has focused on the key grain pest the red flour beetle (*Tribolium castaneum*) and explored the ability of the new form of silica to control the various beetle life stages. The results show that the efficacy of the new silica against larvae was nearly two-to threefold higher than that of adults. The results also show a clear difference in performance between hydrophilic and hydrophobic forms of the silica with hydrophobic forms outperforming the hydrophilic forms for the control of the red flour beetle.

Susceptibility of Stored Grain Insects to the Insect Growth Regulator Methoprene

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Abstract

The insect growth regulator (IGR) methoprene is labeled in the United States (US) for direct application to stored grain commodities, and as a residual surface treatment to empty grain bins and flooring surfaces inside indoor structures. Methoprene is also labeled in the US as an aerosol for use in indoor areas. One of the challenges in research with methoprene and stored product insects is through design of experiments that mimic how methoprene would be used in practical applications. Recent research with methoprene will be used to describe experimental designs to examine efficacy of methoprene when used as a grain protectant.

Keywords: stored products, IGRs, management, efficacy

1. Introduction

Insect pests can cause economic damage to stored grain commodities, especially in warm temperature areas and in the tropics. One of the components of integrated pest management (IPM) for stored grains is the use of protectant insecticides, which are applied as raw commodities are loaded into grain bins or elevator silos. Historically organophosphate insecticides were the primary protectants used by grain managers, but due to concerns with insecticide resistance, along with new regulations, some products in developed countries have been removed from the market (Daglish, 2008; Arthur, 2012). Today there is more emphasis today on using reduced-risk insecticides, including but not limited to pyrethrins, pyrethroids, insect growth regulators (IGRs) (Arthur, 2012), and biological insecticides such as spinosad (Hertlein et al, 2011, Nayak and Daglish, 2017).

Other components of IPM programs for stored grains include the use of aeration, which involves using low airflow rates to cool and modify a grain bin, thus limiting the growth of insect pests Arthur and Casada, 2016). It is not to be confused with grain drying, which utilizes much higher airflow rates to remove excess moisture from grains before they are stored (Navarro et al., 2012). Pre-binning cleaning and sanitation is also important, as the environment in and around grain storage sites can contain residual pockets of grain residues and spillages that will support insect pest development (see references in Arthur, 2018). Finally, the primary component for controlling stored grain insects is the fumigant phosphine. Concerns regarding the development of phosphine resistance (Lorini et al., 2007, Nayak et al., 2015, 2017, Afful et al., 2018) could lead to more extensive use of grain protectants. Thus, when designing experiments to evaluate efficacy of grain protectants, including reduced-risk adulticides and IGRs, there are multiple factors that must be considered

One IGR that is being used in the US as a grain protectant is methoprene, which is also labeled as a residual surface treatment and as an aerosol inside structures to control stored product insects. As an IGR, it does not kill adults, and it has limited efficacy against *Sitophilus species* (Lui et al, 2016). Methoprene can be used alone, or combined with other strategies, including the use of aeration (Arthur, 2016; Lui et al., 2016) and with the pyrethroid deltamethrin (Kavallieratos et al., 2015). Multiple factors should be considered when planning experiments utilizing methoprene as a grain protectant. This paper presents a review and discussion of some of those factors as they relate to

the use of methoprene alone and in combination with other strategies, and will not necessarily follow the standard format of a research paper, though data will be presented to illustrate specific concepts and ideas.

2. Three Simulated field trials

Here at the USDA-ARS Center for Grain and Animal Health Research (CGAHR), we have grain bins of different storage capacities that can be used for studies. These bins are equipped with aeration systems that operate when temperatures fall below set thresholds, using a controller that operates as a thermostat when outside ambient temperatures fall below specified levels (Arthur and Casada, 2005, 2010, 2016). We have also conducted studies with university cooperators that also have small-scale storage bins equipped with similar aeration systems (Lui et al., 2016).

3. Field Trial One: Pitfalls with inconsistent natural populations

One of the problems with field trials involving methoprene is the difficulty assessing treatment effects by examining natural insect populations in treatment and control bins. The first example is a test in which wheat was treated with a target application rate of 2.5 ppm as it was loaded into each of two 110-metric ton (MT) capacity metal grain bins at the CGAHR, using a commercial application system. Two untreated bins served as controls. All bins had aeration systems that were set to operate at an approximate rate 0.206 m^3/min/MT when temperatures fell below 23.9, 15.6, or 7.2 °C (Arthur and Casada, 2005; 2010). After the bin was cooled to the desired temperature the aeration fans were turned off until the next cycle.

