. In each tube, 20 individuals of a given species or strain were added without food. There were four tubes of insects per barrel with one tube for each species and susceptibility. These bioassays were inserted into the top 10 cm of the grain and near the gas sensor and dosimeter tube. After each fumigation trial, the tubes were collected and taken to the laboratory, and the tubes were emptied with insects observed under a microscope. The insect responses were classified as either alive, affected/knocked down, or dead based on Morrison et al. (2018) and Agrafioti et al. (2020). Briefly, dead individuals were completely immobile (no movement or twitching from legs or antennae, even after prodding), alive individuals were fully mobile, without twitching of any sort, and could right themselves when flipped, while affected individuals were somewhere between these two extremes. The insects were placed back in their tubes with ~0.1g of flour and stored in an environmental chamber at 25 °C, 65% RH. After a 3-day recovery period, the insects were re-evaluated and classified as either alive, affected, or dead. For analysis, the combined dead and affected insects after 3 days were used to determine the relationship with the phosphine CT.

2.5. Experimental design and analysis

A total of nine primary trials were performed and yielded 45 phosphine treatment observations (3 holding times * 5 phosphine levels * 3 replications). The resulting data measured phosphine CT dosages with two methods; the WiFi phosphine monitoring system and the dosimeter tubes. The comparison of phosphine measurement methods was analyzed statistically with the Accuracy and Precision routines available in the Method Comparison Add-In of JMP® 13.2.1 (SAS Institute Inc., Cary, NC). The statistical approach follows Clinical and Laboratory Standards Institute guidelines (clsi.org). The two methods are compared for measurement agreement based upon starting phosphine target levels (target PPM * hr) and response level measured after time of exposure (Bland and Altman, 1999). The fall experiment had 6 phosphine treatment observations (2 phosphine levels * 1 holding time * 3 replications).

3. Results

3.1. Fumigation CT dosages

The phosphine CT dosages (ppm*hr) achieved during the experiments, as determined from the WiFi phosphine monitoring sensor data, varied among replications, likely due to variation in chemical reactions and gas leakage from the barrels (Table 1). Overall, the aluminum phosphide pellets performed more consistently than the magnesium phosphide pellets. On at least two occasions, a single magnesium phosphide pellet released very low amounts of phosphine (Table 1). Across the replicates, a wide range of CT dosages were created, which facilitated the evaluation of the dosimeters and the insect bioassays. In control barrels no

Table 1
Experimental phosphine CT dosages obtained with the magnesium phosphide and aluminum phosphide pellets. C.V. is coefficient of variability or standard deviation/mean.

Treatment	Target	Achieved CT Dose (ppm*hr)			Dose	Dose
Exposure time	CT	rep1	rep2	rep3	Avg.	C. V.
1 Mg_3P_2						
24	1680	**391**	2221	1997	1536	65%
48	3360	2891	**293**	5232	2805	88%
96	6720	3995	3771	3666	3811	4%
3 Mg_3P_2						
24	5040	5372	7332	6536	6413	15%
48	10080	17240	11551	12748	13846	22%
96	20160	13558	17561	10812	13977	24%
1 Al P						
24	16800	14509	16892	13537	14979	12%
48	33600	33434	35448	33319	34067	4%
96	67200	54568	66725	58644	59979	10%
2 Al P						
24	33600	26245	43593	33139	34326	25%
48	67200	65576	70428	63925	66643	5%
96	134400	140744	129478	137475	135899	4%

phosphine was detected in any replicates, using either measurement device (data not shown). The Mg3P2 pellets were special items made for use with a phosphine resistance testing kit and are not made in the same size or configuration as most commercial Mg3P2 products.

3.2. High range dosimeter tubes

For most treatments, the high range (LPG-1) dosimeter tubes provided overlapping estimates of CT compared to the WiFi phosphine sensors, except at the highest CT level (96 h, 1400 ppm target) (Fig. 3). Variability was similar between the two phosphine measurement systems. The coefficient of variability average 11% for the gas sensors and 16% for the dosimeter tubes.

