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Impact of accumulated food on survival of *Tribolium castaneum* on concrete treated with cyfluthrin wettable powder

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Abstract

Experiments were conducted to determine if the presence of wheat flour and other substrates would affect the residual efficacy of 20% cyfluthrin wettable powder applied to concrete at the low label rate of 100 mg (20 mg [AI])/m^2. Adults of *Tribolium castaneum* (Herbst), the red flour beetle, were exposed for 2 h on treated concrete covered with wheat flour, then removed and held for 1 week without food. Knockdown decreased and survival increased as the percentage of area covered by flour increased. Covering the concrete with flour for 2 weeks and removing it before *T. castaneum* were exposed for 2 h did not affect knockdown, but subsequent recovery and survival increased in proportion to the area of the concrete that had been covered. *Tribolium castaneum* were also exposed for 2 h on treated concrete, then held in Petri dishes that contained either no food material or 1 g of flour, pine sawdust, or wheat kernels. More beetles survived in Petri dishes containing flour or sawdust than in dishes with wheat kernels. The presence of flour or other food substrates may affect the residual efficacy of cyfluthrin by forming a barrier so that insects do not come in contact with the residues, providing exposed insects with nutrition, or enabling the insects to physically remove insecticide particles after they are exposed on a treated surface. Published by Elsevier Science Ltd.

Keywords: *Tribolium castaneum*; Red flour beetle; Cyfluthrin; Exposure; Treated surface

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1. Introduction

The 20% [AI] wettable powder formulation of the pyrethroid insecticide cyfluthrin is registered for direct application to flooring surfaces inside flour mills, processing plants, and food warehouses for controlling stored-product insects in the United States. The label specifies application at two rates, 9.5 and 19.0 g/94 m^2 (1.9 and 3.8 g [AI]/94 m^2), however, the residual efficacy of cyfluthrin can be dependent upon several biological and environmental conditions, including temperature conditions within the warehouse, the target species, and the application rate. *Tribolium castaneum* (Herbst), the red flour beetle, and *T. confusum* Jacquelin duVal, the confused flour beetle, are common pests of indoor storage facilities and processing plants, with *T. castaneum* being more tolerant to cyfluthrin than *T. confusum* (Arthur, 1998a,b).

Cleaning inside mills and warehouses and eliminating food sources that can support infestations are recommended components of sanitation programs (Hedges and Lacey, 1996). Residual treatments with insecticides such as cyfluthrin may also be considered part of sanitation programs. In addition to providing nutritional support for insect infestations, food material may also affect the residual efficacy of insecticidal treatments should insects encounter this food after they are exposed to an insecticide. *Tribolium castaneum* exposed to cyfluthrin wettable powder on concrete recovered when provided with flour, as opposed to being placed in Petri dishes with no flour after they were exposed (Arthur, 1998c).

There is limited published information concerning the effect of food substrates on residual efficacy of cyfluthrin or any other insecticide, therefore experiments were conducted to determine: (1) if either the presence or accumulation of flour would affect the efficacy of cyfluthrin wettable powder toward *T. castaneum*; or (2) if food substrates would promote recovery of *T. castaneum* after being exposed to cyfluthrin.

2. Materials and methods

2.1. Experiment 1: Survival of T. castaneum when exposed on concrete covered with different amounts of flour

Individual treatment arenas were made by mixing about 3200 g of ready-mix concrete with 1600 ml tap water to create a liquid slurry (Arthur, 1998b), and filling the lids of 30 standard 100 mm disposable plastic Petri dishes (92 mm diameter by 7 mm high, 66.6 cm^2 area). Four days later, specific areas on 20 of the concrete arenas were delineated by drawing circles in pencil that were proportional to the total area of the bottom of the Petri dish (62.0 cm^2). Circles with areas of 49.0, 38.3, 25.6, and 11.3 cm^2, which corresponded to 80, 62, 41, and 18% of 62.0 cm^2, were drawn on each of five concrete arenas. Two sets of five arenas were unmarked to represent 0 and 100% of 62.0 cm^2. A replicate consisted of two unmarked and four marked arenas, and five replicates were treated, giving a total of 30 arenas. Four replicate solutions of 20% cyfluthrin wettable powder were formulated at 100 mg/m^2, and each solution was used in six replicate arenas. Individual treatments were applied by using a Badger 100 artists' airbrush (Franklin Park, IL, USA) to spray each concrete arena at the label spray rate of 40 ml of formulated solution/per m^2 (0.27 ml per dish). The last replicate of six arenas was the untreated control.

