

Lethal and sub-lethal effects from short-term exposure of *Rhyzopertha dominica* on wheat treated with Storicide II®

Frank H. Arthur

Received: 23 May 2011 / Accepted: 28 September 2011 / Published online: 20 October 2011
© Springer-Verlag (outside the USA) 2011

Abstract Hard red winter wheat was treated at 0 (untreated control), 25, 50, 75, and 100% of the label rate of the insecticide Storicide II®, which is chlorpyrifos-methyl and deltamethrin applied at label rates of 3 and 0.5 ppm, respectively. Paired male and female *Rhyzopertha dominica* F., the lesser grain borer, were exposed at 27°C and 60% RH on wheat treated at each of the five rates above for 2, 4, 8, 16, or 32 h, and then transferred to untreated wheat and held for 1 week at the same environmental conditions. After this 1-week holding period, the parental adults were removed, mortality was assessed, and the wheat was then held for 7 weeks at the same environmental conditions to determine progeny production. As the concentration and exposure interval increased, mortality of both sexes approached 100%, but at the intermediate concentration–time combinations male mortality was greater than female mortality. Progeny production also decreased with increasing concentration of Storicide II® as the exposure time increased, with non-linear patterns of decrease at the lower concentrations and time combinations and linear decline at the higher levels of concentration and time. Results seem to indicate greater susceptibility of males to Storicide II®, and also show delayed parental mortality from the insecticide exposure and sub-lethal effects of reduced progeny production.

Keywords *Rhyzopertha dominica* · Insecticides · Chlorpyrifos-methyl · Deltamethrin

Introduction

Storicide II® is a grain protectant registered in the United States (US) for direct application to wheat and small grains. This formulation is a combination of the organophosphate chlorpyrifos-methyl and the pyrethroid deltamethrin, and the label rates are 3 and 0.5 ppm for each insecticide, respectively. The label on the older formulation of chlorpyrifos-methyl that was registered in the US at an application rate of 6 ppm (Reldan®) did not specify control of the lesser grain borer, *Rhyzopertha dominica* (F.), but the label did state that it would control most other stored-grain beetles. Deltamethrin and other pyrethroids are more effective for control of *R. dominica* compared to *Sitophilus* weevils (Arthur 1994, 1995). Hence, the combination product would be expected to give more complete control of the complex of beetles that can attack stored wheat. All life stages of externally feeding stored-grain insects come into contact with a grain protectant, in contrast to *R. dominica* and *Sitophilus* weevils that develop inside a grain kernel.

Grain protectants are sometimes used as surface treatments to the top of a grain mass. However, with diatomaceous earth (DE) and contact insecticides applied to layers of wheat, *R. dominica* was able to penetrate through the treated layer, and oviposit in untreated wheat before dying from the exposure to either DE or the contact insecticides (Vardeman et al. 2006, 2007; Athanassiou et al. 2009, 2011a, b). When grain protectants are used as a surface treatment to the top layer of a grain mass or untreated wheat is mixed with surface wheat, it is possible for the adults to disperse from the treated wheat. Therefore, mortality from short exposure intervals may be important in overall pest control because insects could escape the treated portion of the wheat. This could also occur when

Communicated by C.G. Athanassiou.

F. H. Arthur (✉)
USDA-ARS-Center for Grain and Animal Health Research,
1515 College Avenue, Manhattan, KS 66502, USA
e-mail: frank.arthur@ars.usda.gov

treated wheat is mixed with untreated wheat. There are only a few studies that evaluate mortality and reproduction after short exposure intervals of 2 days or less (Athanasios et al. 2010).

Sub-lethal effects such as reduced oviposition and fecundity, as described for studies with the insect growth regulator methoprene (Daglish and Pulvirenti 1998; Chanbang et al., 2008; Daglish and Nayak 2010), could occur when adult *R. dominica* are exposed to wheat treated with an insecticide. There are no assessments of short-term exposures, or on differential susceptibility of the sexes to Storicide II[®]. Therefore, the purpose of this test was to assess mortality and progeny production of adult male and female *R. dominica* exposed for different times on wheat treated with different concentrations of Storicide II[®].

Materials and methods

Storicide II[®]-emulsifiable concentrate (EC), 216 mg active ingredient [AI] of chlorpyrifos-methyl and 37 mg [AI] deltamethrin/ml of the EC, was obtained from Bayer Crop Science (Research Triangle Park, NC, US). The label specifies application at a field spray rate of 367 ml of product in 18,905 ml of water to treat 27,272 kg of wheat, which gives an application rate of 3.0 ppm for chlorpyrifos-methyl and 0.5 ppm for deltamethrin. The experimental lot of wheat used in our study was 600 g, and the application rate was adjusted to spray this 600 g lot at the same volume rate of formulated spray as specified for the field rate.

