Efficacy of chlorfenapyr against *Tribolium castaneum* and *Tribolium confusum* (Coleoptera: Tenebrionidae) adults exposed on concrete, vinyl tile, and plywood surfaces

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Accepted 10 August 2007

Abstract

The insecticidal pyrrole chlorfenapyr was applied to concrete, vinyl tile, and plywood surfaces, at an application rate of 1.1 g AI m\(^{-2}\). Adult *Tribolium castaneum* (Herbst), the red flour beetle, and adult *Tribolium confusum* (Du Val), the confused flour beetle, were exposed for 2 and 4 h, removed, and held without food for 7 d post-exposure. All beetles survived the initial exposures, but survival of both species decreased during the 7-d holding period, with *T. confusum* being the more susceptible species. Survival was generally lower on concrete than on tile or plywood, and was greatly reduced on all three surfaces after 4 h of exposure compared to 2 h. Survival of *T. castaneum* after 2 h of exposure on concrete, tile, and plywood was 2.5 ± 2.5%, 25.5 ± 15.4%, and 40.0 ± 7.1%, respectively, after 7 d. In contrast, all *T. castaneum* exposed on concrete and tile were dead after 4 and 5 d, respectively, while survival on plywood after 7 d was 20.0 ± 16.8%. After 4 h of exposure, all *T. castaneum* and *T. confusum* exposed on concrete and tile were dead after 2-4 d post-exposure, while survival on plywood after 7 d was 41.5 ± 6.4% and 0 for each species, respectively. Non-linear and linear regressions were fit to the data for both species. Results show exposure to chlorfenapyr is effective against *T. castaneum* and *T. confusum*, but efficacy will vary depending on the surface substrate.

Keywords: *Tribolium*; Chlorfenapyr; Treated surfaces; Efficacy

1. Introduction

When conducting pest control operations in structural facilities or sites containing processed food products, the variety of surface substrates in these facilities represents a challenge to pest management programs. Insecticidal efficacy will often vary depending on the surface substrate, as documented in studies with urban insects (Chadwick, 1985; Braness and Bennett, 1990; Fletcher and Axtell, 1993; Koehler et al., 1996) and with stored-product insects (Williams et al., 1983; Samson and Hall, 1989; Arthur, 1994, 1997; Collins et al., 2000). In general, efficacy is best on non-porous surfaces such as steel or ceramic tile compared with more porous surfaces (Arthur, 1994, 1997).

During the past decade, there has been an introduction of new insecticides in the United States of America (USA) to replace conventional neurotoxicants in insect pest management programs. One such insecticide is chlorfenapyr, an insecticidal pyrrole that uncouples oxidative phosphorylation in the mitochondria, resulting in cell death through inhibition of ATP synthesis (Hunt, 1996; Mascarenhas and Boethel, 1997; McLeod et al., 2002). Tests conducted in the 1990s established efficacy on insect pests of cotton (Brickle et al., 2001), but the compound was never registered for this use. Subsequent tests with other agricultural pest species also demonstrated effectiveness of chlorfenapyr (Waldstein and Reissig, 2000; McLeod et al., 2002), and it is registered as a miticide in Japan, primarily to control *Tetranychus urticae* Koch, the two-spotted spider mite.
spider mite (Uesugi et al., 2002). Chlorfenapyr will control Blatella germanica (L.), the German cockroach and Monomorium pharoasis (L.), the Pharaoh ant (Ameen et al., 2000; Buckzkowski et al., 2005), and is registered in the USA for control of termites, cockroaches and nuisance ants under the trade name Phantom®. The label was recently expanded to include food and feed mills, food handling areas, restaurants, and other areas where food is handled and stored.