The next day, the treated concrete was covered with flour by placing 0.5, 1.0, 1.5, 2.0 and 2.5 g in the circles comprising 18, 41, 62, 80, and 100% of the total area, and spreading the flour out using a small paintbrush. Ten mixed-sex 1 to 2 wk-old adult *T. castaneum* were put on the concrete and covered with the inverted Petri dish. A hole was cut out of the bottom of the dish to provide air exchange. After 2 h, the beetles were removed, classified as running or knocked down (on their backs but able to move), then transferred to new Petri dishes lined with filter paper. These dishes did not contain food. The flour was brushed off the concrete into a trash can.

The clean dishes containing the beetles and the treated dishes were held on a counter inside the laboratory, at approximate conditions of 28°C, 60% relative humidity (r.h.). After 1 week, the beetles were removed and classified as running, knocked down, or dead (not moving when prodded). Residual bioassays were conducted at 2 and 4 weeks by adding flour to the treated concrete and exposing beetles as previously described.

2.2. Experiment 2: Survival of T. castaneum when concrete was covered with flour prior to exposure

Thirty concrete treatment arenas were created, and after 3 days circles of the same dimension as described in Experiment 1 were drawn on the concrete. Cyfluthrin was formulated at 100 mg/m^2 and sprayed on the concrete. After 24 h, 0.5, 1.0, 1.5, 2.0 and 2.5 g of flour were distributed throughout the circles comprising 18, 41, 62, 80, and 100% of the total area. The arenas were held on a laboratory counter for 2 weeks, then the flour was cleaned from the concrete, *T. castaneum* were exposed on the concrete for 2 h, then removed, assessed and transferred as described for Experiment 1. New flour was put back on the concrete, and the entire process was repeated at 2 and 4 weeks. Temperature and humidity conditions inside the laboratory remained at approximately 28°C, 60% r.h.

2.3. Experiment 3: Survival of T. castaneum after beetles were exposed on clean concrete then held on different substrates

In this test 60 individual treatment units were constructed as previously described, and after 3 days four replicate solutions of 100 mg/m^2 cyfluthrin were formulated to treat individual replicates comprising 12 concrete arenas. Concrete was treated with the airbrush as described in Experiments 1 and 2. The untreated replicate in each concentration served as the untreated control. Twenty four hours after application, ten 1 to 2 week-old mixed-sex adult *T. castaneum* were exposed on the treated concrete for 0.5, 1, and 2 h in each of four arenas for each replicate. After exposure, beetles were classified as running or knocked down, then one group of 10 were transferred to Petri dishes containing filter paper, while each of the other three groups of 10 were put in Petri dishes that contained 1 g of either flour, pine sawdust, or wheat kernels.

All dishes were held on a laboratory counter for 1 week, then the beetles were classified as active, knocked down, or dead. The flour, dust, sawdust, and wheat were discarded. The treated concrete was also held on a laboratory counter, and residual bioassays were conducted as described at 2 and 4 weeks. Temperature and humidity conditions inside the laboratory remained at approximately 28°C, 60% r.h.

2.4. Statistical analysis

For all three experiments, beetles that were running when the assessments were made after the 1-week holding period were considered to have survived exposure. Experiments 1 and 2 were analyzed using the General Linear Models Procedure (SAS Institute 1987) with the percentage of treated surface covered by flour as a main effect and bioassay week as a repeated measure, with the percentage of surviving beetles as the response variable. Control mortality was rare, and no corrections were necessary. Lack-of-fit tests (Draper and Smith, 1981) were conducted using Table Curve 2D software (Jandel Scientific, San Rafael, CA, USA) to determine the amount of variation that could be explained by any model fitted to the data (maximum R^2), and the amount of variation explained by the given equation (R^2). Experiment 3 was also analyzed using the GLM procedure with exposure interval, and type of material (none, flour, sawdust, or wheat) inside the Petri dishes during the 1-week holding period as main effects and bioassay week as a repeated measure.