For our study, the application rates used in the test were 0 (untreated control), 25, 50, 75, and 100% of the label rate. These differing rates were used to simulate degradation that would occur from the label rate. There were four replicate 600 g lots for each rate, including the untreated control. Each replicate for each application rate was formulated and applied separately by mixing the appropriate amount of formulation with tap water in separate volumetric flasks to achieve the calculated application rate (20 flasks total). The flasks for the untreated controls contained tap water only. Each replicate lot of 600 g was treated by first spreading the wheat on a piece of heavy paper laid on a 0.6 × 0.3 m surface, then spraying the replicate lot with a volume rate of 0.42 ml of formulated spray using a Badger 100 artists' airbrush (Badger Corporation, Franklin Park, IL, US). After each replicate lot of wheat was treated it was poured into a 0.95-l glass jar and hand-rolled for 30 s.

The experimental unit for the study consisted of approximately 20 g of wheat in a 7-dram plastic vial. After a replicate lot of wheat was treated, each of forty 7-dram vials was filled with about 10 g each from the treated lot, in

order to obtain eight sub-sets of vials for exposure intervals of 2, 4, 8, 16, and 32 h (400 g of treated wheat total). The remaining 200 g was discarded. After all vials for all replicates and concentrations were filled with wheat, a pair of male and female 1-week-old adult *R. dominica*, obtained from colonies maintained on whole wheat at 27°C and 60% relative humidity, were placed in each vial. The adults were sexed according to method described by Stemley and Wilbur (1966). The last abdominal segments of the adult males are slightly darker than the females. Upon completion of the exposure period the pair of *R. dominica* were transferred to a new vial containing 20 g of untreated wheat, which was placed in an incubator set at 27°C and 60% relative humidity. The original vial of treated wheat was also placed in the incubator.

One week after each respective exposure interval was completed for each replicate, concentration, and the eight sub-replicate vials, the vials were removed from the incubator, the wheat was sifted, and the adult males and females classified as live or dead (0 or 1), and then discarded. In some cases individuals of a particular sex were not recovered, and were assumed to be either live or dead inside a cracked wheat kernel. The wheat was placed back into the vials, which were returned to the incubator, and held for an additional 7 weeks.

After the 7-week holding period, the vials containing the original treated wheat and the vials containing the untreated wheat to which the adult male and female *R. dominica* were transferred after exposure, were removed from the incubator. All F₁ progeny in all vials were counted. There were few F₁ progeny adults in the vials that contained the treated wheat, so these numbers were not used in the data analysis. The dataset was first analyzed for differences in parental mortality. In those instances where both individuals of each sex were not recovered after the 1-week holding period, those observations were deleted from the dataset. Because the parental adults were classified as either live or dead, Fisher's exact test under the Frequency Procedure of the Statistical Analysis System (SAS) (SAS Institute 2007) was used to determine if parental mortality differed between sexes. The Mixed Procedure of SAS was used to analyze the number of F₁ adults as a response variable, with exposure and concentration as fixed effects and replicate as the random effect. Data for progeny production from the parental pairs exposed for each time interval were in an ordered sequence with concentration as the independent variable. Therefore, data for progeny production were analyzed by regression analysis and curve-fitting procedures using Table Curve software (SPSS, Chicago, IL, US). This software program calculates the actual R^2 of the equation, along with the maximum R^2 of any equation that can be fit to the dataset, and hence accounts for variability in the dataset. Progeny

production across all concentrations and exposure intervals was determined using the Waller–Duncan *k*-ratio *t* test under the General Linear Model (GLM) Procedure in SAS.

Results

Within each concentration–exposure interval combination, there were some indications that males were more susceptible to Storicide II® than females, with 4 of the possible 20 comparisons in treatments and exposure intervals showing a significant difference (Table 1). The number of F₁ progeny produced by the exposed parental pairs was significant for concentration, exposure interval, and the interaction (Table 2). Within each exposure interval, progeny production declined with concentration of Storicide II® to which the parents were exposed (Fig. 1). The curve-fit equations also were different for the five datasets, indicating a different pattern in progeny production. Linear equations could be fit to the data for 2, 4, and 8-h exposures (Table 2), while the decline in progeny production was non-linear at the higher two-exposure intervals.