The treated and untreated wheat was loaded into the bins the first week of August. After the bins were filled, HOBO temperature cables (Onset Computers, Pocasset, MA, USA) were put into the bins at the North, South, and Center positions, at depths of 0.3, 0.9, and 1.8 m. In mid-August and at the end of the month, each bin was artificially infested with 500 adults each of *Rhyzopertha dominica* (F.), *Tribolium castaneum* (Herbst), and *Cryptolestes ferrigineus* (Stephens) to supplement natural populations. In late August insect pest populations in the bins were assessed by placing five plastic pitfall traps, at each cardinal position and in the center, left for one week, and then removed from the bin. Samples for bioassays, using only *R. dominica*, were also taken as well. Thereafter bins were sampled monthly during autumn except in December, and in January, February, March, and April of the following year (eight total). Data for live adult insects collected in pitfall traps were totaled for all five traps in the two untreated bins. This was also done for all traps in the treated bins as well. Data were compared by a t-test (Statistical Analysis System version 9.2, Cary, NC, USA). Temperatures in the bins were plotted using Sigma-Plot (Systat Software, Version 11, San Jose, CA, USA).

The only species collected in the pitfall traps were *C. ferrugineous*, *Ahasverus advena* (Waltl), *Typhea stercoria* (L.), and *T. castaneum* (Fig. 1). Insects were collected in early autumn, but populations in treated and untreated bins declined during the Winter and except for *C. ferrigineous* did not increase in the Spring. However, there were several sample points were populations were greater ($P < 0.05$) in untreated wheat versus the treated wheat. Temperatures for most of the storage period were below 15 °C, the lower developmental limit for most stored product beetles (Howe, 1965; Fields, 1991) (Fig 2.).

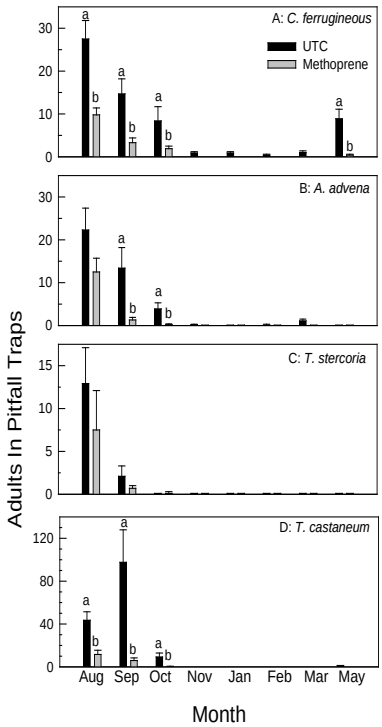

Fig. 1. Average number of adults of four insect species (A-D) collected from probe traps placed in bins with aeration alone (dark bar) versus bins with aeration + methoprene (grey barr), means denoted with different letters indicate significant differences ($P < 0.05$, Proc *t*-test, SAS). The sample in May was taken after aeration monitoring ceased.

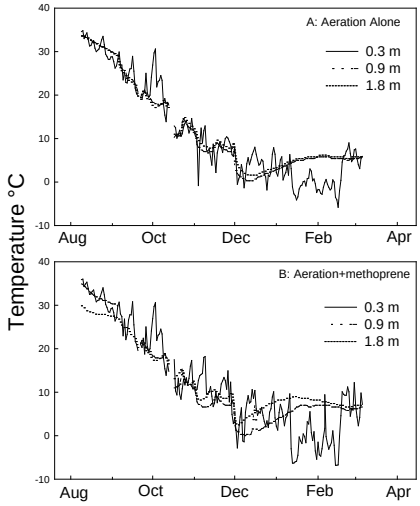

Fig. 2. Temperature at depths of 0.3, 0.9. and 1.8 m in wheat mass in 110 MT bins in wheat with aeration alone (A) and in wheat with aeration + 2.5 ppm methoprene (B).