Insects commonly associated with stored food products can also be present in food handling establishments and storage facilities. Tribolium castaneum Herbst, the red flour beetle, and Tribolium confusum (Du Val), the confused flour beetle, are worldwide insect pests of mills, food warehouses, retail stores, and urban homes (Rees, 2004). They are often more difficult to kill than other stored-product beetles, though the order of toxicity will often vary depending on the particular insecticide (Arthur 1997, 1998a, b). Although there are toxicity data for chlorfenapyr for urban insect pests, there are no comparable data for any stored-product insect species. Since stored-product insects can often be found in the same environment as urban pests, data for susceptibility to chlorfenapyr would be useful for further label modifications. The objectives of this test were to: (1) establish the efficacy of chlorfenapyr on adults of T. castaneum and T. confusum and determine species susceptibility; (2) examine the effects of different surface substrates on pesticide efficacy; and (3) determine the susceptibility of the more tolerant of the two species when exposed to chlorfenapyr at several concentration/time combinations.

2. Materials and methods

The surface substrates chosen for this study were concrete, vinyl floor tile, and plywood, three common surfaces that could be encountered in structural insect pest management. Treatment arenas of each surface were constructed as follows. Individual concrete exposure arenas were created in the bottoms of standard plastic 100 mm Petri dishes (62 cm² in the bottom of the dish) using a concrete patching material (Rockkite®) purchased from a local hardware store. A water-based slurry was prepared by mixing about 2000 g Rockkite® with 1.0 L of tap water, and pouring about 10 mL of this slurry into the bottom portion of a Petri dish to create an individual treatment arena. Individual plywood arenas were made by cutting circular disks from 1.25 cm thick plywood to fit the dish, then caulking the margins to prevent adults from escaping the surface. Tile exposure arenas were created by cutting a circular portion from a standard 0.093 m² piece of vinyl floor tile, also purchased from a local hardware store. The circular disks were cut to fit the Petri dish, then caulked around the outside edge to minimize escape of test insects. An insecticide spray solution was prepared from an emulsifiable concentrate (EC) formulation (Phantom®) containing 240 mg mL⁻¹ (2 pounds per gallon). After dilution, it was applied at the rate of 1.95 L m⁻² to attain a dosage rate of 1.1 g m⁻².

Exposure intervals of 2 and 4 h were selected for each species, based on the results of preliminary testing. Each replicate spray solution was used to treat a group of 12 arenas, four of each type. A Badger 100 artist’s airbrush (Franklin Park, IL, USA) was used to mist the spray volume of 1.2 mL directly onto each individual treatment arena. Untreated control arenas of each surface were sprayed with 1.2 mL of tap water. All treated arenas were allowed to dry overnight. Adult T. castaneum and T. confusum were obtained from pesticide-susceptible cultures maintained at the Grain Marketing and Production Center, Manhattan, KS, USA. These strains were originally collected in Kansas in 1958 and occasionally supplemented with wild individuals collected from field sites. Cultures were maintained on a diet of 95% whole-wheat flour and 5% yeast, and held in continual darkness at 27 °C, 69% relative humidity (r.h.). Ten 1–2-week-old mixed sex adults were exposed for either 2 or 4 h on each set of treated surfaces and the untreated controls for each surface and replicate. Upon completion of the exposure interval, the beetles were transferred to new Petri dishes lined with filter paper, and held for 1 week without food on a laboratory counter. Approximately conditions inside the laboratory were about 25 °C and 50% r.h. Post-treatment survival, knockdown, and mortality were recorded daily among beetles originally exposed for 2 and 4 h on each treated and untreated arena.

Data were analyzed as a multi-factorial experiment with original exposure time of 2 and 4 h, surface, and species as main effects, and day post-treatment as a repeated measure because all of the post-treatment observations were on the same set of beetles. Variables of interest were survival, knockdown, and mortality. Analysis was done using the General Linear Models Procedure of the Statistical Analysis System (SAS Institute, 2001), and raw data were converted to percentages, and transformed to their square roots because of heterogeneity of variances. Means for each treatment surface were separated by exposure interval and beetle species using the GLM Procedure of SAS and the Waller–Duncan k-ratio t-test to determine significant differences (P < 0.05). Data for species were also compared using the t-test (PROC t-test) of SAS. Because the post-treatment data were an ordered sequence from 0 to 7, mean separation tests were not done on the different dates. Equations were fitted to the data using lack-of-fit tests (Draper and Smith, 1981) in Table Curve 2D software (SPSS, Chicago, IL, USA) to determine appropriate linear or non-linear equations with date post-exposure as the independent variable, the maximum R² of any model which could be fitted to the data set, and the R² of the selected model. This approach provides a means of accurately fitting linear and non-linear curves to biological data (Draper and Smith, 1981), and has been used in previous publications (Arthur et al., 2004).
3. Results

