STORED-PRODUCT AND QUARANTINE ENTOMOLOGY

Survival of Red Flour Beetles (Coleoptera: Tenebrionidae) on Concrete Partially Treated with Cyfluthrin

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J. Econ. Entomol. 92(4): 981–987 (1999)

ABSTRACT Different densities of red flour beetles, *Tribolium castaneum* (Herbst), were exposed for 1 h on concrete with 20, 40, 60, or 80% of the total area treated with 200 mg/m^2 of 20% [(AI)] cyfluthrin wettable powder. Residual bioassays were repeated 4 times at monthly intervals. Population density did not affect knockdown or survival of red flour beetles. The percentage of beetles still mobile after they were exposed decreased as the percentage of treated area increased, and there was no significant difference with respect to month. Survival decreased as the percentage of treated concrete increased and increased with each successive monthly bioassay. When beetles were knocked down after exposure, survival was higher for beetles knocked down on untreated concrete compared with treated concrete in 9 out of 16 comparisons. The presence of untreated pockets within a treated area could enable some of the insects to partially survive exposure to cyfluthrin in field applications.

KEY WORDS *Tribolium castaneum*, beetle, cyfluthrin, pyrethroids, population density

RESIDUAL SPRAYS FOR crack and crevice and surface treatments are often used to control insect pests in food storage facilities. These sites usually contain areas that remain untreated and provide refuges for insects to escape the treated surface (Barson 1991, Pinniger 1974). Field and laboratory studies have shown that the ability of stored-product insects to survive in these untreated sites within a larger treated environment can be dependent on population structure (Mason et al. 1997, Cox et al. 1990), strain and temperature (Cox et al. 1989), and susceptibility to a particular pesticide (Mason et al. 1997).

One residual insecticide used to control insect pests in food storage facilities is the pyrethroid insecticide cyfluthrin. The 20% [(AI)] WP (wettable powder) formulation is labeled at 2 application rates, 100 and 200 mg/m^2. Previous tests have shown that when red flour beetles, *Tribolium castaneum* (Herbst), were exposed for 1–2 h on concrete treated with the high label rate, they were knocked down after exposure and did not recover when held for an additional week on untreated filter paper (Arthur 1998a). Beetles that were still active upon completion of the exposure interval also died after they were removed from the treated concrete and held for 1 wk.

Laboratory bioassays with pyrethroid insecticides used to control field crop pests indicate that the presence of untreated areas can enable insects to escape exposure to insecticidal residues. Hall et al. (1989) showed that cabbage looper, *Trichoplusia ni* (Hübner) larvae exposed to pyrethroids on solitary leaf discs were usually killed and caused little feeding damage. However, when untreated discs were included, mortality was reduced because the larvae escaped from the treated leaf onto the untreated leaf. Pest insects also respond to the distribution of pyrethroid residues on a treated leaf and can disperse to areas with low residues (Hall et al. 1990).

The insecticide labels for residual surface treatments often state that the material can be applied at a range of concentrations, depending on the level of infestation. However, there is very little published information concerning the effectiveness of residual surface treatments when insects are exposed at different population densities. Also, there are no published studies with insecticidal surface treatments that evaluate residual efficacy when different proportions of a treated arena are left untreated. The objective of this research was to determine if either population density or the presence of untreated areas affect survival of red flour beetles exposed on concrete treated with cyfluthrin WP.

Materials and Methods

Individual treatment arenas were constructed by mixing ready-mix concrete with tap water to create a slurry (Arthur 1998b), and by filling each of 20 standard 150 by 15-mm disposable plastic petri dishes (14 cm actual diameter, 154 cm^2 area) to an approximate depth of 1 cm. The concrete was allowed to dry for several days, then circles with diameters of 12.4, 10.8, 8.9, and 6.4 cm and areas of 121.0, 91.6, 62.2, and 32.2 cm^2 (≈80, 60, 40, and 20% of the total area, respec-

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tively) were drawn on each of 4 arenas (16 total). The remaining 4 dishes with no circles were untreated controls, and the total 20 dishes comprised 1 complete replicate.

