LONG TERM EFFECTIVENESS OF SEVERAL GRAIN PROTECTANTS ON WHEAT

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Abstract

This study was initiated in order to determine how long several grain protectants will provide acceptable protection against the adults and the progeny of *Sitophilus oryzae* (L.), *Rhyzopertha dominica* (F.), and *Tribolium castaneum* (Herbst), when applied to clean eastern white winter wheat containing 13.6% moisture content. The following insecticides were in the experiment: mixture of diatomaceous earth (DE) and deltamethrin (DM) (DE/DM insecticide) applied at 100 ppm containing 90 ppm of DE and 0.1 ppm of deltamethrin active ingredient (a.i.); spinosad technical 92% powder applied at 1 ppm a.i.; mixture of chlorpyrifos methyl (CM) and deltamethrin (DM), formulation Storicide II applied at 3 ppm CM and 0.5 ppm DM a.i.; pirimiphos methyl, formulation Actellic 5 E applied at 10 ppm ai. In the USA, Actellic 5E is primary registered as a grain protectant on corn and Storicide II on wheat, however, in some European countries, Actellic is registered on wheat, as well. Bioassays were initiated immediately after treatment (zero day), 30, 120 and 180 days after the initial treatment and were conducted under the same conditions. The results demonstrate that, under the grain storage conditions and bioassays implementation, treatment of wheat with 100 ppm of DE/DM mixture and 3 ppm of CM and 0.5 ppm of DM (Storicide II) provided effective protection against the adults and the progeny of *S. oryzae*, *R. dominica* and *T. castaneum* during the investigating period of 6 months. However**,** 10 ppm of pirimiphos methyl (Actellic 5E) didn't control the adults of *R. dominica* and *T. castaneum*, and 1 ppm of spinosad didn't control the adults and the progeny of *S.oryzae* and *T. castaneum*, immediately after the treatment and 180 days after the grain treatment, as well.

Key words: grain protectants, Storicide II, diatomaceous earth and deltamethrin mixture, Actellic 5E, spinosad, *Sitophilus oryzae*, *Rhyzopertha dominica*, *Tribolium castaneum*, wheat, long term protection.

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Introduction

Integrated pest control (IPM) strategy is used to protect stored agricultural commodities. Different measures are included in IPM strategy such as prevention, monitoring and control (Muller, 1998). The use of grain protectant insecticides is an important part of IPM strategy. Grain protectants should be safe with low mammalian toxicity, be easy to apply with minimal residue issues in finished products, have a broad spectrum of activity towards stored-grain insects, reduce progeny production, have low adverse effects on grain handling and quality properties, and a price that is acceptable in terms of efficacy and economic viability (FAO Manual of prevention of post harvest losses – Pest control using insecticides). Fewer options are available for providing long term protection of grain due to concern over pesticide residues in food, insecticide resistance and the loss or restricted use of conventional grain protectants and fumigants due to new regulations (Fields, 1999; Ignatowicz and Olejarski, 2010). Currently there are only a few grain protectant insecticides that are permitted for controlling insects in stored grain products. Grain protectants including fenitrothion, malathion, pirimifos methyl (organophosphorus compounds), deltamethrin (a synthetic pyrethroid), diatomaceous earth (DE), had proven effective against grain insects when used alone and in a combination (White and Leesch 1996; Daglish, 1994, 1998). However, the insect resistance against new protectants soon developed, as well (Collins et al., 1993; Arthur, 1994). Different types of protectants lose activity at different rates depending on factors such as their intrinsic chemistry, development of insect resistance, the grain and storage type, ambient temperature and moisture. As regards the last-mentioned, higher temperatures and moistures generally lead to greater rates of decay of protectant efficacy (Arthur, 1994; Athanassiou et al., 2008a, 2008b). Hence, the amounts of protectants used, the type of storage, the length of storage, and the conditions experienced during storage (such as temperature and moisture), all had to be managed together to attain maximum protection. Pesticides residues in food are recognized as a major safety concern (Fishwick, 1988; Fields, 1999), therefore it was essential to leave a minimum residue of protectant on the grain, and certainly below the maximum residue level (MRL) imposed by each country.

