

Efficacy of surface applications with diatomaceous earth to control *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae) in stored wheat[☆]

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Ab ac

Commercial formulations of diatomaceous earth (DE) products labeled for use as grain protectants usually specify on the label the depth for using them as a surface treatment, which is often 30.5 cm. An experiment was conducted at two temperatures (27 and 32 °C) and three exposure intervals (7, 10 and 14 d), at a relative humidity of 57–60% to determine if *Rhyzopertha dominica* (F.), the lesser grain borer, could penetrate a 30.5-cm layer of wheat treated with the labeled rates of three commercial formulations of DE, and, if so, to measure rates of adult survival and progeny production. When *R. dominica* adults were introduced to this surface layer of 30.5-cm wheat admixed with DE, they were able to penetrate the DE-treated layer and oviposit in the untreated wheat below. Both adult survival and progeny production were significantly lower in wheat with a surface-layer treatment of Dryacide[®] (1000 ppm) as compared to Insecto[™] (500 ppm), Protect-It[®] (400 ppm) or the untreated control. Temperature and exposure interval had no effect on adult survival or progeny production. The vertical displacement patterns of adults were significantly different among DE treatments, but not for temperature or exposure intervals. More *R. dominica* traveled a greater distance in the untreated control, followed by Insecto[™], Protect-It[®], and then Dryacide[®]. Results indicate that *R. dominica* can penetrate a surface layer of DE-treated wheat and reproduce within and below it, but it is possible that pest suppression is dose dependent, or it may depend on a combination of application rate and specific DE formulation. Published by Elsevier Ltd.

Keywords: *Rhyzopertha dominica*; Diatomaceous earth; Wheat; Control; Movement

1. I d c

Diatomaceous earth (DE) is mined and obtained from deposits of fossilized diatoms, which are microscopic plants related to algae. These unicellular plants extract silica from salt and fresh water, which then forms an outer skeletal structure. Upon death, the cell walls are deposited on the ocean floors, river beds, or lake bottoms (Subramanyam and Roesli, 2000; Korunic, 1998). Fossilized deposits are collected and processed for commercial use by drying, crushing, and milling to create a fine powder (Quarles and

Winn, 1996). DE kills insects by adhering to and abrading the insect cuticle, thereby adsorbing lipids in the epicuticle and causing death due to water loss and desiccation (Quarles and Winn, 1996; Korunic, 1998; Subramanyam and Roesli, 2000).

Commercial formulations of DE have been available in the United States since the 1950s (Korunic, 1998). However, the large amount of DE required to adequately control stored-product insects has often resulted in physical problems and mechanical damage to grain handling equipment (Korunic et al., 1996). Newer formulations of DE are more effective than some of the older products, but even at lower application rates physical problems such as decreased flow rate and increased bulk density can occur when entire grain masses are treated (Korunic et al., 1996, 1998). Because of the concern regarding effects on the physical properties of grain, there is interest in using DE

[☆]This paper reports the results of research only. Mention of a proprietary product or insecticide does not constitute a recommendation or endorsement by Kansas State University or the US Department of Agriculture.

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primarily as a surface treatment for insect control in stored grain.

Insecticidal efficacy of DE can be affected by environmental factors, including the source of the deposits (Golob, 1997; Korunic et al., 1996). Generally the efficacy of DE decreases with increases in humidity and grain moisture content, but increases with temperature (Subramanyam and Roesli, 2000). Also, susceptibility to DE can vary among insect species, in general mobile species such as *Cryptolestes ferrugineus* (Stephens), the rusty grain beetle, are more susceptible to DE than less mobile species (Rigaux et al., 2001).