A solution of 20% (AI) cyfluthrin WP (Gustafson, Plano, TX) was formulated in 50 ml of water in proportion to the label spray rate of 200 mg in 40 ml/m^2 (19.0 g in 1 gal to cover 1,000 feet^2). Individual arenas were treated by sizing a piece of cardboard to fit the petri dish, then cutting out a circle to match the area to be treated. The cardboard was then set inside the dish so that it covered the portion of the concrete that was to be left untreated, and 0.49, 0.37, 0.25, and 0.13 ml were sprayed on the circles measuring 121.0, 91.6, 62.2, and 32.2 cm^2, respectively. The arenas were dried overnight, and the next day 10, 25, 50, or 100 unsexed adult red flour beetles (1–2 wk old) were put in each set of 4 treated arenas and the untreated controls. The different densities were exposed for 1 h, then beetles were classified as either mobile (upright and running), knocked down (on their backs but able to move when prodded) on the treated surface, or knocked down on the untreated surface. The beetles in each category were then transferred to separate petri dishes containing filter paper, held for 1 wk without food at ambient conditions in the laboratory, and removed and classified as either having survived exposure (mobile) or dead (on their backs and not moving when prodded).

Because of the large number of beetles that had to be collected, exposed, and transferred, each replicate had to be conducted separately, and the entire process described previously was repeated 3 more times at weekly intervals. All treated arenas were stored in the laboratory and residual bioassays were conducted after 1, 2, and 3 mo for each replicate. The temperature and relative humidity in the laboratory during the entire experiment, as recorded on a hygrothermograph, ranged from ≈25 to 27°C and 40 to 60% RH.

Data for knockdown and survival at each population density were converted to percentage values and the test was analyzed as a randomized complete block by using the analysis of variance (ANOVA) and general linear model (GLM) procedures (SAS Institute 1987) with replicates as blocks, the proportion of treated area and population size as main effects, and monthly residual bioassays as a repeated measure. Response variables were the percentage of beetles that were either mobile, knocked down on the treated concrete, or knocked down on the untreated concrete after exposure, and the percentage of each of these categories that had survived after they were held for 1 wk. The Wilcoxon rank sum test in the NPAR1WAY procedure (SAS Institute 1987) was used to determine significant differences between survival of beetles knocked down on treated concrete compared with survival of beetles knocked down on untreated concrete. Control mortality was rare and no corrections were made. When regressions were significant ($P < 0.05$), lack of fit tests (Draper and Smith 1981) were conducted using Table curve 2 d software (Jandel Scientific 1996) to determine the amount of variation