A final analysis examined progeny production across all concentrations and exposure intervals. When both parental males and females were alive after the 1-week holding period, progeny production was 20.5 ± 0.9. When the female was alive but the male was dead, progeny production was 11.4 ± 1.3, when the reverse was true progeny production was 4.5 ± 2.1, and when both parental adults were dead progeny production was 0.3 ± 0.2. All values were significantly different (*P* < 0.05, Waller–Duncan *k*-ratio *t* test).

Discussion

The results of this test indicate male and female *R. dominica* may respond differentially to Storicide II®. Previous studies with the chlorpyrifos-methyl component of the mixture, formerly sold in the US as Reldan®, did not include tests of differential susceptibility between the sexes of *R. dominica*. However, insecticide resistance of *R. dominica* to chlorpyrifos-methyl was documented in the US within a few years after registration (Zettler and

Table 1 Number of dead parental females (F) and dead parental males (M) 1 week after being exposed for 2, 4, 8, 16, and 32 h on wheat treated with 0, 25, 50, 75, and 100% of the label rate of Storicide II®

% Rate	2 h			4 h			8 h			16 h			32 h		
	F	M	T	F	M	T	F	M	T	F	M	T	F	M	T
0	0	2	22	0	0	22	0	0	25	1	1	24	1	0	28
25	0	2	29	0	0	21	2	3	24	6*	12	23	12	17	29
50	1*	6	21	4	5	24	14*	21	29	6*	13	23	28	30	31
75	7	8	21	8	13	22	21	26	31	25	26	27	29	31	31
100	11	15	21	8	13	22	30	29	32	32	32	32	30	31	32

The total number of paired males and females recovered from a particular set of observations is also listed (T, total possible is 32)

* Significant difference in mortality between the sexes (*P* < 0.05, Fisher’s Exact Test, Frequency Procedure of SAS)

Table 2 Equation parameters for equations fit to the data for F₁ progeny produced by exposure of the parental pairs of *R. dominica*, at each time interval (hours), on wheat treated with 0, 25, 50, 75, and 100% of the label rate of Storicide II®, then transferred to untreated wheat for 1 week

Hours	<i>a</i>	<i>b</i>	<i>c</i>	<i>R</i> ²	Max <i>R</i> ²
2 ^a	29.0 ± 2.3	0.28 ± 0.04		0.29	0.31
4 ^a	23.8 ± 1.6	0.23 ± 0.03		0.39	0.40
8 ^a	20.2 ± 1.4	0.21 ± 0.02		0.35	0.36
16 ^b	−3.1 ± 2.5	29.4 ± 2.7	37.0 ± 9.2	0.60	0.61
32 ^b	−2.5 ± 2.3	28.9 ± 2.7	32.7 ± 8.1	0.52	0.53

The rate is the independent variable (*x*) and progeny is the dependent variable (*y*). The actual *R*² is shown along with the maximum *R*² (max *R*²) of any equation that could be fit to the dataset. Curve-fit lines are shown in Fig. 1

Overall ANOVA values for F₁ progeny for main effects concentration, exposure interval, and their interaction were *F* = 111.6, *df* = 4,684 *P* < 0.01; *F* = 8.7, *df* = 4, 684, *P* < 0.01; *F* = 1.7 *df* = 16, 684, *P* = 0.04 (Proc Mixed, SAS Institute)

^a Linear equations of the form *y* = *a* + *bx*

^b Non-linear equations of the for *y* = *a* + *b* exp(−*x/c*)