Rhyzopertha dominica (F.), the lesser grain borer, is a major cosmopolitan pest of stored wheat (Potter, 1935). It is a strong flier (Dowdy, 1994) and generally enters a grain bin through the headspace, then moves through the grain mass in a slow downward progression (Sharangapani and Pingale, 1957; Surtees, 1965; Keever, 1983; Hagstrum et al., 1994; Vela-Coiffier et al., 1997; Hagstrum, 2001). Both adults and larvae attack whole kernels. Females lay eggs on the outside of the kernel and the first instars bore inside, wher.9(k)11.418.9sceptib-294(e)0(l)1o428(k)(er)13.t(t)14.6(k)(er)13.u.7(t8Do)14-428(

products plus an untreated control. At the end of each exposure interval, the top two rings and the HOBOTM sensor and cables were removed by un-taping them and pulling them out of the tower. Sequentially the duct tape holding the rings together was removed for the entire tower by inserting a metal plate (14.0 × 9.5 cm) between the adjoining rings, allowing for the top ring and plate to be lifted off the cylinder and wheat from that ring transferred to a 177.4 mL clear plastic container (Consolidated Plastics Company, Inc. Twinsburg, OH). Each container had a hole (approximately 2.5 × 2.5 cm) in the side that was covered with a 3-cm diameter round copper mesh screen to allow for airflow. This process was repeated for all rings in all towers. The wheat from each container was sifted to assess adult mortality, wheat was returned to the original container, and held for eight additional weeks under the same temperature as the original exposure. Progeny adults were then sieved from each container, and live and dead insects were counted.

The experiment was set up according to a split-split-plot design, whereby the whole plot factor was temperature and the whole plot unit was incubator; the sub-plot factor was exposure time and the sub-plot unit was the set of four towers assigned to treatments at each exposure interval; and the sub-sub plot factor consisted of the individual DE treatments and the sub-sub plot unit the individual towers. The experiment, comprising two temperatures, four treatments, and three exposure intervals (for a total of 24 treatment combinations), was replicated five times.

2.3. Data analysis

Data were analyzed using the Proc Mixed and General Linear Model (GLM) procedure (SAS Institute, 2002). Raw data were transformed by square root to normalize their distribution, and statistical analyses were performed on the transformed data. Treatment means for the various statistical tests were separated using the Waller–Duncan k-ratio t-test.

Total adult survival was compared across all DE treatments, temperatures, and exposure intervals. A covariance model was used to analyze the distribution of insects among the 10 strata in each tower. To assess the average vertical displacement of beetles from the release point at the top of the wheat mass among DE treatments, temperatures, and exposure intervals, each stratum was assigned a number from 1 to 10, with “1” representing the uppermost stratum. These numbers were multiplied by the number of live insects in the same stratum and then summed over all strata. This sum was then divided by the total number of live insects in each tower to obtain a relative index of net vertical displacement. This is a dimensionless index, the higher the number, the more movement downwards from the release point. These procedures were described in the analysis for a previous study in which these same towers were utilized (Vardeman et al., 2006).

The percentages of live adults within and below the DE-treated layers at each temperature and exposure interval were compared between DE treatments, and again between the DE-treated layer and the untreated wheat below. The untreated control was compared with the DE treatments by separating the tower into the 30.5 cm depth corresponding to the treatment depth, and tabulating percentages of *R. dominica* found in that layer and in untreated wheat below. Progeny production and percentage survival of progeny were compared across all treatments, temperatures, and exposure intervals. To determine if population growth was affected by the DE treatments, a proportion was obtained by dividing the number of progeny from each tower by the initial number of 100 *R. dominica* adults. The distributions of progeny produced were also compared as percentages within and below the DE treatments, between temperatures and among exposure intervals.

3. Results

3.1. Parental survival

Total percentage survival of adult *R. dominica* (all strata within a given tower combined) was significantly different for DE treatments, including the control, ($F = 244.7$, $df = 3$, 92 ; $P < 0.01$), but not temperature ($F = 0.01$, $df = 1$, 92 ; $P = 0.99$) or exposure interval ($F = 0.9$, $df = 2$, 92 ; $P = 0.41$). Survival was greatest in the untreated control and decreased significantly with exposure to InsectoTM, followed by Protect-It[®] and then Dryacide[®], which had the lowest survival (Table 1).