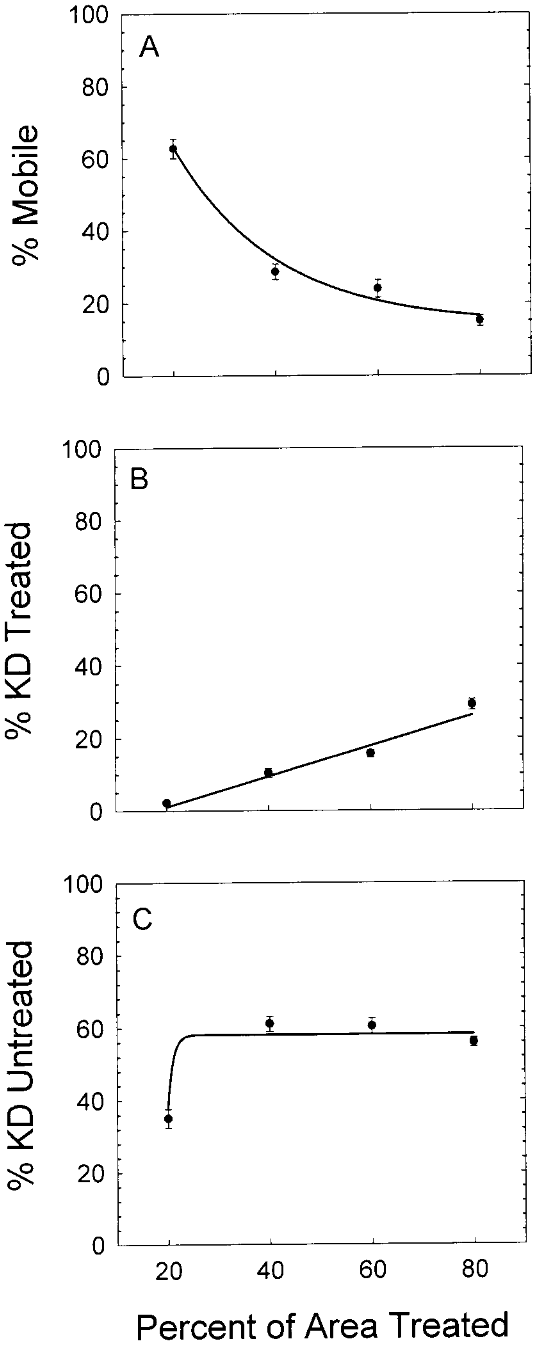

Fig. 1. Percentage (mean ± SEM) of red flour beetles that were (A) still mobile, (B) knocked down (KD) on treated concrete, or (C) knocked down on untreated concrete after exposure for 2 h. Twenty, 40, 60, or 80% of the total area of the concrete was treated with 200 mg/m^2 of 20% (AI) cyfluthrin wettable powder. Curve-fit lines are from the equations in Table 1.

Table 1. Equations describing the percentage of red flour beetles that were either mobile, knocked down (KD) on treated concrete, or knocked down on untreated concrete after exposure for 1 h on concrete that contained 20, 40, 60, and 80% of total area treated with 200 mg/m^2 of 20% (AI) cyfluthrin wettable powder

	Equation parameters ± SE[a]			r^2	Max r^2	% max
	a	b	c			
% mobile	14.0 ± 4.8	133.7 ± 42.8	0.05 ± 0.01	0.87	0.88	98.9
% KD on treated concrete	−7.4 ± 2.8	0.5 ± 0.1	—	0.86	0.87	98.9
% KD on untreated concrete	58.2 ± 1.6	1.0 e^{10} ± 1.5 e^9	—	0.76	0.84	90.4

[a] Equation for percentage mobile, $y = a + b^{(-cx)}$; equation for percentage knockdown on treated concrete, $y = a + bx$; equation for knockdown on untreated concrete, $y = a - be^{-x}$. Dependent variable x for all equations is the percentage area of treated concrete.

that could be explained by any model fit to the data (maximum R^2), and the amount of variation explained by the given equation (R^2), and to fit the appropriate regression equations to the data.

Results

Beetle Mobility After Exposure. The main effect proportion of treated concrete (20, 40, 60, or 80% of the total area treated) was significant with respect to the percentage of beetles that were still mobile upon completion of the 1-h exposure interval ($F = 46.4$; df = 3, 45; $P = 0.01$). The main effect population density was not significant ($F = 0.9$; df = 3, 45; $P = 0.45$). The repeated measure month was significant ($F = 4.5$; df = 3, 144; $P = 0.01$); however, none of the regressions with month as the dependent variable were significant ($P \geq 0.05$), so data for bioassay month were combined. As the percentage of treated area increased relative to the total area of the arena, fewer beetles remained mobile after exposure (Fig. 1A). Data for the percentage of beetles mobile after exposure were described by linear regression. (Table 1).

Beetle Knockdown on the Treated Portion of the Concrete Arenas. The main effect proportion of treated concrete was significant with respect to the percentage of beetles that were knocked down on the treated portion of the concrete arenas ($F = 84.6$; df = 3, 45; $P = 0.01$). Population density was also significant with respect to percentage knockdown ($F = 5.8.2$; df = 3, 45; $P = 0.02$), however, the only specific significant difference ($P < 0.05$) occurred at month 1 (60% treated area). None of the other 15 combinations for percentage of treated area (20, 40, 60, or 80%) or residual bioassay month (0, 1, 2, 3) was significant with respect to density, so data for the various densities were combined. The repeated measure month was significant ($F = 3.6$; df = 3, 144; $P = 0.01$); however, none of the regressions with month as the dependent variable were significant ($P \geq 0.05$). Data for bioassay month were also combined. The percentage of beetles knocked down on the treated portion of the concrete arenas increased as the percentage of treated area increased, but did not exceed 32% (Fig. 1B). Data for percentage knockdown on treated concrete were described by linear regression (Table 1).