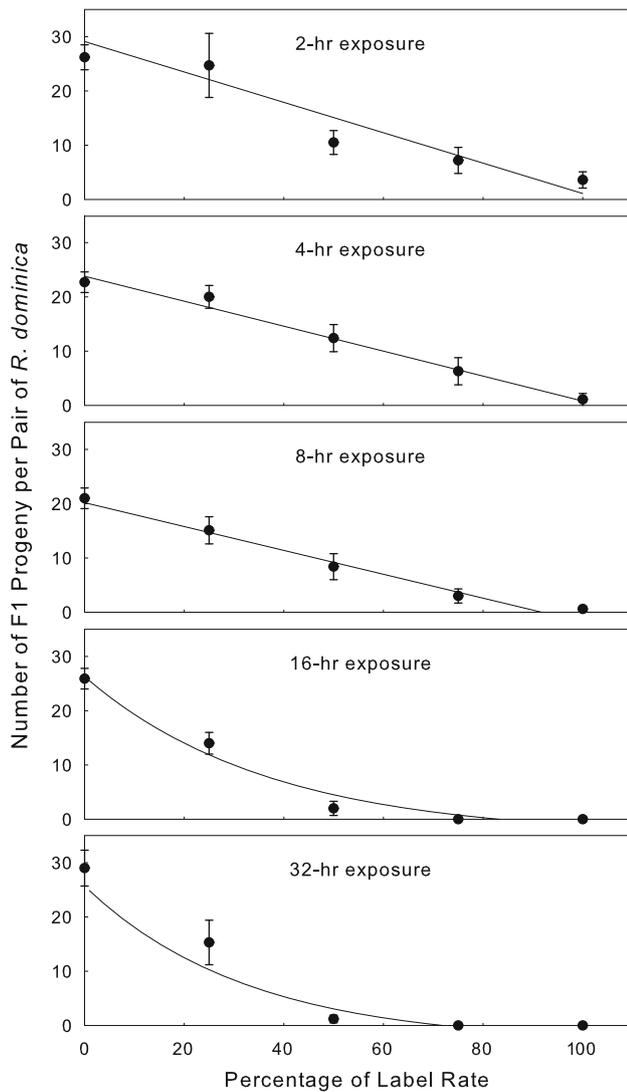


Fig. 1 Average number (means \pm SEM) of F1 progeny produced by paired *R. dominica* males and females on untreated wheat after being exposed for 2, 4, 8, 16, and 32 h on wheat treated with 0, 25, 50, 75, and 100% of the label rate of Storicide II[®]

Cuperus 1990; Guedes et al. 1996), but no tests were done to evaluate resistance between sexes. Similarly, studies with deltamethrin (Arthur 1994, 1995) evaluated efficacy but did not address differential susceptibility between sexes.

Our results also showed that parental adult mortality decreased as either concentration or exposure interval increased, and there was a combined effect of the two factors. This is somewhat analogous to the concentration by time (CT) product approach used for fumigation. Exposure interval is a dosage factor along with actual concentration (Arthur 2009), yet this relationship is not often explored when conducting studies of insecticides on

stored grains. In addition, progeny production also followed the same pattern as the parental exposures, because at the higher concentrations and exposure intervals parental females died before oviposition occurred. However, at the intermediate levels of concentration and exposure interval, parental females may have been able to oviposit before they died.

The methodology used in the current test can be compared to that used in a study described by Daghli and Nayak (2010), in which adult *R. dominica* were exposed on wheat treated with methoprene and mixed with untreated wheat. In one method, differing parcels of treated wheat were mixed with untreated wheat to obtain an average treatment dose for a given wheat lot, and compared to treating wheat at differing target doses and levels of application to achieve an uneven application. Progeny production from exposed parental adults decreased as the dose increased and the percentage of treated wheat increased. In our test, the wheat was treated with differing percentages of active ingredient of Storicide II[®], with exposure interval as the dose factor instead of concentration, which would be representative of an uneven application. Perhaps the CT approach used for fumigants could be expanded for use in studies with grain protectants involving partial or uneven methods of application and resulting insect exposures.

In the current test, various application rates were used to simulate insecticide degradation during storage. The degradation of chlopyrifos-methyl increases with increases in grain temperature and moisture content, while residues of deltamethrin and other pyrethroids are more persistent on grains (Arthur et al. 1991, 1992; Afridi et al. 2001; Morton et al. 2001). The various exposure times, followed by removal of adults from the treated wheat, simulated movement of *R. dominica* from grain parcels that were treated to parcels that were untreated. Recent related studies with other insecticides have also shown delayed mortality of parental *R. dominica* and resulting decreases in progeny when the parental adults were exposed for short time periods, then transferred to untreated grains (Athanasios et al. 2009). This could easily occur as grains are mixed together from farms to elevator storage in the US, and would also be comparable to exposure to partial or uneven insecticidal applications.

Delayed mortality after *R. dominica* has been removed from wheat treated with an insecticide has been observed in previous tests, along with oviposition before death (Vardeman et al. 2006, 2007). However, differential susceptibility of sexes is not normally assessed in evaluations of protectant insecticides and efficacy toward *R. dominica*. Our study indicates the reduced susceptibility of female *R. dominica* compared to males should warrant further consideration and exploration.

Acknowledgments The author thanks B. Barnett for technical assistance with this research, and C. Athanassiou for reviewing the manuscript prior to journal submission. This paper reports the results of research only. 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 US Department of Agriculture (USDA). USDA is an equal opportunity provider and employer.