The percentage of live parental adults found below versus in the DE-treated layer was significantly affected by which DE treatment was employed ($F = 574.6$, $df = 2$, 92 ; $P < 0.01$), but not temperature or exposure interval ($F = 3.4$, $df = 1$, 92 ; $P = 0.07$; $F = 1.8$, $df = 2$, 92 ; $P = 0.17$, respectively). Significantly higher percentages of live adults were found below the DE-treated layer for all DE products than in it. All DE treatments were significantly different from the untreated control (Table 1).

Percentages of dead parental adults below the DE-treated layer differed significantly depending on the DE treatment ($F = 109.4$, $df = 2$, 66.2 ; $P < 0.01$). However, neither temperature nor exposure interval produced significant differences ($F = 0.1$, $df = 1$, 21.1 , $P = 0.71$; $F = 0.3$, $df = 2$, 20.8 ; $P = 0.73$, respectively). Generally, higher percentages of dead adults were found within the DE-treated layer for all DE products than below it. All DE products were significantly different from each other and from the untreated control with respect to dead adults within and below the DE layer (Table 1). The greatest percentage of dead *R. dominica* within the DE-treated layer was in the Dryacide[®] treatment; the lowest was within the equivalent depth of the DE layer in the untreated control (Table 1).

Table 1
Percentages of live and dead parental *R. dominica* within and below the layers of wheat treated with three commercial formulations of diatomaceous earth (DE) at their labeled rate (and the comparable survival in the equivalent depth of untreated wheat in the controls), and the total percentage survival within the entire towers for all DE treatments and the untreated control (mean \pm SEM)^a

Treatment	Live <i>R. dominica</i>		Dead <i>R. dominica</i>		Percent total survival
	Within DE	Below DE	Within DE	Below DE	
Untreated control	96.7 \pm 3.3	3.3 \pm 3.3 c	3.3 \pm 3.3	96.6 \pm 3.3 a	96.7 \pm 3.3 a
Insecto TM 500 ppm	9.0 \pm 1.6	91.0 \pm 1.6 b	53.8 \pm 4.7	46.2 \pm 4.7 b	27.4 \pm 3.7 b
Protect-It [®] 400 ppm	5.7 \pm 1.1	94.3 \pm 1.1 ab	72.4 \pm 4.9	27.6 \pm 4.9 c	16.6 \pm 2.8 c
Dryacide [®] 1000 ppm	2.2 \pm 0.4	97.8 \pm 0.4 a	97.5 \pm 2.3	2.5 \pm 2.3 d	1.9 \pm 0.3 d

^aMeans within columns for below DE followed by different letters are significantly different ($P < 0.05$, Waller–Duncan k-ratio t-test, SAS Institute). Analysis is based on percentages, therefore data for within DE are the inverse of the data for below DE.

3.2. Parental distribution

The distribution of live adult *R. dominica* among the ten strata was significantly affected by DE or control treatments ($F = 95.4$, $df = 3$, 936; $P < 0.01$) and strata ($F = 8.5$, $df = 9$, 936; $P < 0.01$). There was also a significant DE treatment by strata interaction ($F = 3.8$; $df = 27$, 936; $P < 0.01$). However, neither temperature ($F = 0.04$, $df = 1$, 4; $P = 0.84$) nor exposure interval ($F = 0.39$, $df = 2$, 16; $P = 0.68$) had a significant effect on spatial distribution. Because temperature and exposure interval were not significant, data for live insects in each stratum within each treatment were combined for all exposure intervals (Fig. 1). Most of the live *R. dominica* were found below the DE-treated wheat in the 500 ppm InsectoTM treatment, and the fewest live adults were found in the 1000 ppm Dryacide[®] treatment (Figs. 1(B) and (D), respectively).