3. Results

3.1. Survival of *T. castaneum* when exposed on concrete covered with flour

Knockdown and survival were significant with respect to both the percentage of the treated concrete covered by flour when *T. castaneum* were exposed (F = 34.8, df = 5,10, P = 0.0001 for knockdown; F = 19.4, df = 5,10, P = 0.0001 for survival) and bioassay week (F = 61.3,

Table 1
Equations describing knockdown and survival of *T. castaneum* when different percentages of concrete treated with 100 mg/m^2 20% [AI] cyfluthrin wettable powder were covered by flour when beetles were exposed[a]

	Equation parameters ± SE					
Week	a	b	c	R^2	Maximum R^2	% of maximum
			Knockdown (KD)			
0	88.1 ± 5.2	88.6 ± 7.2	−3.2 ± 2.4	0.73	0.76	96.5
2	89.1 ± 10.3	32.1 ± 4.3	−7.2 ± 3.0	0.82	0.83	98.8
4	106.8 ± 17.0	19.0 ± 4.1	−8.1 ± 3.7	0.88	0.88	100
			Survival[b]			
0	99.7 ± 6.9	15.3 ± 4.8	11.5 ± 4.4	0.65	0.66	98.5
2	98.5 ± 3.8	61.0 ± 9.3		0.66	0.67	95.5

[a] All equations sigmoidal, $y = a/(1 + \exp(-x - b/c))$, except for week 2, survival, which was $y = a - be^{-x}$. y = % knockdown, x = % of concrete covered by flour when *T. castaneum* were exposed.
[b] Week 4, regression non-significant ($P \geq 0.05$), survival = 97.9 ± 0.02%.

df = 2,36, P = 0.0001 for knockdown; F = 7.2, df = 2,36, P = 0.0025 for survival). Knockdown and survival at each week was described by sigmoidal and non-linear regression (Table 1). At week 0, 75 to 100% of the beetles were knocked down when 20, 40, 60, and 80% of the concrete was covered, but knockdown decreased to 2.3% when the entire surface was covered (Fig. 1A). After 2 h, the beetles were removed from the concrete, and 1 week later nearly all that were exposed on the 40, 60, and 80% coverage treatments had recovered and survived (Fig. 1B). Recovery and survival after 1 week was positively related to the percentage of the treated area that had been covered by the flour.

Residual activity of cyfluthrin was sharply decreased at 2 and 4 weeks (Fig. 1C–F). At week 2, only 20% of the beetles were knocked down when exposed on concrete with 40% of the area covered by flour, and less than 5% were knocked down on concrete with higher proportions of the area covered by flour (Fig. 1C). Approximately 90% of the beetles exposed on bare concrete were knocked down, but many recovered during the 1-week holding period (Fig. 1D). At week 4, knockdown for beetles exposed on concrete covered with flour continued

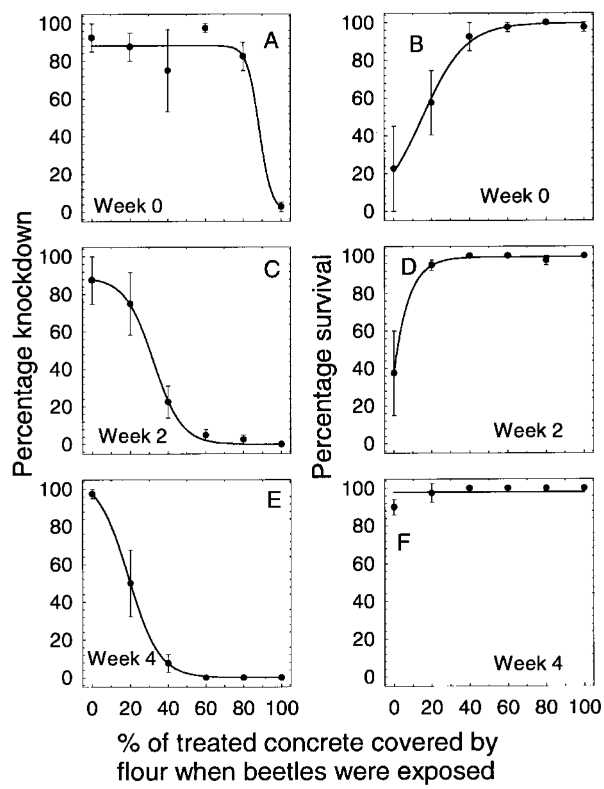

Fig. 1. Percentage knockdown (KD 0, 2, and 4 weeks) and survival (Survival 0, 2, and 4 weeks), means ± SEM for *T. castaneum* exposed for 2 h at bi-weekly intervals on concrete treated with 100 mg/m^2 20% [AI] cyfluthrin wettable powder. Concrete was either clean or covered with flour (20, 40, 60, 80, or 100% of the total area) when beetles were exposed. Curve-fit lines were plotted using equations in Table 1.

to decrease (Fig. 1E). Although knockdown on bare concrete was nearly 100%, the beetles recovered during the holding period. Survival of beetles was virtually 100% for all of the exposures (Fig. 1F).