The knockdown category was a transitional step between survival and mortality, and often survival and mortality were direct reciprocals. For this reason, only data for survival are presented to simplify explanation. Also, survival in untreated controls was virtually 100%, and no corrections for mortality were necessary. These data were eliminated from the statistical analysis. Also, all beetles were considered to have survived the initial 2 and 4 h exposures; none were knocked down or dead. These data points for initial survival (day 0) were not analyzed in the general analysis because survival was 100%; however, this 100% value was used in the curve-fitting procedure.

The ANOVA showed that main effects species, surface, and original exposure time, plus the repeated measure day post-treatment, were all significant for survival at $P < 0.01$ ($F = 13.7, \ df = 1, 36; F = 15.4, \ df = 2, 36; F = 9.6, \ df = 1, 36; \text{ and } F = 3.1, \ df = 6, 216$; respectively). Because the data for day post-treatment were repeated measures, the error term used to test for significance was $\text{rep} \times \text{treatment} \times \text{species} \times \text{surface}$. Assorted interactions were also significant at $P < 0.05$, including species $\times$ surface, treatment $\times$ species $\times$ surface, species $\times$ day, treatment $\times$ species $\times$ day, and species $\times$ surface $\times$ day, indicating differential survival with respect to the main effects.

Survival of $T. castaneum$ after 2 h of exposure on treated concrete was $52.5 \pm 17.0\%$ after 1 d, and then sharply declined to $2.5 \pm 2.5\%$ by 6 d (Fig. 1A). In contrast, survival on tile and plywood after the 2 h exposures was $95 \pm 2.9\%$ and 100%, respectively, after 1 d and $25.5 \pm 15.4\%$ and $40.0 \pm 7.1\%$, respectively, after 7 d. Survival decreased on all three surfaces during the 7 d holding period, but the patterns of declining survival were much more gradual on tile and plywood compared to the
Table 1
Comparison of survival between T. castaneum and T. confusum from 0 to 7 d after exposure for either 2 or 4 h on concrete, tile, and plywood treated with chlorfenapyr at the rate of 0.11 mg AI cm$^{-2}$

<table>
<thead>
<tr>
<th>Surface</th>
<th>Exposure</th>
<th>Day post-treatment</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Concrete</td>
<td>2 h</td>
<td>*</td>
</tr>
<tr>
<td>Vinyl tile</td>
<td></td>
<td>x</td>
</tr>
<tr>
<td>Plywood</td>
<td></td>
<td>*</td>
</tr>
<tr>
<td>Concrete</td>
<td>4 h</td>
<td>x</td>
</tr>
<tr>
<td>Vinyl tile</td>
<td></td>
<td>x</td>
</tr>
<tr>
<td>Plywood</td>
<td></td>
<td>x</td>
</tr>
</tbody>
</table>

Significance at $P < 0.05$ for greater survival of T. castaneum, PROC $t$-test, SAS Institute 2001. Post-treatment day and surface marked with an asterisk (*) indicate greater survival of T. castaneum than T. confusum; those marked with a cross (x) indicate no significance ($P \geq 0.05$).

The lowest survival usually occurred on the concrete surface, and generally there was no difference in survival on tile and plywood.

The effect of increased exposure interval is shown by the lower survival of T. castaneum exposed for 4 h on all three surfaces (Fig. 1B). At 1-d post-exposure, survival of T. castaneum exposed on concrete was 29.4 ± 14.2%, and all were dead at 2-d post-exposure. Similarly, all beetles exposed on tile were dead after 3 d. However, in contrast survival of beetles exposed on plywood was 72.5 ± 13.8% after 1 d and ranged from 35.0 ± 6.5% to 70.0 ± 7.1% during the remaining 6 d.