The mean vertical displacement of live *R. dominica* adults was significantly affected by the DE treatment or the untreated control ($F = 62.6$, $df = 3$; $P < 0.01$); those found alive in the untreated control had moved the farthest, and those in the Dryacide[®] treatment had moved the least (Table 2). Temperature and exposure interval had no effect on displacement ($F = 2.88$, $df = 1$; $P = 0.09$; $F = 0.8$, $df = 2$; $P = 0.46$, respectively). There was a significant difference in vertical displacement of adult *R. dominica* that had moved through the towers prior to death (dead beetles) between the three DE treatments and untreated control ($F = 84.1$, $df = 3$; $P < 0.01$). Dead beetles were found closer to the surface in the untreated control than in any of the DE treatments (Table 2). Temperature and exposure interval had no significant effect ($F = 2.0$, $df = 1$; $P = 0.15$; $F = 1.54$, $df = 2$; $P = 0.21$).

3.3. F₁ production

Total production of F₁ adults varied significantly among the DE and control treatments only ($F = 11.94$, $df = 3$, 72, $P < 0.01$). Temperature and exposure interval had no effect ($F = 0.5$, $df = 1$, 4, $P = 0.53$; $F = 1.1$, $df = 2$, 16, $P = 0.34$, respectively). Progeny production was equally

high — approximately 400, which represented a four-fold increase — in the InsectoTM treatment and the untreated control. It was significantly lower (about a two-fold increase) in the Protect-It[®] treatment, and extremely low where Dryacide[®] was used (Table 3).

3.4. F₁ survival

Total percentage survival of F₁ adult *R. dominica* was significantly affected by DE or control treatment ($F = 89.5$, $df = 3$, 92; $P < 0.01$). Survival was highest in the untreated control, followed by InsectoTM, Protect-It[®], and Dryacide[®] in descending order (Table 3). Neither temperature nor exposure interval had a significant influence on *R. dominica* survival ($F = 0.33$, $df = 1$, 92; $P = 0.56$; $F = 3.13$, $df = 2$, 92; $P = 0.05$, respectively).

3.5. F₁ distribution

The percentages of live F₁ adults found below and within the DE-treated layer were significantly affected by DE or control treatment ($F = 0.6$, $df = 3$, 72; $P < 0.01$), but not temperature or exposure interval ($F = 4.1$, $df = 1$, 24; $P = 0.05$; $F = 1.6$, $df = 2$, 24; $P = 0.23$, respectively). There were no significant differences among the three DE treatments in the distribution of live *R. dominica* progeny (~2–3% of progeny were found in the DE layer). However, significantly ($P < 0.05$) more (~97%) of live progeny were found within the depth of wheat corresponding to the DE layer in the untreated control (Table 3).

The percentages of dead F₁ adult lesser grain borers found within and below the DE-treated layers were not significantly affected by DE treatment, temperature, or exposure interval ($P > 0.05$) with 85–100% of those progeny recovered from within the DE-treated layer (Table 3).

4. D c

Dryacide[®] is a freshwater DE product that has been used in Australia since 1985 (Quarles, 1992; Quarles and Winn, 1996). The present study showed that application of