The WiFi sensor CT dosage and LPG-1 (high range tubes) showed both positive and negative differences among observations with most of the differences being less than ± 25% for CT dosages less than 70,000 ppm*hr (Fig. 4). At the lower CT dosages, the two measurement methods were most similar with average percent difference of −7% with a standard deviation of 14%. At CT dosages greater than 70,000 ppm*hr, the average percent difference was 44% and the dosimeter tubes measured greater CT dosages than those calculated using the WiFi phosphine monitoring sensor data.

3.3. Low range dosimeter tubes vs phosphine monitoring sensors

As the CT dosage increased, the LPG-2 dosimeter tended to give higher CT values than those based on the WiFi phosphine gas monitoring sensors (Fig. 5). Overlap of measurements from the two methods only occurred in the lowest phosphine treatments of 70 ppm. On a couple occasions, the dosimeter's chemical reaction and dark indications went beyond the highest scale line of 20,000 ppm*hr and were estimated as 25,000 ppm*hr. However, none of the CT calculations from the WiFi sensors were evaluated beyond 18,000 ppm*hr. Some of the variability within each device is due to the varied phosphine gas concentrations generated at 70 and 200 ppm target concentrations as shown in Table 1.

Regardless of the phosphine CT, the LPG-2 dosimeter tube readings were higher than the calculated CT from the WiFi phosphine gas sensors (Fig. 6). The percent difference averaged 87% with a standard deviation of 46% and there was no apparent trend in differences across the tested CT ranges.

3.4. Autumn experiment

For the barrels treated with 3 Mg_3P_2 pellets to produce a target CT dosage of ~10,000 ppm*hr, the mean CT dosage for the phosphine gas sensors was 8744 ppm*hr. The mean CT dosage for LPG-1, high-range dosimeter tubes, was 9,667 ppm*hr while the mean from the WiFi sensors 8,744. A standard T-test comparison yielded T-ratio of 0.93 and Prob>F of 0.4, thus these measurements were not statistically different. However, the mean CT dosage for LPG-2, low-range dosimeters, was 23,667 ppm*hr and significantly different that the WiFi data with a T-ratio of 9.9 and Prob>F of 0.001. For barrels with a single Al P pellet and the target CT dosage of 34,000 ppm*hr, the high-range dosimeter LPG-1 mean reading was 27,000 ppm*hr while the mean of the phosphine gas sensor of 35,300 ppm*hr (Table 2) These values were somewhat different with the T-ratio of 3.0 and the Prob>F of 0.04.

3.5. Insect responses to phosphine CT dosage

The susceptible strain of *R. dominica* was controlled at almost all phosphine levels, even below 5000 ppm*hr, while the response of the resistant strain varied considerably across CT levels and even within similar CT levels (Fig. 7). Although highly variable, for the resistant *R. dominica* it appears that below 1000 ppm*hr negative effects on the species are very low, between 1000 and 5,000 negative effects increase, and above 10,000 generally have high percentage with negative effects. However, even at very high concentrations some replicates had no or a low percentage of affected insects, possibly from experimental errors.

The susceptible strain of *T. castaneum* was well controlled, even below 5,000 ppm*hr. The resistant *T. castaneum* had variable survival between 5,000 and 25,000 ppm*hr, with complete control reached at CT levels above 25,000 ppm*hr (Fig. 8). These tests were limited to studying the affects on adult insects. We did not study the affects on larvae or eggs of these species. The term %dead relates directly with the adult insect mortality. The term %KD or knock down related to potential fatality and potential reduction in insect reproductivity. The %KD valve was determined after 3 days of languishing. After 3 days of fresh air and still being knocked down, does not indicate rapid recovery, but rather continued ill-effects.

Insect response to phosphine changed with holding period and phosphine concentration, with response varying between species and between resistant and susceptible individuals (Fig. 9). The susceptible *T. castaneum* strain was controlled with ~100% categorized as nonresponsive, apparently dead, for even the minimal fumigation of 70 ppm at 24 h and this was stable out to 96 h post-exposure. The control of susceptible *R. dominica* was around 80% mortality at the lower phosphine concentration at 24 h, but at 48 and 96 h, mortality of the susceptible *R. dominica* was 100%. Over time after exposure, mortality continued to increase in both the susceptible and resistant *T. castaneum* strains. By contrast, the resistant *R. dominica* strain apparently recovered somewhat by 48 h compared to 24 h, before finally succumbing at 96 h. Resistant *R. dominica* reached 100% mortality at 700 and 1400 ppm concentration 96 h after exposure.