Survival of T. confusum exposed for 2 h was much lower than survival of T. castaneum on all three surfaces (Fig. 1C). All beetles exposed on concrete and tile were dead after 4 and 5 d, respectively, while survival on plywood occurred during the entire 7-d period. As the exposure interval increased to 4 h (Fig. 1D), survival was reduced at 1 d on all three surfaces, but it still took 3–4 d for complete mortality of beetles exposed on concrete and tile. All exposed on plywood were dead after 5 d. When data for each species were compared, survival was greater in T. castaneum versus T. confusum at 2 of 14 comparisons on concrete, 4 of the 14 on tile, and 11 of 14 comparisons on plywood (Table 1). Linear and non-linear equations were fitted to the raw data for survival of each species (Fig. 2A–D; Table 2). The adjusted $R^2$ values indicated that the equations fit the data set for most of the equations.

4. Discussion

During the past 30–40 years there have been a number of tests in which stored-product insects have been exposed on various treated surfaces, including but not limited to filter paper, glass, ceramic and vinyl tile, hardboard, concrete, plywood, polyethylene, burlap, paint, fiberboard, and more. There are published reports where tests were conducted on 5–10 different surfaces, with varying and differing results in the order of efficacy, depending on the species, specific insecticide and formulation, and the surface substrates that were tested (Slominsky and Gojmerac, 1972; Williams et al., 1983; Jain and Yadav, 1989; Samson and Hall, 1989; Arthur, 1997). This inherent variation often causes difficulties when trying to compare results from different tests. Chadwick (1985) reviewed tests with pyrethroids on different surfaces, and formulated some general conclusions, two of which are relevant to this study: surfaces greatly affect initial activity and residuality, and much of the loss of activity is due to the migration of the insecticide into the substrate and also degradation of the insecticide.

These surface effects are seen in our test with chlorfenapyr. Concrete is generally considered to be a porous surface, yet control of both Tribolium species was greater on this surface compared to vinyl tile and plywood. The specific interactions of the insecticide with the surface, and uptake of residues from that surface, even after just 2 and 4 h of exposure, could relate to either the characteristics of the insecticide formulation or the specific composition of the materials used in this study. Previous studies have shown that wettable powder (WPs) formulations of organophosphates and pyrethroids are more effective than ECs applied to concrete and other porous surfaces (White, 1982; Williams et al., 1983; Chadwick, 1985; Barson, 1991), possibly because the ECs are absorbed within the surface, while the WPs are held more on the surface (Chadwick, 1985). The chlorfenapyr formulation used in our study was an EC, but there are no comparable tests in which a pyrrole insecticide has been evaluated as a surface treatment for stored-product insects. It is possible that the uptake and distribution of this material on concrete may be different from other insecticides.

In this particular test, it seemed that the chlorfenapyr EC was more readily absorbed into the plywood and vinyl tile compared to the concrete, which accounted for the greater efficacy on concrete. The type of material and construction of the surfaces used in this study may be considered unique and therefore not easily compared with results from other studies involving concrete. For example, there is considerable variation in what has been described as a concrete or cement surface (Cogburn, 1972; White, 1982; Williams et al., 1983; Arthur, 1994; Collins et al., 2000; Toews et al., 2003). There is no standardization of materials and testing procedure, and any conclusions from a particular study must be evaluated carefully. Concrete, tile, and wood are all surfaces that can be readily encountered in normal pest management operations in structural facilities where stored-product insects are present, and results from one study with a specific set of surfaces may not be easily transferred to other types of material in the same class.