The objective of this study was to determine if grain protectants Storicide II (mixture of chlorpyrifos methyl and deltamethrin), Actellic 5E (pirimifos methyl), spinosad technical powder and the mixture DE/DM (diatomaceous earth and deltamethrin) applied at registered and recommended concentrations can protect wheat grain during 6 months of storage controlling the

adults and the progeny of *Sitophilus oryzae* (L.), the rice weevil, *Rhyzopertha dominica* (F.), the lesser grain borer and *Tribolium castaneum* (Herbst), the red flour beetle.

Materials and method

Mixed-sex adult of *Sitophilus oryzae* (L.), the rice weevil, *Rhyzopertha dominica* (F.), the lesser grain borer and *Tribolium castaneum* (Herbst), the red flour beetle, 7 to 21 days old, were used in the experiment. *S. oryzae* and *R. dominica* were cultured on wheat with approximately 14% Moisture content (m.c.). *T. castaneum* was cultured on white flour with 5% brewer's un-activated yeast. Rearing was conducted at $30+1^0$C and 70+5% air relative humidity (r.h.).

Un-infested clean eastern white wheat from Ontario, Canada, with 13.6% moisture content (m.c.) was used in the experiment. Grain m.c. was measured using a dielectric moisture metre (AACC method 44-11). Dockage was removed by sieving grain for 45 seconds in a sieve with 2.36 mm openings (8 mesh).

The insecticides studied in the experiment were as follows:

- chlorpyrifos-methyl + deltamethrin (Storicide II, 216 mg active ingredient (a.i.) of chlorpyrifos-methyl in 1 ml and 37 mg a.i. of deltamethrin in 1 ml (Bayer Crop Science, Research Triangle Park, NC), which is registered in the USA on wheat and rice at the rates of 3 ppm of chlorpyrifos-methyl and 0.5 ppm deltamethrin;
- spinosad technical materials 92% a.i. , which has in the USA a label rate of 1 ppm for wheat, maize and rice (the producer BioSeen, China);
- pirimiphos-methyl (Actellic 5E, 480 mg a.i. in 1 ml, which is registered in the USA on maize at 8 ppm.) (Agriliance, St Paul, MN), and in some European countries, and
- mixture DE/DM (formulation developed by Z. Korunic) at 100 ppm containing 90 ppm of DE and 0.1 ppm of DM a.i.

The concentrations applied were:

- Storicide II at 3 ppm of chlorpyrifos methyl active ingredient (a.i.) and 0.5 ppm of deltamethrin active ingredient (a.i.);
- Actellic 5E at 10 ppm of pyrimiphos methyl (a.i.);
- Spinosad Technical at 1 ppm (a.i.), and
- DE/DM mixture at 100 ppm.

At the beginning of the test, 5-14 kg groups of wheat were weighed, and the grain m.c. was determined. Four groups were treated with insecticides and one group served as untreated (control) group.

The initial treatment was performed by adding the determined concentrations of the insecticides to the grain and then mixing thoroughly in a plastic container for 5 minutes. Immediately after the initial treatment, 600g of grain was removed from each container containing treated and untreated grain. This 600g grain was evenly divided between 3-500mL jars (3 replicates containing 200g per jar). After introducing 50 adult insects of each species into jars, jars were maintained at 30° ± 1.0°C and 70% ± 5% r.h.

The containers with treated and untreated grain (groups) during the six months were maintained under the same conditions (30° ± 1.0°C and 70% ± 5% r.h.).

Bioassays had been initiated 0, 30, 120, 180 days after the initial treatment.

To determine mortality in each treatment, grain was sieved 7, 14 and 21 days after insects were introduced, and the number of dead and live insects were recorded. All dead insects were removed 7 and 14 days post-introduction and all dead and live insects were removed after 21 days post-introduction and jars were maintained under the same conditions for an additional 21 days (total of 56 days post-introduction) before being sieved again to determine the number of adult offspring generated.