References

- Afridi AK, Parveen Z, Masud SZ (2001) Stability of organophosphate and pyrethroid pesticides on wheat in storage. *J Stored Prod Res* 37:199–204
- Arthur FH (1994) Efficacy of unsynergised deltamethrin and deltamethrin + chlorpyrifos-methyl combinations as protectants of stored wheat and stored corn. *J Stored Prod Res* 30:87–94
- Arthur FH (1995) Degradation and efficacy of deltamethrin + chlorpyrifos-methyl and cyfluthrin + chlorpyrifos-methyl as protectants of wheat stored in southeast Georgia. *J Entomol Sci* 30:397–405
- Arthur FH (2009) Efficacy of chlorfenapyr against adult *Tribolium castaneum* exposed on concrete: effects of exposure interval, concentration, and the presence of a food source after exposure. *Insect Sci* 16:157–163
- Arthur FH, Throne JE, Simonaitis RA (1991) Chlorpyrifos-methyl degradation and efficacy toward maize weevils (Coleoptera: Curculionidae) on corn stored at four temperatures and three moisture contents. *J Econ Entomol* 84:1926–1932
- Arthur FH, Throne JE, Simonaitis RA (1992) Degradation and biological efficacy of chlorpyrifos-methyl on wheat stored at five temperatures and three moisture contents. *J Econ Entomol* 85:1994–2002
- Athanassiou CG, Arthur FH, Opit GP, Throne JE (2009) Efficacy of spinosad in layer-treated wheat against five stored-product insect species. *J Stored Prod Res* 45:236–240
- Athanassiou CG, Arthur FH, Throne JE (2010) Effects of short exposures to spinosad-treated wheat or maize on four stored-grain insects. *J Econ Entomol* 103:197–202
- Athanassiou CG, Arthur FH, Throne JE (2011a) Efficacy of layer treatment with methoprene for control of *Rhyzopertha dominica* (F.) (Coleoptera: Bostrychidae) on wheat, rice, and maize. *Pest Manag Sci* 67:380–384
- Athanassiou C, Kavallieratos N, Arthur FH, Throne JE (2011b) Efficacy of spinosad and methoprene, applied alone or in combination, against six stored-product insect species. *J Pest Sci* 84:61–67
- Chanbang Y, Arthur FH, Wilde GE, Throne JE (2008) Control of *Rhyzopertha dominica* (F.) in stored rough rice through a combination of diatomaceous earth and varietal resistance. *Insect Sci* 15:455–460
- Daglish GJ, Nayak MK (2010) Uneven application can influence the efficacy of *s*-methoprene against *Rhyzopertha dominica* (F.) in wheat. *J Stored Prod Res* 46:250–253
- Daglish GJ, Pulvirenti C (1998) Reduced fecundity of *Rhyzopertha dominica* (F.) (Coleoptera: Bostrychidae) following exposure of adults to methoprene. *J Stored Prod Res* 34:201–206
- Guedes RNC, Dover BA, Kambhampati S (1996) Resistance to chlorpyrifos-methyl, pirimiphos-methyl and malathion in Brazilian and US populations of *Rhyzopertha dominica* (Coleoptera: Bostrychidae). *J Econ Entomol* 89:27–32
- Morton R, Brayon JG, Desmarchelier JM, Dilli S, Haddad PR, Sharp GJ (2001) Statistical analysis of decay of organophosphorus and pyrethroid insecticides and carbaryl on paddy rice, maize, sunflowers and field peas. *J Stored Prod Res* 37:277–385
- SAS Institute (2007) The SAS System version 9 for windows. SAS Institute, Cary
- Stemley PG, Wilbur DA (1966) A color characteristic for sexing live adult lesser grain borers. *J Econ Entomol* 59:760–761
- Vardeman EA, Arthur FH, Nechols JR, Campbell JF (2006) Effect of temperature, exposure interval and depth of diatomaceous earth on distribution, mortality, and reproduction of the lesser grain borer *Rhyzopertha dominica* (F.) (Coleoptera: Bostrychidae) in stored wheat. *J Econ Entomol* 99:1017–1024
- Vardeman EA, Arthur FH, Nechols JR, Campbell JF (2007) Efficacy of surface applications with diatomaceous earth to control *Rhyzopertha dominica* (F.) (Coleoptera: Bostrychidae) in stored wheat. *J Stored Prod Res* 43:241–335
- Zettler JL, Cuperus GW (1990) Pesticide resistance in *Tribolium castaneum* (Coleoptera: Tenebrionidae) and *Rhyzopertha dominica* (Coleoptera: Bostrychidae) in wheat. *J Econ Entomol* 83:1677–1681