Efficacy of dinotefuran (Alpine® spray and dust) on six species of stored product insects

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Abstract
Dinotefuran, an agonist of insect nicotinic acetylcholine receptors, was evaluated both as a 0.5% active ingredient aerosol spray and a dust combined with diatomaceous earth (DE), 5 g/m² and 10 g/m², at 45% r.h. and 75% r.h. Target species were six adult stored product insect species: Tribolium castaneum (Herbst), Rhyzopertha dominica (F.), Oryzaephilus surinamensis (L.), Tribolium confusum Jacqueline du Val, Dermestes maculatus (DeGeer), and Mezium affine Boieldieu. Adults were continually exposed for 4 d on the dusts, and assessments were done after 8 h and after 1, 2, 3, and 4 d to determine knockdown and adult survival/mortality. Mortality of T. castaneum, R. dominica, and O. surinamensis generally increased with exposure interval, and was 90% or more after three days of exposure at both dust rates and r.h. levels. Mortality of D. maculatus and T. confusum after three days ranged between 60 and 70% and 50 and 60%, respectively. Mortality of M. affine was 5% or less even after 4 d of exposure. Mortality of all species except M. affine was generally lower when exposed to the spray rather than the dust. No late stage larvae of T. castaneum, T. confusum, O. surinamensis, exposed to either the spray or the dusts emerged as adults, and only 3% of exposed D. maculatus emerged as adults. Results show that dinotefuran could be incorporated into management plans for control of stored product insects.

1. Introduction
There are many species of insects that can be pests of stored products (Rees, 2004). Sanitation and other cultural control methods, such as product rotation, in-bound inspection and insect monitoring are all components of management programs for stored product insects, but in many cases the use of an insecticide may be required (Arthur, 2008). Low risk insecticides such as neonicotinoids can be used to eliminate infestations of traditional urban insect pests in homes and retail stores. The efficacy of the neonicotinoids thiamethoxam and imidacloprid (Yue et al., 2003; Arthur et al., 2004) has already been evaluated against a few insect pests of stored products. Dinotefuran is a third-generation neonicotinoid belonging to the furanicotinyl group (Wakita et al., 2003). The mode of action of this group is to disrupt synapses in the central nervous system, functioning as an agonist of the nicotinic acetylcholine receptor (Tomizawa and Yamamoto, 1993). This chemical has a wide spectrum of insecticidal activity and low mammalian and avian toxicity (Wakita et al., 2003). Insect species from several orders have been examined for their susceptibility to this new neonicotinoid, including Nephrotettix cincticeps (Uhler), the green rice leafhopper; Laodelphax striatellus (Fallén), the small brown planthopper; Spodoptera litura (F.), the common cutworm (Wakita et al., 2003); Anoplophora glabripennis (Motschulsky), the Asian long-horn beetle (Wang et al., 2005); the mosquitoes Anopheles gambiae (Giles), Culex quinquefasciatus (Say), Aedes aegypti (L.), (Corbel et al., 2004); and Periplaneta americana (L.) (the American cockroach (Kiriyama and Nishimura, 2002). However; there are no published reports in the scientific literature regarding susceptibility of stored product insects to dinotefuran.

Diatomaceous earth (DE) is a natural product consisting of the fossilized cell walls of diatoms. Commercial formulations are sold world-wide for use as insecticides either on raw grains or as a surface treatment in interior structures. There are many research publications and recent reviews on the characteristics of different DE products worldwide, and efficacy often varies widely depending on the biological and environmental factors, the target insect species, and the specific DE product (Arthur, 2000; Jeschke and Nauen, 2008; Athanassiou et al., 2009a; Iatrou et al., 2010; Wakil et al., 2010; Kavallieratos et al., 2012).

There is a commercial product in the United States that contains both products, dinotefuran and DE, sold under the trade name Alpine®.
Alpine® (BASF, Research Triangle Park, NC, USA), as a spray containing 0.5% active ingredient [a.i.] dinotefuran, and as a dust containing 0.25% a.i. dinotefuran combined with Disuac® DE (Boise, ID, USA). The dust formulation has low and high label rates of 5 g/m² and 10 g/m², respectively. The objectives of this test were to: 1) determine effectiveness of the spray and dust formulations on a range of stored product insect species, 2) evaluate how quickly the insecticide kills adult insects, and 3) evaluate effects of relative humidity (r.h.) on product efficacy.