The bioassay data gave a clearer picture of methoprene efficacy. These bioassays were conducted by placing 20 mixed-sex 1 to 2-week old adults on ca. 30 grams of wheat in a 37-ml capacity vial for two weeks, then removing the adults and holding the wheat in an incubator at about 27°C and 60% r.h. for about 6 weeks. At each sample point five 250-g samples were taken from each bin, and then subdivided into 30-gram lots for each of 5 vials. The remainder of the wheat was discarded. The wheat in the vials was warmed in the laboratory for several days before the parental adults were introduced. Thus, there were a total of 10 treated and 10 untreated samples. Average progeny production over all sample months was 99.9 ± 23.5, while progeny production in the methoprene bioassays averaged less than 0.1. This test indicated that strict reliance on natural populations of stored product insects may not be an appropriate method for assessing residual efficacy of grain protectants.

4. Field Trial Two: Supplemental introductions and sampling with traps and trier samples combined with bioassays

This test was conducted in sixteen 13.6 MT capacity metal bins, equipped with aeration systems, at the SPREC Research Center (Oklahoma State University, Stillwater, OK, USA). The test has been reported previously (Lui et al., 2016), so only the essential details will be summarized here. There were four treatments with four replications each: wheat with aeration alone at about 0.206 m^3/min/MT with no methoprene treatment, 1.0 ppm methoprene applied to the entire grain mass

with no aeration, aeration + methoprene applied to the entire grain mass, and aeration + 1.0 ppm methoprene applied only to the top 50-cm of the grain mass. For this test, grain temperatures were monitored and recorded hourly using an aeration control system from OPI Systems Inc. (Calgary, Alberta, CA). Fans were set to operate when outside air temperatures were 5°C lower than the grain temperature at 30 cm below the grain surface. This is different from the test above, where discreet aeration cycles were used.

Wheat was loaded into the bins in July. At 7, 14, 21, and 28 days after bins were filled, 100 mixed sex adults of each of the species described above were introduced into the bins. The bins were sampled at every two months after bins were filled, by placing one probe trap in each bin for seven days, then removing the trap and counting and separating adults by species. Grain samples of about 1 to 2 kg were also taken at the same time from the bins using a grain trier. Samples were also taken at 0.25, 4, and 10 months post-treatment for bioassays of *T. castaneum*, *R. dominica*, and *Plodia interpunctella* (Hübner). For the bioassays of beetles, 50 adults of each species were placed into 240-ml jars with about 100 g of wheat, then held in an incubator at 28°C and 60% r.h. *For P. interpunctella*, 20 eggs were placed on a mixture of ground and whole wheat (20 g each). The test ended after 10 months.

Total numbers of *T. castaneum*, *R. dominica*, and *C. ferrugineous* collected in the probe traps during the entire storage period are listed in Table 1 (complete data for bi-monthly bioassays given in Lui et al., 2016). Data were compared using Chi-Square analysis (SAS Institute). It is apparent that the most optimal treatment was the combination of the entire wheat mass treated with methoprene combined with aeration. Also, far more adults of the three species were collected in probe traps versus trier samples (Table 2). The probe traps provided a measure of relative abundance, and gave an indication of relative species susceptibility: *R. dominica* < *C. ferrugineous* < *T. castaneum*. However, it should be emphasized that the four separate introductions of the beetles helped to establish the populations, and strict reliance on natural populations may not have yielded successful results. Bioassay data for beetles (Table 3) and *P. interpunctella* (Table 4) also showed the residual efficacy of methoprene.

Table 1. Total numbers of three species collected from pitfall traps in wheat held under four treatment regimens from July to May in Stillwater, OK, USA (n=6). Sum totals within columns followed by different lower-case letters are significantly different (Chi Square, $P< 0.05$)

Treatment	*T. castaneum*	*R. dominica*	*C. ferrugineous*
Aeration only	3138a	18ab	173a
Methoprene top+aeration	1868b	24a	89b
Methoprene total-no aeration	1143c	10b	80b
Methoprene total+aeration	564d	13ab	79b

Table 2. Total numbers of three species collected from trier samples in wheat held under four treatment regimens from July to May in Stillwater, OK, USA (n=6). Sum totals within columns followed by different lower-case letters are significantly different (Chi Square, $P< 0.05$).