4. Discussion

The dosimeter tubes were relatively inexpensive and fairly easy to use. The dosimeter tube continuously reacts as long as it is exposed to phosphine gas and the chemical inside the tube is not saturated, thereby continuing to turn to a dark blue. However, after removal from phosphine exposure, this color fades with time, so the dosimeter tube should be collected shortly after the fumigation treatment and possibly photographed for a more permanent

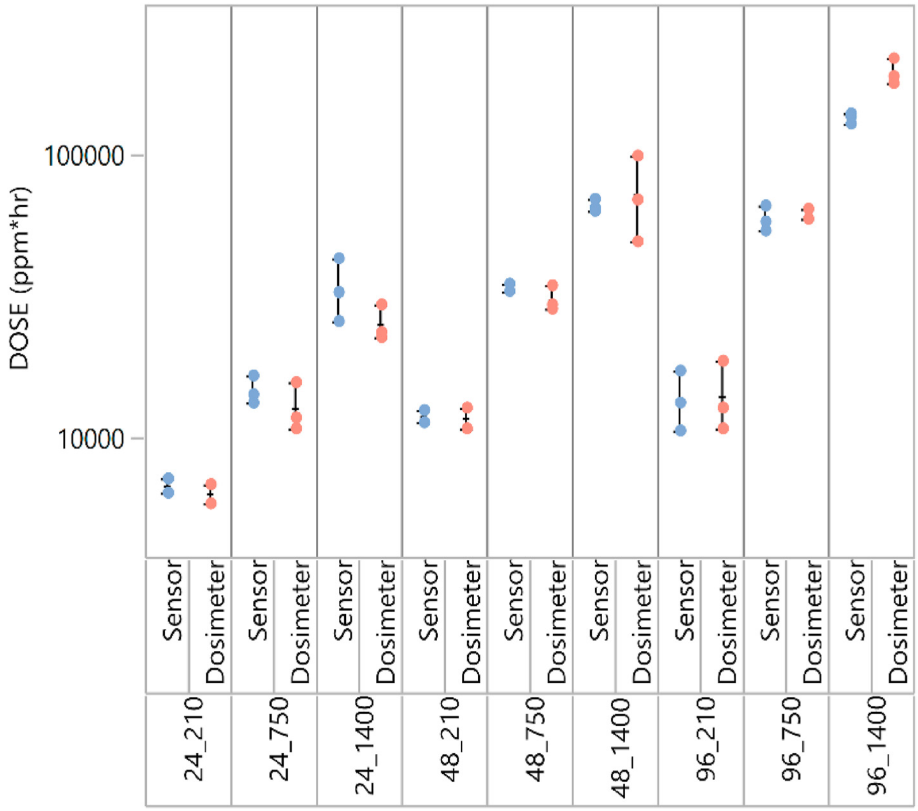

Fig. 3. Comparison of CT dose (ppm*hr) calculations between the WiFi phosphine sensor (blue dots) and high range LPG-1 dosimeter tube (red dots) at different combinations of hold times (24, 48, and 96) and target phosphine concentrations (210, 750, and 1400 ppm). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