The results also show variation between T. castaneum and T. confusum in regards to their susceptibility to chlorfenapyr. In previous tests with these same laboratory strains, T. castaneum was more tolerant to the pyrethroid cyfluthrin compared to T. confusum (Arthur, 1998a, b), but
the reverse was true for the pyrethroid deltamethrin in a dust formulation (Arthur, 1997), different formulations of diatomaceous earth (DE) (Arthur, 2000a) and the insect growth regulator (IGR) hydroprene (Arthur, 2001, 2003). Other researchers have also noted differential susceptibility of these same species exposed on wheat treated with various insecticides in different classes. In studies with residual bioassays of wheat treated with 8 ppm of chlorpyrifos, residues remaining after 12 months killed 83% of the exposed adult T. confusum compared to 98% of the exposed adult T. castaneum (LaHue, 1977). Ardley (1976) exposed both species on wheat treated with 4 ppm bioresmethrin + 20 ppm piperonyl butoxide, and reported lower efficacy for adult T. confusum compared to T. castaneum. In contrast, Bengston et al. (1980) reported lower mortality of two malathion-susceptible strains of T. confusum exposed on wheat treated with the organophosphates fenitrothion and malathion, compared to a T. castaneum strain with slight malathion resistance. While many exposure studies on different surface substrates show that Tribolium spp. may be more difficult to kill than other stored-product species, the order of susceptibility between T. castaneum and T. confusum cannot be automatically assumed. It is also likely that differences between individual laboratory and field strains of these species contribute to the differential susceptibilities obtained from insecticide tests.

The time for which Tribolium spp. or any other stored-product insect is exposed on a treated surface is a dosage factor, along with the actual concentration of active ingredient applied to that surface. If an insecticide is labeled as a general surface treatment, the entire floor area may be treated, but as the floor space increases, it may be more practical in actual operations to do spot or limited scale treatments. This would lead to opportunities for insects to escape exposure. However, even if the entire floor
area is treated, stored-product environments offer numerous refugia where insects can escape exposure (Barson, 1991; Campbell and Arthur, 2007). The results of this study show that if T. castaneum and T. confusum are exposed on a surface treated with chlorfenapyr and then escape, the residual effects could eventually lead to death, depending on the length of the original exposure period and the specific surface. A cautionary note is that the mortality takes some time to occur, because chlorfenapyr affects the metabolism of the insect through inhibition of ATP synthesis, as opposed to a conventional neurotoxicant. This delayed mortality could be compromised by other physical and biological factors, such as the presence of food or trash material, which could lead to increased survival (Arthur, 2000b, c).

Acknowledgments

The author thanks the BASF Corporation for providing product samples and partial funding for the research. He also thanks B.D. Barnett and E.A. Jensen for technical assistance with the research, and X. Hou and K.Y. Zhu for reviewing the paper prior to journal submission.

References

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Table 2

Parameters (mean ± SE) for linear and non-linear equations for survival (Y) of T. castaneum and T. confusum 1–7 d (x) after exposure for 2 and 4 h on concrete, floor tile, and plywood treated with chlorfenapyr at the rate of 0.11 mg AI cm⁻², plus the R² values of the equations and maximum mR² obtainable from the data set

<table>
<thead>
<tr>
<th>Species</th>
<th>Surface</th>
<th>Time (h)</th>
<th>a</th>
<th>b</th>
<th>R²</th>
<th>mR²</th>
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<tr>
<td><em>T. castaneum</em></td>
<td>Concrete</td>
<td>2⁺</td>
<td>100.4</td>
<td>7.8</td>
<td>1.4</td>
<td>0.22</td>
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<tr>
<td></td>
<td>Tile</td>
<td>2⁺</td>
<td>94.5</td>
<td>8.8</td>
<td>11.8</td>
<td>2.1</td>
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<tr>
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<td>9.2</td>
<td>9.2</td>
<td>0.8</td>
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<tr>
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<td>4.4</td>
<td>0.7</td>
<td>0.08</td>
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<tr>
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<td>8.9</td>
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<td>5.3</td>
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<tr>
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<tr>
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<td>10.23</td>
</tr>
<tr>
<td></td>
<td>Tile</td>
<td>4⁻</td>
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<td>105.8</td>
<td>6.1</td>
<td>1.5</td>
<td>0.17</td>
</tr>
</tbody>
</table>

a Two-parameter non-linear exponential equation of the form Y = a e⁻ᵇˣ.
b Linear equation of the form Y = a – b x.
c Non-linear exponential equation of the form Y = b c⁻ˣ.


Slominsky, J.W., Gojmerac, W.L., 1972. The Effect of Surfaces on the Activity of Insecticides. Research Report 2376, College of Agricultural and Life Sciences University of Wisconsin, Madison, WI.