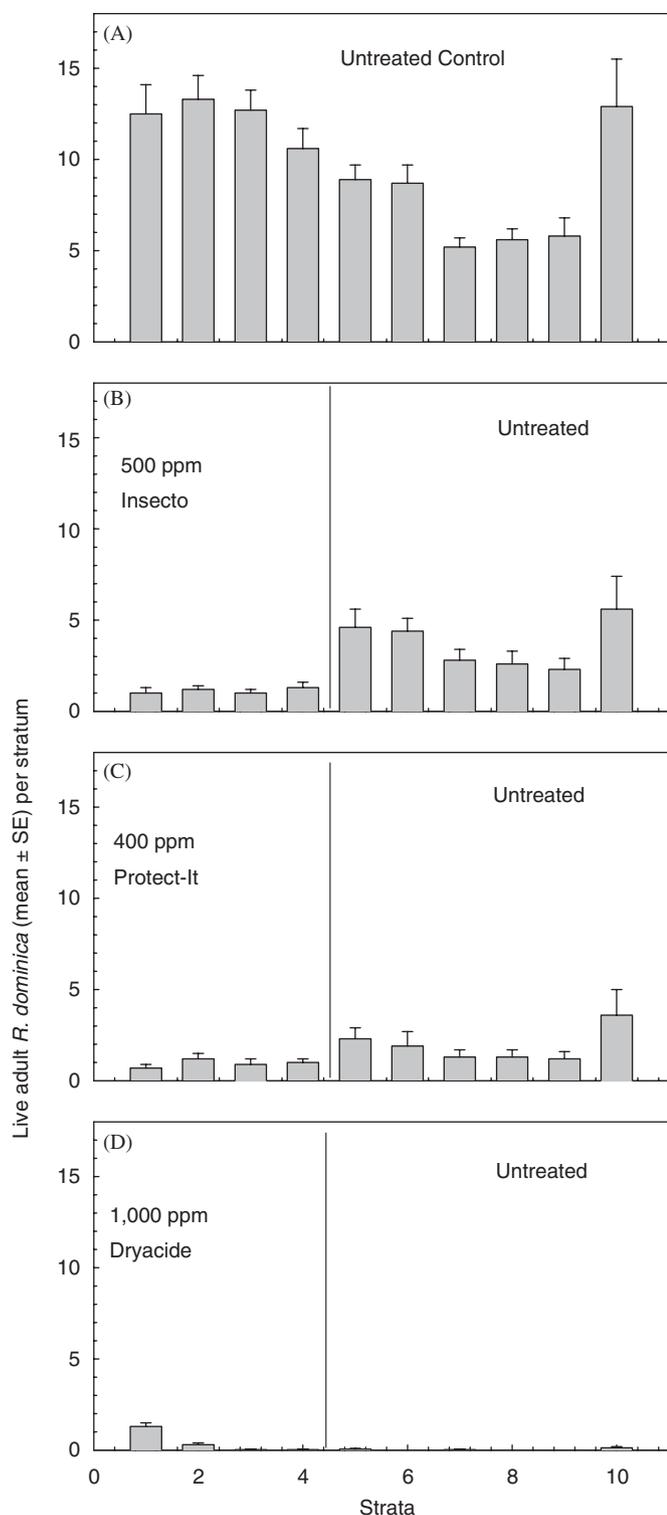


Fig. 1. The number of live adult *R. dominica* in each stratum in untreated wheat (A), and in wheat with three treatments of DE: Insecto™ (B), Protect-It® (C), or Dryacide® (D) layered on top of untreated wheat. Data combined for exposure interval and temperature. The vertical line in (B)–(D) shows the depth of the treated layer; the surface and treated wheat is to the left of the line.

Table 2

Index of vertical displacement (mean ± SEM) of live and dead adult *R. dominica* in each DE treatment^a

Treatment	Vertical displacement		
	Live <i>R. dominica</i>	Dead <i>R. dominica</i>	Total
Untreated control	4.8 ± 0.3 a	0.2 ± 0.2 c	5.0 ± 0.3 a
Insecto™ 500 ppm	1.8 ± 0.3 b	2.5 ± 0.1 ab	4.3 ± 0.2 b
Protect-It® 400 ppm	1.0 ± 0.2 c	2.8 ± 0.1 a	3.8 ± 0.2 c
Dryacide® 1000 ppm	0.04 ± 0.01 d	2.2 ± 0.1 b	2.2 ± 1.0 d

The greater the number, the more movement downwards through the grain mass from the release point (see text for details).

^aMeans followed by different letters are significantly different ($P < 0.05$, Waller–Duncan k-ratio t-test, SAS Institute).