Statistical analysis

Data were subjected to analysis of variance (ANOVA) for insects mortality post- treatments and progeny according to the GLM (general linear model). Significant differences were shown by LSD test (least significant difference) and entered in tables. Data processing was conducted by SAS/STAT software 9.1.3.

Results

In order to present the changes in the effectiveness of the insecticides used in the experiment during 6 months and to reduce the numbers of the tables, the results of the effectiveness against *S. oryzae*, *R. dominica* and *T. castaneum* immediately after treatment (zero day) and after 180 days

post-treatment and their exposure of 7, 14 and 21 days to treated and untreated grain are presented and discussed in this manuscript.

Table 1. The mortality of *Sitophilus oryzae*, *Rhyzopertha dominica* and *Tribolium castaneum* at zero day and 180 days post-treatment after 7,14 and 21 days of adults' exposure to treated and untreated grain

Formulation	Concentr. (ppm)	***Sitophilus oryzae* average adults' mortality (%) after days**					
		Zero day post-treatment* **			**180 days post-treatment**** **		
		7 days $\overline{X} \pm SE$	14 days $\overline{X} \pm SE$	21 days $\overline{X} \pm SE$	7 days $\overline{X} \pm SE$	14 days $\overline{X} \pm SE$	21 days $\overline{X} \pm SE$
Untreated	0	0.6±0.6^{d}	6.0±3.0^{cd}	10.0±3.0^{c}	10.6±3.5^{c}	21.3±4.6^{bc}	28.6±2.4^{b}
*DE/DM	100	99.3±0.6^{a}	100.0±0.0^{a}	100.0±0.0^{a}	98.6±1.3^{a}	100.0±0.0^{a}	100.0±0.0^{a}
Actellic E5	10	100.0±0.0^{a}	100.0±0.0^{a}	100.0±0.0^{a}	29.3±2.9^{b}	90.0±4.1^{a}	96.0±2.0^{a}
**Storicide II	3CP;0.5DM	100.0±0.0^{a}	100.0±0.0^{a}	100.0±0.0^{a}	100.0±0.0^{a}	100.0±0.0^{a}	100.00±0.0^{a}
Spinosad	1	71.3±6.3^{b}	86.0±3.0^{b}	88.6±2.9^{b}	92.0±4.6^{a}	96.0±2.3^{a}	97.3±1.7^{a}
Formulation	Concentr. (ppm)	***Rhyzopertha dominica* average adults' mortality (%) after days**					
		Zero day post-treatment* **			**180 days post-treatment**** **		
		7 days $\overline{X} \pm SE$	14 days $\overline{X} \pm SE$	21 days $\overline{X} \pm SE$	7 days $\overline{X} \pm SE$	14 days $\overline{X} \pm SE$	21 days $\overline{X} \pm SE$
Untreated	0	2.0±1.1^{c}	2.0±1.1^{c}	2.6±0.6^{c}	4.0±1.1^{e}	19.3±1.7^{d}	24.6±1.7^{d}
*DE/DM	100	97.3±1.7^{a}	100.0±0.0^{a}	100.0±0.0^{a}	78.0±1.1^{b}	100.0±0.0^{a}	100.0±0.0^{a}
Actellic E5	10	7.3±1.7^{c}	24.6±3.5^{b}	38.0±7.2^{b}	23.3±5.8^{d}	45.3±5.2^{c}	45.3±6.9^{c}
**Storicide II	3CP;0.5DM	98.6±1.3^{a}	100.0±0.0^{a}	100.0±0.0^{a}	69.3±5.4^{bc}	88.0±4.1^{ab}	100.0±0.0^{a}
Spinosad	1	100.0±0.0^{a}	100.0±0.0^{a}	100.0±0.0^{a}	85.3±6.9^{ab}	100.0±0.0^{a}	100.0±0.0^{a}
Formulation	Concentr. (ppm)	***Tribolium castaneum* average adults' mortality (%) after days**					
		Zero day post-treatment* **			**180 days post-treatment**** **		
		7 days $\overline{X} \pm SE$	14 days $\overline{X} \pm SE$	21 days $\overline{X} \pm SE$	7 days $\overline{X} \pm SE$	14 days $\overline{X} \pm SE$	21 days $\overline{X} \pm SE$
Untreated	0	0.6±0.6^{e}	0.6±0.6^{e}	5.3±1.3^{d}	3.3±1.7^{e}	15.3±5.2^{d}	15.3±5.2^{d}
*DE/DM	100	68.6±5.8^{b}	100.0±0.0^{a}	100.0±0.0^{a}	50.6±3.7^{b}	96.6±1.7^{a}	100.0±0.0^{a}
Actellic E5	10	100.0±0.0^{a}	100.0±0.0^{a}	100.0±0.0^{a}	11.3±1.3^{d}	36.6±2.9^{c}	40.6±2.4^{bc}
**Storicide II	3CP;0.5DM	100.0±0.0^{a}	100.0±0.0^{a}	100.0±0.0^{a}	56.0±5.0^{b}	98.6±0.6^{a}	100.0±0.0^{a}
Spinosad T	1	0.0±0.0^{e}	10.0±1.1^{d}	22.0±1.1^{c}	11.3±1.3^{d}	34.0±3.0^{c}	41.3±2.4^{bc}