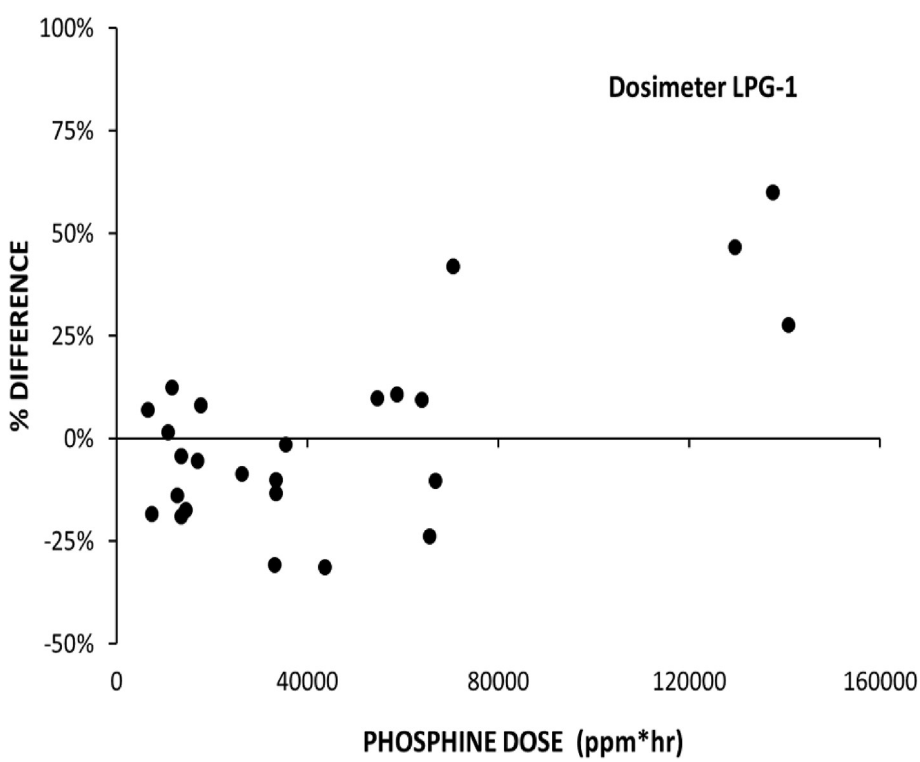

Fig. 4. Plot comparing the percent difference between the CT computed from the WiFi phosphine monitoring sensors and CT dosage recorded by the LPG-1 high-range dosimeter tubes (difference between two method divided by the WiFi phosphine monitoring sensor CT).

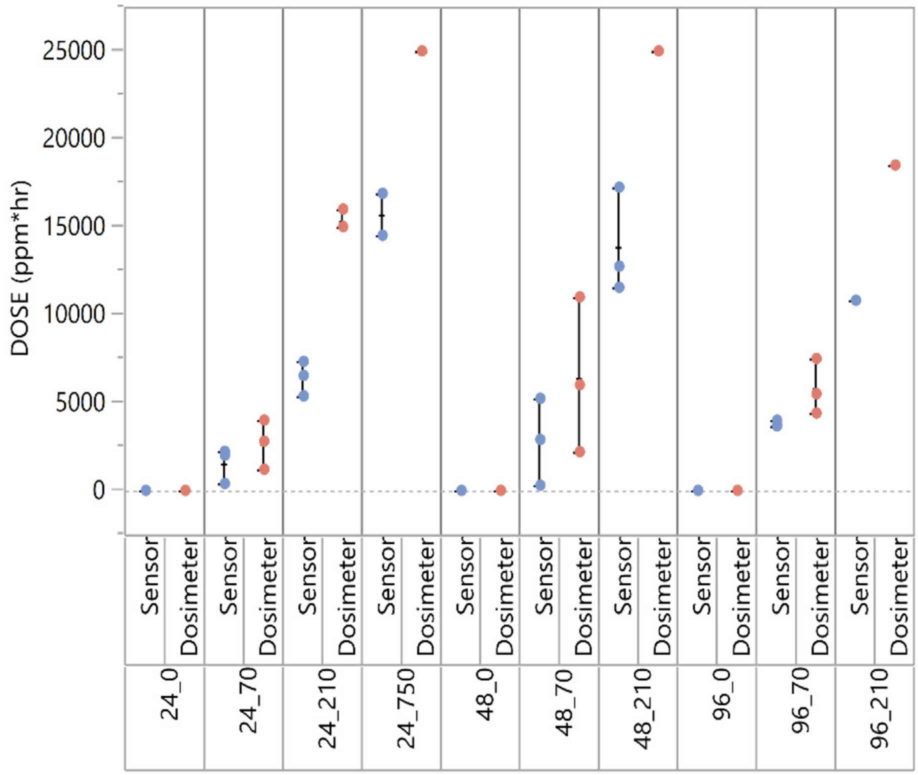

Fig. 5. Comparison of CT dose (ppm*hr) calculations between the WiFi phosphine sensor (blue dots) and low range LPG-2 dosimeter tube (red dots) at different combinations of hold times (24, 48, and 96) and target phosphine concentrations (0, 70, 210, and 750 ppm). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