Beetle Knockdown on the Untreated Portion of the Concrete Arenas. The main effect proportion of treated area was significant with respect to the percentage of beetles knocked down on the untreated portion of the concrete arenas ($F = 15.8$; df = 3, 45; $P = 0.01$). Neither population density ($F = 0.2$; df = 3, 45; $P = 0.87$) nor bioassay month ($F = 2.0$; df = 3, 144; $P = 0.12$) was significant with respect to knockdown, so data for population density and bioassay month were combined. The number of beetles knocked down on the untreated portion of the concrete averaged 34.8 ± 2.6 (mean ± SEM) and 61.2 ± 2.1%, respectively, on concrete with 20 and 40% total treated area, but as the proportion of treated concrete increased to 60 and 80%, knockdown remained constant (Fig. 1C). Data for percentage knockdown on untreated concrete were described by nonlinear regression (Table 1).

Survival of Beetles Still Mobile upon Completion of the Exposure Interval. Survival of these beetles was significant with respect to both the main effect pro-

Table 2. Equations describing survival of red flour beetles that were either mobile, knocked down (KD) on treated concrete, or knocked down on untreated concrete after they were exposed for 1 h on concrete that contained 20, 40, 60, and 80% of total area treated with 200 of mg/m^2 20% (AI) cyfluthrin wettable powder

Survival	% treated area of concrete	Equation parameters ± SE		r^2	Max r^2	% max
		a	b			
% survival, beetles mobile after exposure	40	22.3 ± 7.0	14.9 ± 3.8[a]	0.20	0.21	95.2
% survival, beetles KD on treated concrete	60	14.3 ± 6.0	2.4 ± 0.6[b]	0.23	0.27	85.2
	80	—	16.4 ± 3.1[c]	0.32	0.34	94.1
% survival, beetles KD on untreated concrete	40	53.1 ± 4.5	1.5 ± 0.4[b]	0.18	0.25	72.0
	60	31.3 ± 5.2	2.3 ± 0.5[b]	0.27	0.29	93.1
	80	18.3 ± 4.9	2.7 ± 0.5[b]	0.36	0.38	94.7

[a] Linear equation, $y = a + bx$, y = % survival, x = month.

[b] Nonlinear equation, $y = a + be^x$, y = % survival, x = month.

[c] Linear equation with intercept not significant, $y = bx$, y = survival, x = month.

portion of treated concrete relative to the total area of the arena (F = 31.1; df = 3, 45; P = 0.01) and the repeated measure bioassay month (F = 17.4; df = 3, 144; P = 0.01). Percentage survival was not significant with respect to population density (F = 1.1; df = 3, 45; P = 0.33), so data for density were combined. Although month was significant, regressions for month as the dependent variable were significant ($P < 0.05$) only for concrete with 60% treated area relative to the total arena. At month 0, ≈78% of the beetles survived when exposed on concrete with 20% treated area (Fig. 2A). Survival decreased to 67% on concrete with 40% treated area (Fig. 2B) and was ≈25% on concrete with 60 and 80% treated area (Fig. 2 C and D). The number of beetles that survived exposure at each residual monthly bioassay generally decreased as the percentage of treated area increased. Except for concrete with 60% treated area, survival at month 1 was less than survival at month 0. Survival on concrete with 60% treated area was described by linear regression (Table 2). Survival on all treatments increased at month 2, and by month 3 at least 67% of the beetles survived exposure on all arenas.