2. Materials and methods

The following insects were used as the test species: Tribolium castaneum (Herbst), the red flour beetle, Tribolium confusum Jacqueline du Val, the confused flour beetle, Rhyzopertha dominica (F.), the lesser grain borer, and Oryzaephilus surinamensis (L.), the saw-toothed grain beetle. Dermestes maculatus (DeGeer), the hide beetle, infests a variety of dried animal products, but can also be present in processing plants (Olsen et al., 1987). The final insect species selected was Mestium affine (Boieldieu), the American spider beetle. This species is distributed throughout the USA and Canada, and is considered a minor pest (Rees, 2004). However, it is present in flour mills and warehouses and there are no data concerning susceptibility of M. affine to insecticides relative to other stored product insects.

All species used in the study were obtained from cultures reared at the USDA-ARS Center for Grain and Animal Health Research (CGAHR), Manhattan, KS, USA. The colonies of T. castaneum and T. confusum had been maintained for about 30 years, and were reared on a mixture of 95% unbleached-white wheat flour and 5% brewer’s yeast. The cultures of R. dominica and O. surinamensis culture have also been maintained for about 20 years at CGAHR, and were reared on whole wheat and rolled oats, respectively. The D. maculatus colony originated from individuals collected in 2009 from a processing plant in the state of Missouri, and it was reared and maintained on ground commercial dog food. The colony of M. affine was received at CGAHR in 2000 from the Ohio State University, and was reared on the same media as the flour beetles. Cultures of all species were maintained at 27 °C–60% r.h. in continual darkness.

Individual test arenas were created using the bottom portion of a plastic Petri dish, which was 14 cm diameter × 1.25 cm in height, and about 62 cm² in area. Arenas were created using a driveway patching material (Rockite®, Hartline Products, Cleveland, OH, USA). This powder was mixed with tap water in an approximate ratio of 0.5 g/1 ml of water to create a slurry, which then was used to fill the arena to a depth of about 0.5 cm. A total of 192 arenas were created, which were allowed to cure in open air for 2 d. The sides of the arenas to be used for O. surinamensis, D. maculatus, and M. affine were lined with Fluoron® (Northern Products, Woonsocket, RI, USA) to minimize escape of adults. A replicate consisted of 48 arenas, for the six species, four treatments (untreated controls, the dinotefuran spray, and the low and high label rates for the dust), and two r.h. levels of 45% and 75%. The replicates were conducted separately as individual blocks (Randomized Complete Block) at 27 ± 1 °C.

For each replicate, the dust was applied in proportion to the label rates using a flour sifter (1.18 mm diameter mesh openings) to dispense 77 mg per arena for the low rate (5 g/m²) and 154 mg per arena for the high rate (10 g/m²). The spray was a 0.5% active ingredient product packaged in a pressurized 557 g aerosol can. The insecticide was dispensed by attaching a plastic extension tube to the nozzle, and applying to the surface area of the arena for about 2 s, as specified on the product label. The next day after the treatments were applied, 25 one to two-week old adults of each of the six species were placed on an individual arena, eight arenas for each species. After treatment these arenas were placed in an incubator set at 27 °C and 45% r.h. Another set of four control arenas of each species were placed in second incubator also set at 27 °C and 75% r.h. Arenas in both incubators were maintained in complete darkness. At 8 h and at 1, 2, 3, and 4 d post-treatment, each arena was removed from the incubator, and adults classified as having “survived” exposure (upright and capable of normal motor movement), knocked down (on their backs and incapable of upright movement), or dead (no movement when touched with a probe). Whenever mortality was complete, no further counts were made on that arena. After each post-treatment assessment, arenas were immediately returned to the incubator. After the assessments at 96 h (4 d) the insects and arenas were discarded.

Tests were also conducted against late-stage larvae but procedures were modified. Tests were not done with larvae of R. dominica, as this is an internal feeder of bulk grains, hence the total number of arenas created for this portion of the study was 160, 40 for each of four replicates. For each of the five species, 25 late-instar larvae were placed on the treated arenas, along with 2 g of the media used for the colonies of each species, as previously described. After one week, adult emergence was monitored every 2–3 days on the arenas. The percentage less than adult emergence that completed in the untreated controls, the individuals in the treatment arenas were classified as live larvae, dead larvae, live pupae, dead pupae, live adults, and dead adults. The majority of dead adults were those that completed eclosion to the adult stage but died after emergence.