* DE/DM – diatomaceous earth/chlorpyrifos methyl;
**3ppm chlorpyrifos; 0.5 ppm deltamethrin
*** means in the columns for zero day post-treatment followed by the same letters are not significantly (P>0.05) different as determined by the LSD-test
****means in the columns for 180 days post-treatment followed by the same letters are not significantly (P>0.05) different as determined by the LSD-test

Table 2. The progeny of *Sitophilus oryzae*, *Rhyzopertha dominica* and *Tribolium castaneum* at zero day and 180 days post-treatment

Formulation	Concentr. (ppm)	***Sitophilus oryzae* average adults number (progeny)**	
		Zero day post-treatment* after 56 days $\overline{X} \pm SE$	**180 days post-treatment**** after 56 days $\overline{X} \pm SE$
Untreated	0	429.3 ± 37.7^{c}	500.3 ± 41.3^{c}
*DE/DM	100	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}
Actellic E5	10	0.3 ± 0.3^{a}	1.3 ± 0.3^{a}
**Storicide II	3CP;0.5DM	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}
Spinosad	1	163.6 ± 11.8^{b}	181.0 ± 7.1^{b}
Formulation	Concentr. (ppm)	***Rhyzopertha dominica* average adults number (progeny)**	
		Zero day post-treatment after 56 days $\overline{X} \pm SE$	**180 days post-treatment** after 56 days $\overline{X} \pm SE$
Untreated	0	229.3 ± 18.0^{c}	279.6 ± 10.0^{c}
*DE/DM	100	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}
Actellic E5	10	7.3 ± 1.7^{b}	7.3 ± 2.0^{b}
**Storicide II	3CP;0.5DM	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}
Spinosad	1	1.3 ± 0.6^{a}	1.0 ± 0.5^{a}
Formulation	Concentr. (ppm)	***Tribolium castaneum* average adults number (progeny)**	
		Zero day post-treatment after 56 days $\overline{X} \pm SE$	**180 days post-treatment** after 56 days $\overline{X} \pm SE$
Untreated	0	101.0 ± 4.0^{c}	99.6 ± 8.7^{d}
*DE/DM	100	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}
Actellic E5	10	0.6 ± 0.6^{a}	2.0 ± 0.5^{b}
**Storicide II	3CP;0.5DM	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}
Spinosad T	1	40.0 ± 17.0^{b}	24.0 ± 4.0^{c}

* DE/DM – diatomaceous earth/chlorpyrifos methyl;
**3ppm chlorpyrifos; 0.5 ppm deltamethrin
*** means in the columns for zero day post-treatment followed by the same letters are not significantly (P>0.05) different as determined by the LSD-test
****means in the columns for 180 days post-treatment followed by the same letters are not significantly (P>0.05) different as determined by the LSD-test

Immediately after the treatment (zero day) and 180 days, the mixture DE/DM and Storicide II controlled *S. oryzae*, *R. dominica* and *T. castaneum* and their progeny successfully (100%) (Table 1 and 2).