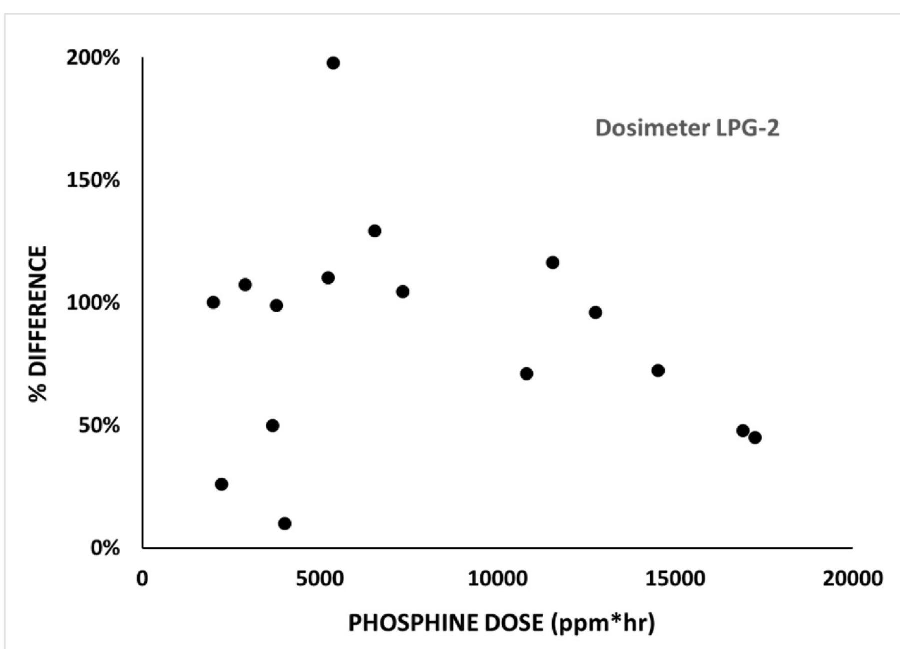

Fig. 6. Plot comparing the percent difference between the CT computed from the WiFi phosphine monitoring sensors and CT dosage indicated in the LPG-2 low-range dosimeter tubes (difference between two method divided by the WiFi phosphine monitoring sensor CT).

record. The scale on the dosimeter tube is non-linear and the lower portion of the scale is easier to read, because the upper scale demarcation lines are spaced closely together. But, at those higher CT levels, especially above 100,000 ppm*hr, the insects are controlled and accuracy of readings is less of a concern.

The dosimeters are made of glass and usage of glass materials in and around grain and processing facilities is discouraged because of the potential for broken glass entering the product stream, which is

Table 2
Autumn experiment results. Phosphine concentration*time (CT) dosages obtained from two fumigation treatments. The WiFi phosphine sensor was compared to both the LPG-2, low-range dosimeter tubes, and LPG-1, high-range dosimeter tubes at 10,000 ppm*hr. The fumigation treatments were generated by using the magnesium phosphide and aluminum phosphide pellets.

Treatment	barrel	Sensor	LPG-1	LPG-2
Target CT		ppm*hr	ppm*hr	ppm*hr
3 Mg_3P_2				
10,000	1	8,256	9,000	25,000
10,000	2	10,193	11,000	25,000
10,000	3	7,785	9,000	21,000
	mean	8,744	9,667	23,667
	s.e.	737	667	1333
1 Al P				
34,000	4	32,688	23,000	na
34,000	5	35,600	27,000	na
34,000	6	37,644	31,000	na
	mean	35,311	27,000	
	s.e.	1438	2309	

difficult to catch or remove. Glass is one of the many potential physical contaminants that are addressed in hazard analysis and critical control point plans, HACCP (Fowler, 2012). The dosimeter tubes attempt to address this issue with the protective plastic case to hold each tube. Careful documentation of placement and removal of dosimeter tubes during fumigations should be practiced or possibly restricting applications to where the dosimeter tubes can be placed outside of the bulk materials may be prudent.