For the adult study, data for adult survival, knockdown, and mortality at the exposure intervals of 8 h and 1, 2, 3, and 4 d post-treatment (date) were analyzed using the General Linear Models Procedure (GLM) of the Statistical Analysis System (SAS, SAS Institute, v9.1, Cary, NC, USA) with species, treatment, r.h., exposure interval, and date as main effects. Associated two-way interactions were also estimated. Because all observations at the daily intervals were done on the same individual units, this initial data analysis was done with date (8 h and 1–4 d post-treatment) as a repeated measure. After this initial analysis, data were then analyzed with treatment and r.h. as main effects, again with date (1–4 d post-treatment) as a repeated measure. The error term used in the repeated measure was the replicate by species by treatment by r.h. error term, with a denominator df value of 36, instead of the overall error denominator df value of 469, which gave a more conservative measure of main effects and interactions. One-way ANOVA analysis was then done using the GLM Procedure in SAS to determine treatment effects within exposure intervals for each species for survival and mortality, and also differences in both of these variables between the r.h. levels. Both survival and mortality were used because they are not the inverse of each other, as individual adult beetles were also classified as knocked down.

For the larval study, observations were recorded at approximately 3 weeks after adult emergence was completed in untreated controls. Data were recorded as live and dead larvae, live and dead pupae, and live and dead adults (those individuals able to emerge as adults but died within 24–48 h). Because of excessive control mortality in larvae of M. affine, this species was eliminated from the analysis. Adult emergence was analyzed using the Waller–Duncan option under the GLM Procedure to determine differences between treatments and between treatments and untreated controls. As only one measurement was made, the test was not analyzed as a repeated measure in this case. The order of species susceptibility was determined by adding the percentage knockdown with the percentage of non-responsive insects at the conclusion of the test to create the variable percentage affected for each of the three treatments.
3. Results

The overall GLM analysis for percentage survival showed significant differences for main effects treatment, species, r.h., and day, all at $P < 0.001$ ($F = 18.2$, $df = 2.36$; $F = 38.2$, $df = 5.36$; $F = 6.8$, $df = 1.36$; $F = 31.8$, $df = 4.469$, respectively). There were significant model differences ($P < 0.01$) in knockdown percentage for species ($F = 11.5$, $df = 5.36$) and day ($F = 327.8$, $df = 4.469$) but not for treatment ($F = 18.2$, $df = 2.36$, $P = 0.09$) or r.h. ($F = 6.8$, $df = 1.36$, $P = 0.07$). Mortality was significant ($P < 0.01$) for treatment ($F = 6.2$, $df = 2.36$, species ($F = 5.3$, $df = 5.36$), and day ($F = 704.2$, $df = 4.469$) but not for r.h. ($F = 6.8$, $df = 1.36$, $P = 0.11$). Data for all three variables at the conclusion of the four-day exposure period were then analyzed to determine relative susceptibility of all species, within the two r.h. levels. For the purpose of this analysis, knockdown and mortality were combined (percentage affected) to determine relative species susceptibility for all three variables. At the conclusion of the test, few $M. \textit{affine}$ were affected by any treatment (Table 1). There was no difference in mortality between r.h. levels for any species ($P > 0.05$), though at 75% r.h. there were fewer affected $D. \textit{maculatus}$ compared to $T. \textit{castaneum}$, $T. \textit{confusum}$, $O. \textit{surinamensis}$, or $R. \textit{dominica}$.

Data were then analyzed by species for each r.h. level to determine if there was a difference with respect to survival, knockdown, and mortality at each sample time, by the order of species that is presented in Table 1. Although for each species the overall analysis showed there was a significant difference between r.h. levels for each of three analysis variables ($P < 0.05$), in most cases it was for only one or two of the possible 15 comparisons (five for each variable). Therefore, for further analysis, data for both r.h. levels were combined for each species, and data re-analyzed to determine significant differences among the three insecticide treatments at each exposure assessment to determine how quickly the insecticide killed adults of the various species.

Knockdown of $T. \textit{castaneum}$ after 8 h was 97%–100%; it was lower in the spray treatment after 2 d compared to the two dust treatments, and at 3 and 4 d knockdown was greater in the spray treatments than in the two dust treatments (Fig. 1A). After 1 d survival was greater in the spray treatment than the dust treatments, and by days 3 and 4 no adults survived in either of the two dust treatments or in the spray treatment (Fig. 1B). After 3 and 4 d mortality was 98–100% in the dust treatments, and while mortality in the spray treatments was increasing during the test after 2 d it was less than the corresponding mortality in either of the two dust treatments (Fig. 1C).

All $R. \textit{dominica}$ were knocked down after 8 h of exposure in all treatments, but after 1 d only 57% of the adults exposed to the spray treatment were knocked down compared to 99–100% in the two dust treatments (Fig. 2A). By day 4 there was no knockdown of adults exposed to the dust treatment, in contrast to spray treatment. Survival was low in the two dust treatments and declined to 0 by day 3, which contrasted to the continued survival in the spray treatment (Fig. 2B). In addition, there was evidence of some initial recovery from knockdown in the spray treatment. Mortality in the spray treatment began increasing at day 2, but was lower than corresponding mortality in the dust treatments, which was 100% after 3 d (Fig. 2C).