Actellic applied at 10 ppm of a.i. at zero day successfully controlled *S. oryzae* and *T. castaneum* (100%). Probaly such a high effectiveness on freshly treated grain is caused with significant fumigant property of pirimiphos methyl. However, the same concentration didn't control the adults and the progeny of *R. dominica* (38% adult's mortality after 21 days and 97% progeny reduction).

The initial effectiveness of Actellic 5E on 180 days old deposit on grains (7 days of the exposure time) was greatly reduced, from 100% at zero day to 29% only at 180 days (S. oryzae) and from

100% (at zero days to 11% only at 180 days (Table 1). Such a great difference among the effectiveness on freshly treated grain and 189 days treated grain was probably due to the absence of the fumigant property of a.i.on 189 days old deposit on grain. However after the prolonged exposure time of 14 and 21 days the effectiveness against *S. oryzae* was acceptable (96% after 21 days) but was still low against *T. castaneum* (40% only) (Table 1). However, Actellic 5E didn't control completely the progeny of *S.oryzae, R. dominica* and T. *castaneum* at zero and 189 days, as well (Table 2).

Spinosad applied at 1 ppm didn't control the adults (88% at zero day and 97% at 180 days) and the progeny of *S. oryzae* (38% progeny reduction) and the adults (22% at zero day and 41% at 180 days) and the progeny (40% reduction) of *T. castaneum* (Table 1 and 2).

Discussion

Insecticides from organophosphate and pyrethroids group have their advantages and disadvantages of use. Unlike organophosphates, pyrethroids do not rapidly break down at high temperature and moisture contents. However, the concentrations of pyrethroids that control *R. dominica* do not control completely *S. oryzae* and *T. castaneum*, but the concentrations of organophosphates that control many species do not control *R. dominica.* Pyrethroids synergised with pyperonil butoxide applied in a combination with organophosphates have been used in control program in Australia for several years (Daglish, 1994, 1998).

According to Arthur (1994) and Duguet et al. (1990) the mixture of the individual pyrethroid with orhanophosphate seems to be a logical approach and may be desirable for economic considerations. According to the study of Frank and Zehner (1994) the degradations of the active ingredients in the mixture is similar to their degradations when they are applied alone. Daglish (1994) evaluated a mixture of chlorpyrifos methyl, deltamethrin and PBO against a malathion resistant strain of *S. oryzae, S. zeamais* and *R. dominica*. He found that the minimum effective application rates of chlorpyrifos methyl applied alone for control of *S. oryzae* in freshly treated rice was from 2.5 to 5 ppm (adults) and 5 to 10 ppm (progeny). In mixtures with deltamethrin and PBO, the minimum effective concentrations that caused 100% adults mortality, and prevented the production of live progeny, were 0.25 ppm or 0.5 ppm of deltmethrin and 2.5 to 5 ppm of chlorpyrifos methyl (adults), and 0.25 ppm or 0.5 ppm of deltamethrin and 1.25 to 2.5 ppm of chlorpyrifos methyl (progeny).

Storicide II, the mixture of chlorpyrifos-methyl and deltamethrin is registered in the USA for use on small grains (barley, oats, rice, sorghum and wheat). According to the producer's label, it is effective against a broad spectrum of stored grain pests, including lesser grain borer. Daglish (1994) determined a high effectiveness of the mixture of chlorpyrifos methyl, deltamethrin and PBO against adults and the progeny of *S. oryzae* and demonstrated the synergistic effect of these two a.i.

The results of our experiment showed an excellent effectiveness of Storicide II. However, we think that the recommended concentrations of both active ingredients in the mixture are too high (3 ppm of chlorpyrifos- methyl a.i. and 0.5 ppm of deltamethrin a.i.) and because of the stability of both active ingredients on grain, the residues may be of safety concern.