Monitoring fumigations is essential for estimating the resulting CT dosage and potential insect control. Two methods of monitoring fumigations were used in this study, namely dosimeter tubes and WiFi phosphine monitoring sensors. These methods provide phosphine measurements for a particular location within a grain bin or container but do not describe the distribution of phosphine within a container. For example, a single measurement at the top of a grain bin may not describe phosphine gas concentration at points of 2 m below the grain surface (Brabec et al., 2019). From multiple phosphine sampling locations and time data, mathematical models can be used to help estimate the fumigation gas distribution and treatment of the entire volume. Several groups have developed fumigation gas modeling such as Centuar Analytics as well as university researchers (Isa et al., 2016; Kaloudis et al., 2018; Plummer et al., 2018).

These trials were done with adults only to estimate potential insect control given a range of possible phosphine CT values from these trials. For resistant insects, our data shows that 10,000 ppm*hr of phosphine would provide only 60%–80% control of these adults, and also provides an explanation of how resistant species persist and multiply under normal fumigation conditions in the postharvest supply chain (Opit et al., 2012; Kaur and Nayak, 2015). Grain storage specialists at Oklahoma State University recommend that phosphine fumigations maintain at least 200 ppm for 100 h or a minimum CT of 20,000 ppm*hr (Jones et al., 2017). To fumigate and maintain 200 ppm over 100 h requires that the grain

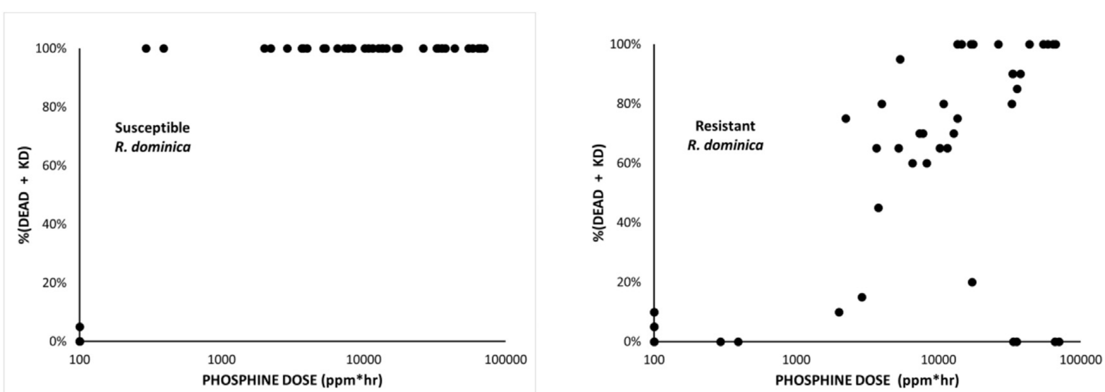

Fig. 7. Percentage of dead and affected (KD) adults for susceptible (left) and resistant (right) *Rhyzopertha dominica* strains at different experimental phosphine CT dosages as determined with the WiFi sensors.