Nearly all $O. \textit{surinamensis}$ exposed to the dust were knocked down after 8 h, and by day 3 knockdown was 1% or less (Fig. 3A). Knockdown at the early time assessments of 8 h and 1 d was less in the spray compared to the high dust treatment. There was little survival in the dust treatments at 2 d or later, while some were still surviving in the spray treatment (Fig. 3B). By the end of the test all were dead in the dust treatments, and mortality in the spray treatment was 83% (Fig. 3C).

Knockdown of $T. \textit{confusum}$ was above 80% in all three treatments until day 2 but was not different until day 4, knockdown was greater in the spray versus the two dust treatments (Fig. 4A). At 3 and 4 d survival was greater in the spray versus the dust treatments (Fig. 4B). Mortality in the low and high dust treatments at day 3 was 45% and 52% respectively, which was less than corresponding mortality at this same time for the previous three species (Fig. 4C). Mortality in the spray treatment at day 4 was only 26%, which again was less than corresponding mortality of the previous three species.

Knockdown of $D. \textit{maculatus}$ in the dust treatments did not exceed 70%, while knockdown in the spray treatment did not exceed 20% (Fig. 5A). Percentage survival in all treatments declined with assessment time and was usually greater in the spray treatment compared to the two dust treatments (Fig. 5B). However, the percentage survival after 8 h was far greater than survival of the previous four species at this same exposure interval. Mortality in the low and high dust treatments at day 3 increased to 68% and 72.5%, respectively, and increased further to 92 and 98%, respectively, at day 4 (Fig. 5C). Mortality increased in the spray treatment with assessment time but was still only 22.8% after 4 d.

There was little knockdown of $M. \textit{affine}$ at any time during the test in any treatment (Fig. 6A). Survival was above 80% at all times (Fig. 6B), and there was little morality (Fig. 6C). Neither the spray nor the dust was effective on this species, and mortality did not exceed 5% in any treatment after 4 d of exposure. This species was far more tolerant than the others, and the dinofeturan did not provide any control of the adults.

Adult emergence from exposed larvae in untreated controls of $T. \textit{castaneum}$, $T. \textit{confusum}$, and $O. \textit{surinamensis}$ was 87.0 ± 12.4%, 98.7 ± 0.7%, 75.9 ± 5.6%, respectively. No larvae of these species exposed to the dinofeturan spray or the two dust rates emerged as adults. Adult emergence of $D. \textit{maculatus}$ larvae in untreated

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<td>Percentage (mean ± SE) of adults of each species affected (total of those knocked down + dead) by 0.5% dinofeturan spray, or 5 g/m² and 10 g/m² of dinofeturan dust (low dust and high dust) after four days of exposure at 45 and 75% r.h. Means within columns followed by the same lower case letters are not different ($P &gt; 0.05$, Waller–Duncan k-ratio t-test).</td>
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a No difference between r.h. for any species ($P > 0.05$, Waller–Duncan k-ratio t-test).
b Percentage affected of untreated controls after four days was 0 for $T. \textit{castaneum}$, $T. \textit{confusum}$, and $M. \textit{affine}$ at both r.h. levels. Mortality of untreated controls of $R. \textit{dominica}$, $O. \textit{surinamensis}$, and $D. \textit{maculatus}$ after four days at 45 and 75% r.h. was 7.0 ± 4.1 and 2.0 ± 1.1; 1.0 ± 1.0 and 2.0 ± 2.0; and 19.0 ± 9.7 and 5.0 ± 3.0, respectively.
controls was 71.7 ± 8.2% compared to 3.5 ± 1.3% for all three insecticide treatments combined.

4. Discussion

The dust formulation of dinotefuran insecticide was more effective both in terms of knockdown and mortality of adults for all species except M. afine when compared to the spray treatment. In addition, there was no negative effect of the higher r.h., in contrast to many tests with DE alone (Athanassiou et al., 2007; Vayias and Stephou, 2009). Oryzaephilus surinamensis has been cited as being slightly more susceptible to DE compared to R. dominica and T. castaneum (Kljajic et al., 2010), but in our test mortality of all three species exposed to both dust formulations was above 90% after three days. The lower effectiveness of the spray could also be due to the fact that it was difficult to get an accurate dosage from the spray can. In addition, there are several studies that indicate an additive effect of DE when it is combined with a conventional contact insecticide (Athanassiou et al., 2006; Chanbang et al., 2007), the biological insecticide spinosad (Chintzoglou et al., 2008; Vayias et al., 2009) or with natural products including DE (Arthur, 2004; Vayias et al., 2006; Athanassiou et al., 2008, 2009a). This additive effect may explain the higher efficacy with the combination product.