Pirimiphos-methyl is a broad spectrum, non-cumulative organophosphorus pesticide; a cholinesterase inhibitor with fast acting fumigant, contact, and stomach action; and slightly toxic to mammals. Pirimiphos-methyl has a long persistence on inert surfaces, including stored grain. The insecticide based on pirimiphos methyl, Actellic 5E, is registered in the USA for use on corn and grain sorghum. In some European countries pirimiphos methyl is registered for use on different grains, as well. In this study even pretty high concentration of 10 ppm of pirimiphos methyl (Actellic 5E) didn't control the adults and the progeny of *R. dominica* at zero and 180 days. The effectiveness against adults and the progeny of *T. castaneum* was significantly reduced with time, from 100% (zero day) to 40% (180 days). The progeny was reduced by 100% at 0 day and 98 % at 180 days. These results are in agreement with data published by FAO Manual of prevention of post harvest losses – Pest control using insecticides.

Spinosad is a mixture of the metabolites of the bacterium *Saccharopolyspora spinosa* Mertz and Yao (Bacteria: Actinobacteridae). This is a broad-spectrum insecticide with low mammalian toxicity, and it is effective against many stored-grain insect species (Subramanyam et al., 1999; Fang et al., 2002; Nayak et al., 2005, Vayios et al., 2010). Spinosad gives a good residual control, which makes it an ideal protectant for stored grain commodities (Fang et al., 2002; Fang and Subramanyam, 2003). Although spinosad is registered for use in stored products in the USA (Subramanyam et al., 2003), as an alternative to traditional grain protectants, it is still not in use. Some studies documented that the effectiveness of spinosad is highly affected by several factors, biotic or abiotic such as the target species, the type of commodity, and the dose rate and the registered rate of 1 ppm of spinosad a. i. was not high enough to control all insects species (Fang

et al., 2002; Subramanyam et al., 2003; Toews and Subramanyam, 2003; Chintzoglou et al., 2008; Athanassiou et al., 2008a, 2008 b; Vayios et al., 2010; Bonjour and Opit, 2010).

In this study the results of the effectiveness of spinosad are in the agreement with the published results. Spinosad applied at 1 ppm didn't control the adults and the progeny of *S.oryzae* and *T. castaneum*, immediately after grain treatment and 180 days after the grain treatment, as well.

Diatomaceous earths (DE) are registered for all grains. Applications of these materials to the entire grain mass can lower test weight and reduce the flowability of the grain. In many cases, these products are only applied to the bottom and top layers of the grain (Korunic, 1998; Korunic and McKay, 2000; Subramanyam and Roesli, 2000). One of the potentially valuable usages of diatomaceous earth is in the admixture of DE, impregnated with insecticide, with grain. Insecticide concentrations can be significantly reduced in this form and the insecticides then become partly removable. Without the loss of the effectiveness, according to Desmarchelier (1998, unpublished data) it is possible to halve the applied dose of insecticides such as chlorpyrifos methyl and deltamethrin by simultaneous application of small amount (100g/t) of diatomaceous earth.

The mixture of DE and deltamethrin (DE/DM) was developed to mitigate the disadvantages of DE and reduce deltamethrin residues in grains (Korunic and Rozman, 2010). The main advantage of using the DE/DM formulation is an acceptable efficacy in grain treated with very low doses of chlorpyrifos methyl and deltamethrin. These doses are much lower in comparison with the doses required for effective control when these two insecticides are applied either alone or in a mixture (Korunic and Rozman, 2010; Korunic and Kalinovic, 2010). The results in this study confirmed the previous published results regarding to the high and long-term effectiveness of the mixture DE/DM.

Conclusions

Under the conditions of wheat grain storage (30°C; 12.6 to 13.6% grain moisture content), treatment of wheat grain with 100 ppm of DE/DM mixture and 3 ppm of CM and 0.5 ppm of DM (Storicide II) provided the effective protection against the adults and the progeny of *S. oryzae*, *R. dominica* and *T. castaneum* for at least 6 months of storage.

However, 10 ppm of pirimifos methyl (Actellic 5E) didn't control the adults of *R. dominica* and *T. castaneum* at zero days and 180 days post-treatment.

Spinosad applied at 1 ppm didn't control the adults and the progeny of *S.oryzae* and *T. castaneum*, immediately after grain treatment and 180 days post-treatment, as well.

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