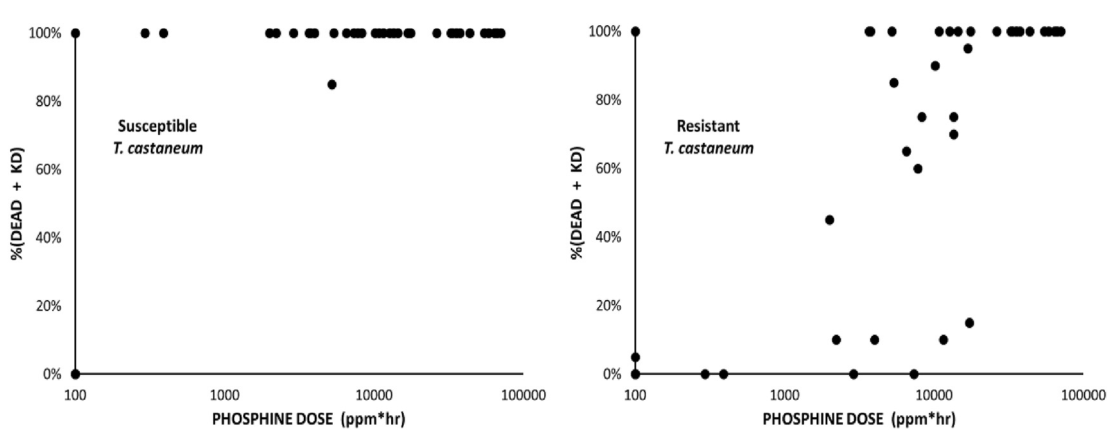

Fig. 8. Percentage of dead and affected (KD) adults for susceptible (left) and resistant (right) *T. castaneum* strains at different experimental phosphine CT dosages as determined with the WiFi sensors.