The lack of efficacy on M. afine could relate to the fact that spider beetles in general can be resilient to desiccation (Benoit et al., 2005) and this may help to explain why M. afine survived...
exposure to the dust formulations. During the observation periods it was apparent that the DE particles clung to the head of individual adults, which seemed to affect locomotion but did not have any effect on mortality.

One factor to consider when evaluating insecticides is how mortality relates to initial knockdown from exposure and the transition from knockdown to either recovery or mortality. For all species in our test, mortality increased with exposure interval, though the effect on *M. affinis* was negligible, as the adults that were knocked down remained in the exposure arenas and continued contact with the insecticide. This result is common when insects are exposed to DE on a treated surface without food material (Collins and Cook, 2006a,b), or on treated raw grains (Vayias et al., 2009), as exposure interval is a dose factor along with the actual concentration (Seghal et al., 2013). However, recovery from knockdown often occurs when insects are provided with food during exposure to DE or other insecticides, or exposed for short time intervals and given food after exposure (Arthur, 2008). Similar results have also been noted for contact insecticides (Arthur, 2008, 2009) and also the biological pesticide spinosad (Athanassiou et al., 2009b; Toews et al., 2003; Getchell and Subramanyam, 2008). In our test we did not evaluate food effects on insecticidal efficacy, and instead focused on relative susceptibility of the test species to the dinotefuran spray and dust.

Larvae of many stored product species are generally more susceptible to DE-based products compared to adults (Kavallieratos et al., 2013).
et al., 2007; Vayias et al., 2009), though Mewis and Ulrichs (2001) report the opposite for *Tenebrio molitor* (L.), the yellow mealworm. Similarly, larvae are usually more susceptible than adults to contact insecticides and also aerosols (Arthur and Campbell, 2008; Arthur and Fontenot, 2012). In our test no exposed larvae of *T. castaneum*, *T. confusum*, or *O. surinamensis* emerged as adults, even on the spray treatment, which was far less effective than the two dust rates. Similarly, only a low percentage of *D. maculatus* larvae were able to emerge as adults. Many tests with DE and DE-combination product focus on grain treatments, but another sector where these types of products could be used is as crack and crevice treatments in mills, food production sites, and food storage facilities. Many of these sites contain refugial areas where populations can persist, and recent studies with *T. castaneum* in simulated field studies and actual sites show the bulk of the population is in the immature stages, and with the percentage of adults ranging from 5 to 15% (Campbell et al., 2010a,b; Arthur et al., 2013). Presumably this would be true of other stored product insect species as well that inhabit milling and processing facilities. The greater susceptibility of the larval stage to insecticides compared to the adult stage will affect population development even when adults may survive exposure. Also, adults that encounter dinotefuran may be eventually knocked down and incapacitated, and therefore unable to escape exposure and will eventually die.

A potential use of the combination product evaluated in our study is as a residual crack and crevice or spot treatment in

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**Fig. 5.** Percentage (mean ± SEM) of *D. maculatus* that were knocked down after 8 h (0.3 d on x axis), 1, 2, 3, and 4 d of exposure to 0.5% dinotefuran spray, 5 g/m² (Low DE) of dinotefuran dust, or 10 g/m² (High DE) of dinotefuran dust (A), had survived (B), or were dead (C). Means for treatments at each assessment for each category with the same letters are not different from each other (*P* ≥ 0.05, Waller–Duncan k-ratio t-test, Statistical Analysis System).

**Fig. 6.** Percentage (mean ± SEM) of *M. affinis* that were knocked down after 8 h (0.3 d on x axis), 1, 2, 3, and 4 d of exposure to 0.5% dinotefuran spray, 5 g/m² (Low DE) of dinotefuran dust, or 10 g/m² (High DE) of dinotefuran dust (A), had survived (B), or were dead (C). Means for treatments at each assessment for each category with the same letters are not different from each other (*P* ≥ 0.05, Waller–Duncan k-ratio t-test, Statistical Analysis System).
structures containing bagged or processed grain-based products. This type of treatment would be particularly effective if the diotefuran—DE combination could be enhanced with an attractant. Such studies would be an extension of research utilizing methodology to determine population effects, and could also incorporate additional research with the spray product to determine if an increase in application rate would lead to increased knockdown and eventual mortality.

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