3.2. Survival of T. castaneum when concrete was covered with flour prior to exposure

Knockdown was not significant for the percentage of concrete covered by flour prior to exposure (F = 2.5, df = 5, 10, P = 0.0659) but was significant for bioassay week (F = 24.5, df = 2,36, P = 0.0001), therefore data were combined for the percentage of flour coverages. Knockdown at weeks 0, 2, and 4 was 98.3 ± 0.8, 97.5 ± 1.4, and 87.9 ± 2.0%, respectively. Survival was significant for both percentage of concrete covered by flour (F = 10.0, df = 5,10, P = 0.0001) and bioassay week (F = 24.5, df = 2,36, P = 0.0001), and survival at each bioassay week was described by sigmoidal regression (Table 2). At week 0 no beetles survived when exposed on bare concrete or concrete that had 20% of the area covered with flour prior to exposure, but survival gradually increased as the percentage of area covered by flour increased (Fig. 2A). Even with these relatively high percentages of knockdown compared with Experiment 1, survival steadily increased for each successive bi-weekly bioassay, and by week 4 it approached 100% for beetles exposed on the concrete covered with flour 2 weeks prior to exposure, regardless of the percentage of area that was covered (Fig. 2B, C).

3.3. Survival of T. castaneum after beetles were exposed on clean concrete then held on different food substrates

The main effect, exposure interval, and the repeated measure, week, were significant for red flour beetle knockdown after exposure on concrete treated with cyfluthrin (F = 55.2, df = 2,12, P = 0.0001; F = 34.1, df = 2,72, P = 0.0081). Knockdown increased as exposure interval increased at each bioassay week, and decreased within exposure interval at each successive bioassay week (Table 3). The main effect, recovery substrate, and the repeated measure, week, were significant for survival (F = 33.8, df = 3,12, P = 0.0001; F = 8.2,

Table 2
Equations describing survival of *T. castaneum* when different percentages of concrete treated with 100 mg/m^2 20% [AI] cyfluthrin wettable powder were covered by flour for 2 weeks before beetles were exposed

	Equation parameters ± SE[a]					
Week	a	b	c	R^2	Maximum R^2	% of maximum
0	55.2 ± 25.3	55.0 ± 23.8	15.1 ± 17.4	0.29	0.31	93.5
2	89.2 ± 5.6	26.4 ± 4.5	4.6 ± 2.8	0.82	0.83	98.9
4	98.2 ± 3.9	2.7 ± 2.7	7.1 ± 3.5	0.62	0.63	98.4

[a] All equations sigmoidal, $y = a/(1 + \exp(-(x-b)/c))$, y = % survival, x = % of concrete covered by flour prior to the time *T. castaneum* was exposed.

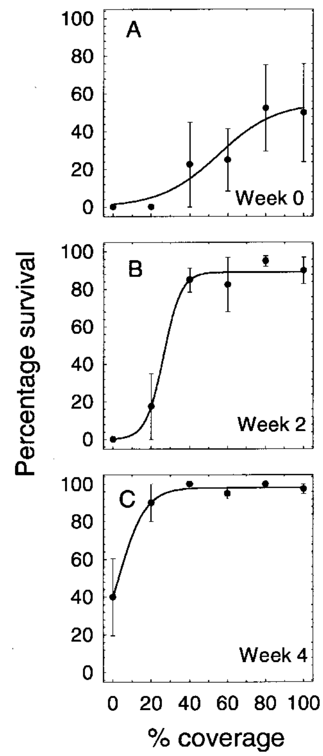

Fig. 2. Percentage and survival (Week 0, 2, and 4), means ± SEM for *T. castaneum* exposed for 2 h at bi-weekly intervals on concrete treated with 100 mg/m^2 20% [AI] cyfluthrin wettable powder. Concrete was covered with flour (20, 40, 60, 80, or 100% of the total area) 2 weeks prior to the time *T. castaneum* were exposed. Curve-fit lines were plotted using equations in Table 2.

df = 2,72, P = 0.0008), but exposure interval was not significant (F = 0.9, df = 2,12, P = 0.4058), therefore the data for the three exposure intervals were combined. Survival of *T. castaneum* was greater on flour and sawdust than on wheat or filter paper at weeks 0 and 4, but there were no significant differences at week 2 (Table 4).