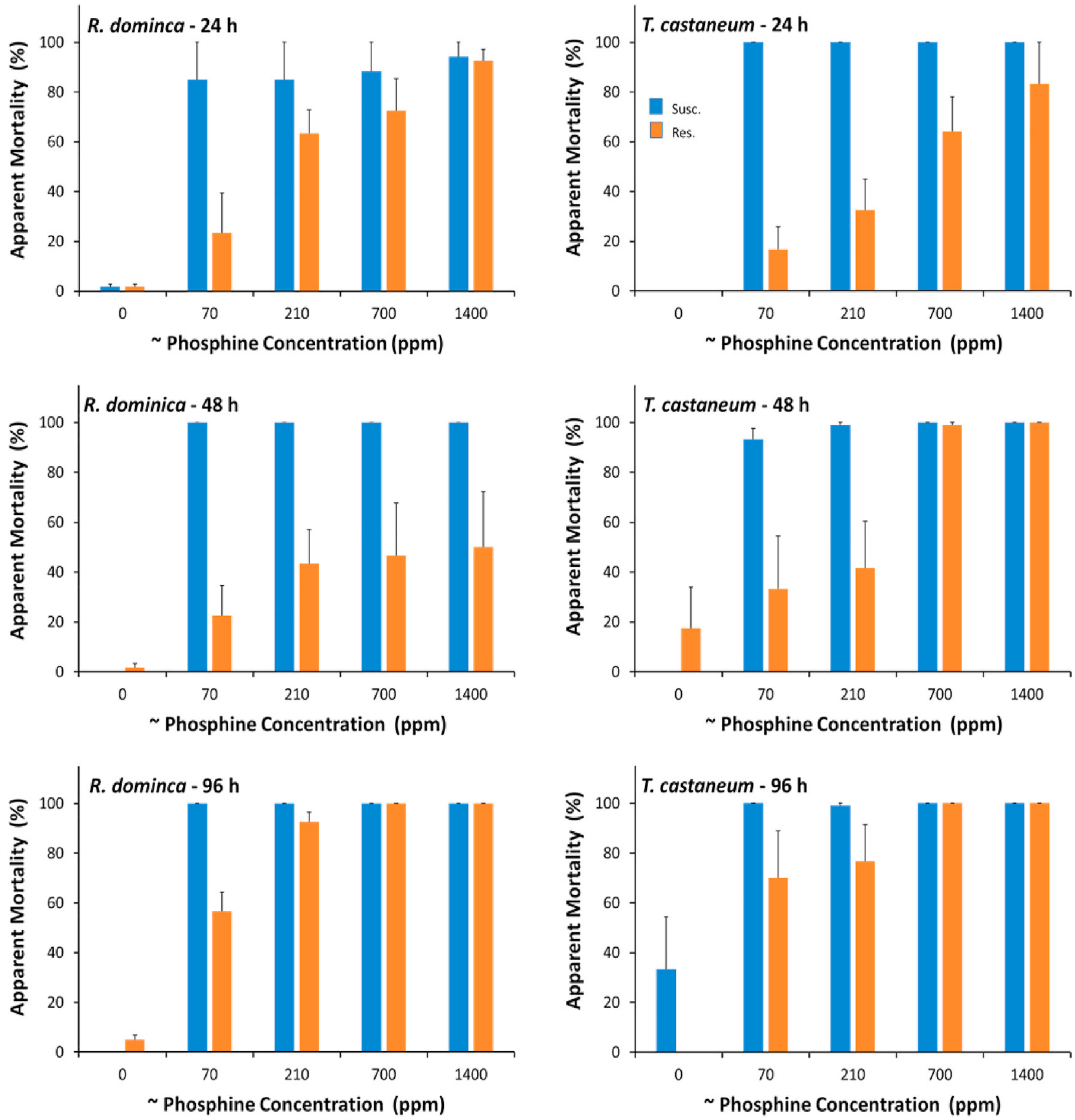

Fig. 9. Insect mortality after exposure to different phosphine concentrations after holding times of 24, 48, and 96 h for *R. dominica* (left) and *T. castaneum* (right) susceptible (dark grey) and resistant strains (light grey).

structures or transport vessels are well-sealed and have minimum gas leakage. If real-time phosphine monitoring is used and it indicated low phosphine levels, fumigators could use chemical delivery systems that automatically inject more gas or tablets periodically during the holding period to maintain target phosphine concentrations.

Other researchers have tested mix-aged colonies of *R. dominica* during phosphine fumigations (Collins et al., 2005). They determined the time to population extinction (TPE) and developed a nonlinear mathematical relationship of phosphine concentration and fumigation holding time which model those relationships. The lethal time for control (LT_{99}) for resistant strain fit the equation $C^{0.54}*T = 3.85$ using concentration values in mg/liter and time in days. Using their equation and for concentrations of 1.0, 0.3, and 0.2 mg/L, the resulting treatment times were 3.9, 7.4, and 9.2 days. The corresponding CT products of those phosphine treatments were ~66400, ~38100, and ~31700 ppm*hr, respectively. These CT products reflect the levels necessary to kill both adults and eggs and to extinguish the population by 8 weeks, provided there is not any re-infestation, an assumption that is almost never true given what is known about stored product insect movement and landscape dispersal. Daglish et al. (2002) found mix-aged colonies of resistant *Sitophilus oryzae* had an LT_{99} of ~86000 ppm*hr (1 mg/L * 5 days). Gautam et al. (2016) found the lethal concentrations (LC_{95}) during 72-h fumigation periods for several strains of *T. castaneum* adults and eggs. The varied resistant strains of adults required CT dosages from ~2100 to ~13500 ppm*hr for 95% control while the resistant eggs required ~10,000 to 30,000 ppm*hr.

There was a clear contrast between the susceptible and the resistant strains of both insect species. These contrasting insect responses might encourage fumigators or applicators to test a subsample of insects that they are trying to eradicate prior to treatment for phosphine tolerance. There are methods to estimate insect resistance by using a 30-h FAO test procedure (Collins et al.,

2017) or by using an abbreviated 1-h method with a test kit that is available from Detia-Degesch (Athanassiou et al., 2019; Cato et al., 2019).

Dosimeter tubes are less expensive the electronic sensing systems. Dosimeter are easy-to-use and come with a protective plastic sleeve, thus they could potentially be deployed in areas of fumigation where rough handling and breakage is not a concern. Overall, the LPG-1 dosimeter tubes provided good correlations and estimates of 'phosphine concentration' x 'holding time' product (CT) with the WiFi sensors up to ~60,000 ppm*hr. The LPG-2 dosimeter tubes were designed for CT below 20,000 ppm*hr. Associated bioassays revealed that CT dosages over 25,000 ppm*hr are required to significantly control our adult resistant lesser grain borer and red flour beetles. Also, the LPG-2 dosimeter tubes tended to significantly overestimate CT dosages as compared to the WiFi sensors. For producing the recommended 25,000 ppm*hr dosage, an average of 250 ppm phosphine would need to be contained within the product being fumigated for 4 days or ~100 h. The specific insect control for a given fumigation event undoubtedly varies with species and susceptibility/resistance of insects located at the site of the fumigation. Rapid screening of insects beforehand as available with the Detia-Degesch phosphine resistant test kit would help determine fumigation requirements for significant control.

Author contributions

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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.

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Appendix A. Supplementary data

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