Dryacide® at the labeled rate of 1000 ppm could effectively control *R. dominica* in stored wheat. The observed mortality level was about 98%, which is not complete kill but could provide an acceptable level of control. This rate was double or more than double the rates of the other two commercial DE products, but direct comparisons are difficult because the rates are different. However, we chose to apply the products in accordance with label rates to be consistent with the way these products would be applied in actual field use. The actual amount of DE in the Dryacide® formulation could have accounted for the greater efficacy compared to the other two DE products, but qualitative differences in the products and/or their formulations may also have contributed to differences in efficacy.

Our findings are consistent with those published previously for *R. dominica* and other stored-product insect pests. Quarles (1992) reported that Dryacide® caused a 100% mortality rate for populations of *R. dominica*, *Sitophilus oryzae* (L.) (rice weevil), and *Tribolium castaneum* (Herbst), the red flour beetle, at a rate of 1000 ppm at 65% r.h. and 20 °C. In another test where Dryacide® was applied at various rates on the grain surface, and combined with cooling, few insects of any species were trapped during a nine-month study, and no progeny developed from incubated samples (Nickson et al., 1994). In the present study, Dryacide® resulted in ~98% mortality of adult P₁ *R. dominica*, as well as ~95% mortality of adult F₁ *R. dominica* produced.

Stathers et al. (2004) evaluated the efficacy of Dryacide® and Protect-It® against *Prostephanus truncatus* (Horn) (larger grain borer), *Sitophilus zeamais* Motschulsky (maize weevil), *Callosobruchus maculatus* (F.) (cowpea weevil), and *Acanthoscelides obtectus* (Say) (common bean weevil) at different rates and humidities, and a storage period of up to six months on stored maize, cowpeas, and red kidney beans. The insecticidal value differed among the species, and it was suggested that dosages of the product should be based on the diversity of insects within the grain bin environment. In our study, using Dryacide® and Protect-It® at their labeled rates (1000 and 400 ppm, respectively),

Table 3
Percentages of live and dead *R. dominica* progeny within and below the layers of wheat treated with three commercial formulations of DE at their labeled rate, including the untreated control at a depth corresponding to that of the DE layer, the percentage total survival for the entire tower, and the actual number of progeny produced for the entire tower (mean \pm SEM)^a

Treatment	Percentage live <i>R. dominica</i>		Percentage dead <i>R. dominica</i>		Percentage survival	Total number of live + dead progeny
	Within DE	Below DE	Within DE	Below DE		
Untreated control	96.6 \pm 3.3	3.3 \pm 3.3 b	96.7 \pm 3.3	3.3 \pm 3.3 a	96.7 \pm 3.3 a	438.0 \pm 80.3 a
Insecto TM 500 ppm	2.3 \pm 1.1	97.7 \pm 1.1 a	90.8 \pm 3.6	9.2 \pm 3.6 a	55.8 \pm 6.0 b	464.1 \pm 110.4 a
Protect-It [®] 400 ppm	3.2 \pm 2.2	96.8 \pm 2.2 a	85.4 \pm 5.9	14.6 \pm 5.9 a	34.0 \pm 6.3 c	200.5 \pm 61.9 b
Dryacide [®] 1000 ppm	2.6 \pm 0.9	97.3 \pm 0.9 a	100.0 \pm 0.0	0.0 \pm 0.0 a	4.7 \pm 2.5 d	19.7 \pm 6.1 c

^aMeans within columns for below DE followed by different letters are significantly different ($P < 0.05$, Waller–Duncan k-ratio t-test, SAS Institute). Analysis is based on percentages, therefore data for within DE are the inverse of the data for below DE.

there were significant differences with respect to total survival of parents and the progeny they produced.

Dryacide[®] is considered an enhanced DE product due to the addition of silica gel (Subramanyam and Roesli, 2000). This coating of silica gel to the DE particles and the relatively small particle size of the dust may add to its insecticidal value. Protect-It[®] is also an enhanced DE product; however, the silica gel is admixed with the DE product, and not coated on the dust particles as is done with the Dryacide[®] formulation (Subramanyam and Roesli, 2000). Protect-It[®] was developed mainly in response to one of the physical problems associated with DE, which is an increased test weight. The silica gel was added to increase the efficacy of the product as an insecticide (Quarles and Winn, 1996).