Table 3
Percentage knockdown of *T. castaneum* (mean ± SEM) after exposure for 0.5, 1, 0 and 2.0 h on concrete treated with 100 mg/m^2 20% [AI] cyfluthrin wettable powder. Bioassays conducted at 0, 2, and 4 weeks after treatment[a]

Exposure (h)	Week 0	Week 2	Week 4
0.5	58.7 ± 7.2c	30.6 ± 7.2c	10.0 ± 2.9c
1.0	76.9 ± 5.7b	68.7 ± 5.0b	48.7 ± 6.6b
2.0	98.7 ± 0.8a	88.7 ± 4.2a	71.2 ± 7.3a

[a] Means for exposure at each week were separated using the Waller–Duncan *k*-ratio *t*-test, means followed by different letters are significantly different from each other (P < 0.05).

Table 4
Percentage of *T. castaneum* survival (means ± SEM) after they were exposed on concrete treated with 100 mg/m^2 20% [AI] cyfluthrin wettable powder. After exposure, beetles were transferred to untreated concrete containing either 1 g of sawdust, wheat kernels, wheat flour, or plain concrete with no material (none), and held for 1 week[a]

Substrate	Week 0	Week 2	Week 4
Flour	95.0 ± 2.9a	96.7 ± 2.6a	90.8 ± 3.8a
Sawdust	84.2 ± 4.7a	94.2 ± 3.8a	80.0 ± 5.5a
Wheat	51.7 ± 12.3b	61.7 ± 12.7a	30.0 ± 12.3b
None	42.5 ± 12.8b	77.5 ± 10.2a	17.5 ± 10.6b

[a] Means for survival at each week on different substrates were separated using the Waller–Duncan *k*-ratio *t*-test, means followed by different letters are significantly different from each other ($P < 0.05$).

4. Discussion

The presence of flour while *T. castaneum* was exposed on the concrete led to decreased knockdown and increased survival compared to knockdown and survival on concrete that did not contain any flour. Partially covering the concrete with flour may have either created a barrier so that *T. castaneum* could not come into contact with cyfluthrin residues, or enabled the beetles to physically remove the insecticide particles that they absorbed from the portion of the concrete that was not covered by flour. *T. castaneum* could only absorb the residues either by tarsal contact or ingestion of the flour until they were knocked down by the insecticide and their backs were in contact with the treated surface, but even when knocked down they may not have received a lethal dose because of the barrier created by the flour. Covering the concrete with flour prior to the exposure of beetles also led to increased survival compared to uncovered concrete. The flour could have possibly either absorbed the insecticide particles or accelerated residue degradation on the concrete.

T. castaneum also survived when exposed on concrete treated with cyfluthrin, then placed in Petri dishes containing either flour or sawdust. Published reports have documented that absorption of insecticide particles through the cuticle is a slow process even when insecticides are topically-applied to individual insects (Little et al., 1989; Lagadic et al., 1993). The cyfluthrin residues were probably confined to the exterior cuticle after the exposure interval was completed, and the flour and sawdust enabled the beetles to physically remove the insecticide particles. Also, the flour could have provided nutrition for the beetles so that the effects of insecticidal exposure were reduced.

In a previous study, *T. castaneum* were exposed for 2 h or less on concrete treated with cyfluthrin, then put in Petri dishes that either did or did not contain flour (Arthur, 1998b). Survival on filter paper that did not contain flour was <20% until 8 weeks after treatment, but in this test, survival on concrete that did not contain flour was >40% after 4 weeks in two of the three experiments. One possible explanation for the discrepancy in the results is that these three experiments were conducted during the summer months, and the temperature inside the laboratory was about 28°C. In contrast, the earlier test (Arthur, 1998b) was conducted during the winter, and the internal temperature of the laboratory was about 22°C. A related test (Arthur, 1999) showed that the residual efficacy of cyfluthrin wettable powder decreases as the temperature increases, and there is a marked decrease in toxicity at temperatures above 25°C. This negative temperature relationship for cyfluthrin is similar to the results from toxicity studies with other pyrethroid insecticides that also show decreased toxicity with increasing temperature (Johnson, 1990).

The results of this study show that if target application sites in stored product environments are not kept clean after they are treated with cyfluthrin, the presence of food material may affect the residual efficacy, either by providing exposed insects with nutrition or forming a barrier so that insects do not come in contact with the residues. Food and extraneous material within the storage facility could also promote recovery by providing the insects with a means for physically removing insecticide particles after they are exposed on a treated surface.[1]

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