Treatment	*T. castaneum*	*R. dominica*	*C. ferrugineous*
Aeration only	36a	19a	96a
Methoprene top+aeration	9b	10ab	9b
Methoprene total-no aeration	4b	4b	2b
Methoprene total+aeration	4b	5b	4b

Table 3. Total numbers of progeny produced in bioassay samples from wheat held under four treatment regimens from July to May in Stillwater, OK, USA (n=6). Sum totals within columns followed by different lower-case letters are significantly different (Chi Square, $P< 0.05$).

Treatment	*T. castaneum*	*R. dominica*
Aeration only	659a	1694a
Methoprene top+aeration	0b	54b
Methoprene total-no aeration	0b	1c

Methoprene total+aeration	0b	1c

Table 4. Average percentage (mean ±SE) of adult *P. interpuctella* from eggs placed on wheat from four treatment regimens from wheat in Stillwater, OK, USA (n=3). Sum totals within columns followed by different lower-case letters are significantly different (*t*-test, $P< 0.05$).

Treatment	
Aeration only	98.7± 0.03a
Methoprene top+aeration	11.3± 5.48b
Methoprene total-no aeration	0
Methoprene total+aeration	0

5. Field Trial Three: Assessing residual efficacy assessed only with bioassays

Given the difficulty of determining methoprene efficacy based on actual infestation, another option for collecting data, and for determining species susceptibility, is to rely on bioassay data. This approach was utilized in Arthur (2016), a two-year study in which wheat, corn, rough rice, and brown rice was treated with 1.25 and 2.5 ppm methoprene, and stored for two years under natural conditions on the floor of a grain bin. The commodities were bioassayed every two months, using different insect species depending on the commodity. Data for rough rice and brown rice highlight the differences between grain commodities, in terms of assessing insecticidal efficacy. Data also show differences in susceptibility between two internal feeders, *R. dominica* and *Sitotroga cereaella* (Oliver). Adult females of both species lay eggs on the interior of the grain kernel, and the neonate larva bores inside the kernel, and completes development. Adult *R. dominica* chew their way out of the kernel, while adult *S. cereallella* push their way out through the feeding holes created by the larva. While *R. dominica* causes physical damage to the kernel, and *S. cerealella* does very little feeding, high populations of *S. cereallella* may still present a contamination issue.

The rough rice and brown rice was treated in August by treating four individual replicate lots of 11 kg with either 1.25 or 2.5 ppm methoprene, then storing the replicate lots in buckets held on the floor of an empty grain bin at the CGAHR. Every two months two aliquot samples of about 80 g each were taken from an individual replicate bucket, placed in a 120-ml capacity plastic vial and 10 parental adults of either *R. dominica* or *S. cerealla* was exposed on the aliquots from each treatment and on companion sets of untreated controls. The individual vials were held for 3 months at 27°C and 60% r.h. to determine progeny production.

There was very little development of *R. dominica* even on untreated rough rice, presumably because the husk offers protection from larval penetration. There was some development of *S. cerealla* on rough rice treated with the two methoprene rates, but far less compared to untreated controls. On brown rice, there was extensive progeny production of *R. dominica* in untreated controls, but none in treatments. As with rough rice, there was some progeny production in the treatments, but far less than the controls. The bioassay data were then used to compare percentage weight loss of samples, of *R. dominica* and *S. cerealella* feeding damage on brown rice. Progeny production was correlated with feeding damage for *R. dominica* but not for *S. cerealella*.

6. Conclusions

Data for these studies show there are multiple methods for assessing residual efficacy of methoprene, and other contact insecticides, when used as grain protectants. IPM in stored grains could be considered more as a multi-component management strategy, and grain protectants, especially reduced-risk products, could play a more important role in the future. However, when conducting experiments, many factors should be considered. In actual or simulated field tests, bioassays must be paired with grain sampling through probe traps and pheromone traps, as natural infestations may be inconsistent. Even seeding experimental bins with insects may not yield reliable results, as the introductions may not be successful, or the populations could decline and die out during winter months, especially in temperate climates. Bioassays offer the best option for evaluation of residual efficacy of methoprene. Relating progeny production to physical grain

damage, such as weight loss, frass production, and presence of insect-damaged kernels, should also be done as well. **8.**

7. Acknowledgements

Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture. The US Department of Agriculture is an equal opportunity provider and employer**.**

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Comparative efficacy of spinetoram, chlorfenapyr, cypermethrin, beta-cyfluthrin against *Tribolium castaneum* (Herbst) and *Trogoderma granarium* (Everts)

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