In previous studies, Protect-It[®] has been effective in controlling insects in laboratory trials. Fields and Korunic (2000) tested six different DE formulations against five different stored-product insects, including *R. dominica*. Among the six DE products tested in this study were earlier formulations of Protect-it[®] and Dryacide[®] as well as InsectoTM. They reported that ranking between the DE formulations was closely related to moisture content of the wheat, the temperature at which the wheat was stored, and whether or not a dust or spray was used (the dust being more efficacious). In their study *R. dominica* was considered one of the least susceptible species to DE. but they documented a 90% mortality rate on wheat treated with 300 ppm of Protect-it[®] after a 14-d exposure at 25 °C and a grain moisture content of 11.8%. It was also noted that *R. dominica* was twice as sensitive to Dryacide[®] at 30 °C than at 20 °C. In our study Dryacide[®] was the most effective DE product in suppressing *R. dominica* when used at the labeled rate, followed by Protect-It[®] and then InsectoTM.

There are very few published reports on the insecticidal value of InsectoTM. McLaughlin (1994) tested six DE products, including silica aerogels and the modified DE products Dryacide[®] and InsectoTM, against *Sitophilus granaris* (L.) and *S. oryzae* in grain and structural treatments. In the grain treatments, the most effective DE product was Dryacide[®], and InsectoTM fell just below that ranking and the ranking of Insectogone[®] but above the

least effective product Permaguard. In the structural treatments, Dryacide[®] again seemed to be the most effective against the species tested.

Subramanyam et al. (1994) studied InsectoTM in small-scale field tests and in laboratory tests against six stored-product insects, one of which was *R. dominica*. In barrel studies with surface-treated wheat, mortality ranged from 55% to 70%, and this value was not significantly different among treatments. In the laboratory trials, InsectoTM was applied throughout the wheat and barley at three rates, two label rates and one slightly higher rate, for two weeks. Mortality of all test species was 100% at all three doses after the first 7 d. This suggests that a difference in insecticidal value between laboratory assays and barrel studies may result from the presence of untreated wheat below the treated layers, and that this untreated wheat may aid in the survival of *R. dominica*. These findings are supported by this current study. Specifically, the presence of both live and dead individuals in the untreated portion of the wheat below the DE-treated layer indicates that adult *R. dominica* were capable of surviving exposure to DE long enough to reach the untreated wheat below and oviposit before they died.

The fact that *R. dominica* was able to move following exposure to DE, and that differential mortality may have had additional effects on the distribution of live adults makes it difficult to interpret the percentages of beetles found within and below the DE layer. However, the consistent pattern of percentages of live beetles found in the DE layer and dead beetles below it suggests that deaths occurred more quickly with the most effective product, Dryacide[®], and least quickly in the untreated wheat (Table 1). A somewhat different pattern was evident for live F₁ adults (progeny) where a uniformly low percentage was found in the DE-treated layer and a very high percentage was found within the upper, untreated wheat layer. Likewise, the percentages of dead beetles below the DE-treated layer were similar for the three DE products (Table 3). Survival rates were consistently lowest for Dryacide[®] and highest for InsectoTM. Therefore, percentages of live and dead progeny found within and below the DE-treated layers may have been influenced by where oviposition occurred.

In conclusion, this study showed the label rate of 1000 ppm Dryacide[®] reduced penetration of *R. dominica* through the treated grain mass, as shown by the level of mortality in the initial adult population, and the relatively low numbers of live adults collected after each exposure interval; the resultant population of live progeny was also very low. While increasing the label rates of Insecto[™] or Protect-It[®] may or may not yield comparable results to those obtained for Dryacide[®], the current rate of 1000 ppm Dryacide[®] could be used as a surface treatment or in combination with other control strategies to suppress *R. dominica* populations in stored wheat.

Ac e d e

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