Survival of Beetles Knocked Down on the Treated Portion of the Concrete Arenas. Survival was significant with respect to month (F = 12.1; df = 3, 144; P = 0.01), but was not significant for either the proportion of treated area relative to the entire arena (F = 0.8; df = 3, 45; P = 0.31) or population density (F = 2.0; df = 3, 45; P = 0.13). Data were combined for density, but were not combined for treated area to determine significant differences in survival between beetles knocked down on treated concrete compared with those knocked down on untreated concrete. Survival of beetles knocked down on the treated portion of the concrete arenas ranged from 7 to 27% at months 0 and 1, from 25 to 47% at month 2, and 35 to 60% at month 3 (Fig. 3 A–D). Data for survival of beetles knocked down on concrete with 60 and 80% treated area were described by nonlinear and linear regression (Table 2).

Survival of Beetles Knocked Down on Untreated Portion of the Concrete Arenas. Survival was significant with respect to proportion of treated area relative to the entire arena (F = 14.4; df = 3, 45; P = 0.01) and bioassay month (F = 18.8; df = 3, 144; P = 0.01) but not for population density (F = 1.1; df = 3, 45; P = 0.36). Data for population density were combined. At month 0, ≈69% of the beetles survived after they were knocked down on the untreated portion of concrete that contained 20% treated area (Fig. 3E). As the proportion of treated area increased, survival generally decreased (Fig. 3 E–H). Survival of beetles knocked down on the untreated portion of concrete with 40, 60, and 80% treated area increased with month, and data were described by nonlinear regression (Table 2).

Survival of Beetles Knocked Down on Treated Compared with Untreated Concrete. With 1 exception, survival of beetles knocked down on untreated concrete compared with treated concrete was significantly higher on the arenas with 20 and 40% total

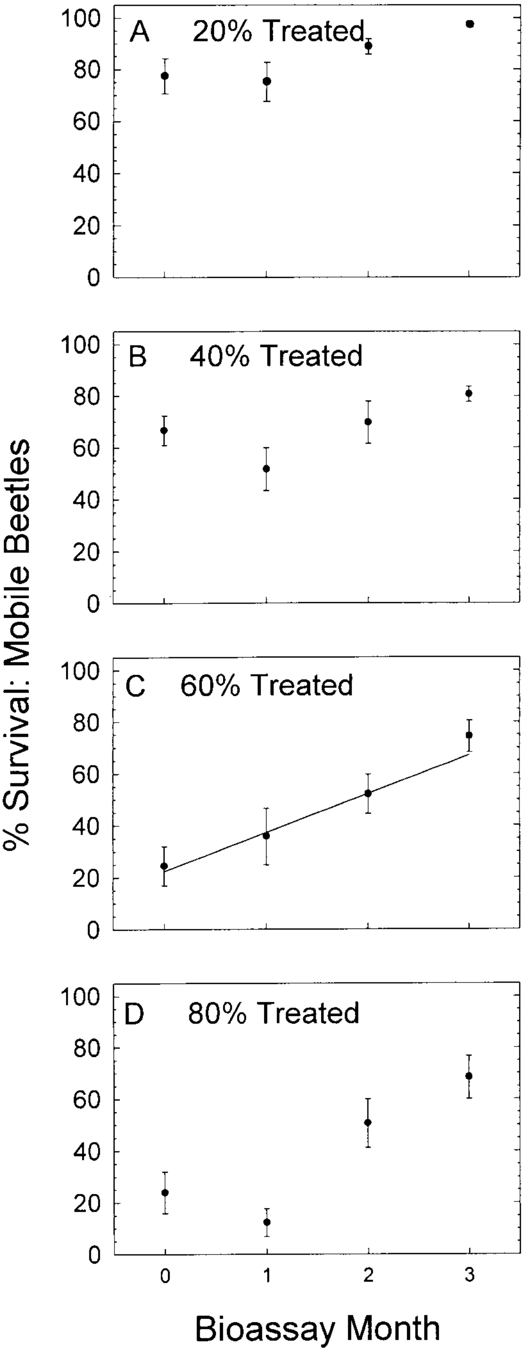

Fig. 2. Percentage of survival (means ± SEM) of red flour beetles that were still mobile after they were exposed on concrete with (A) 20%, (B) 40%, (C) 60%, or (D) 80% of the total area treated with 200 mg/m^2 of 20% (AI) cyfluthrin wettable powder. Curve-fit lines are from the equations in Table 2.

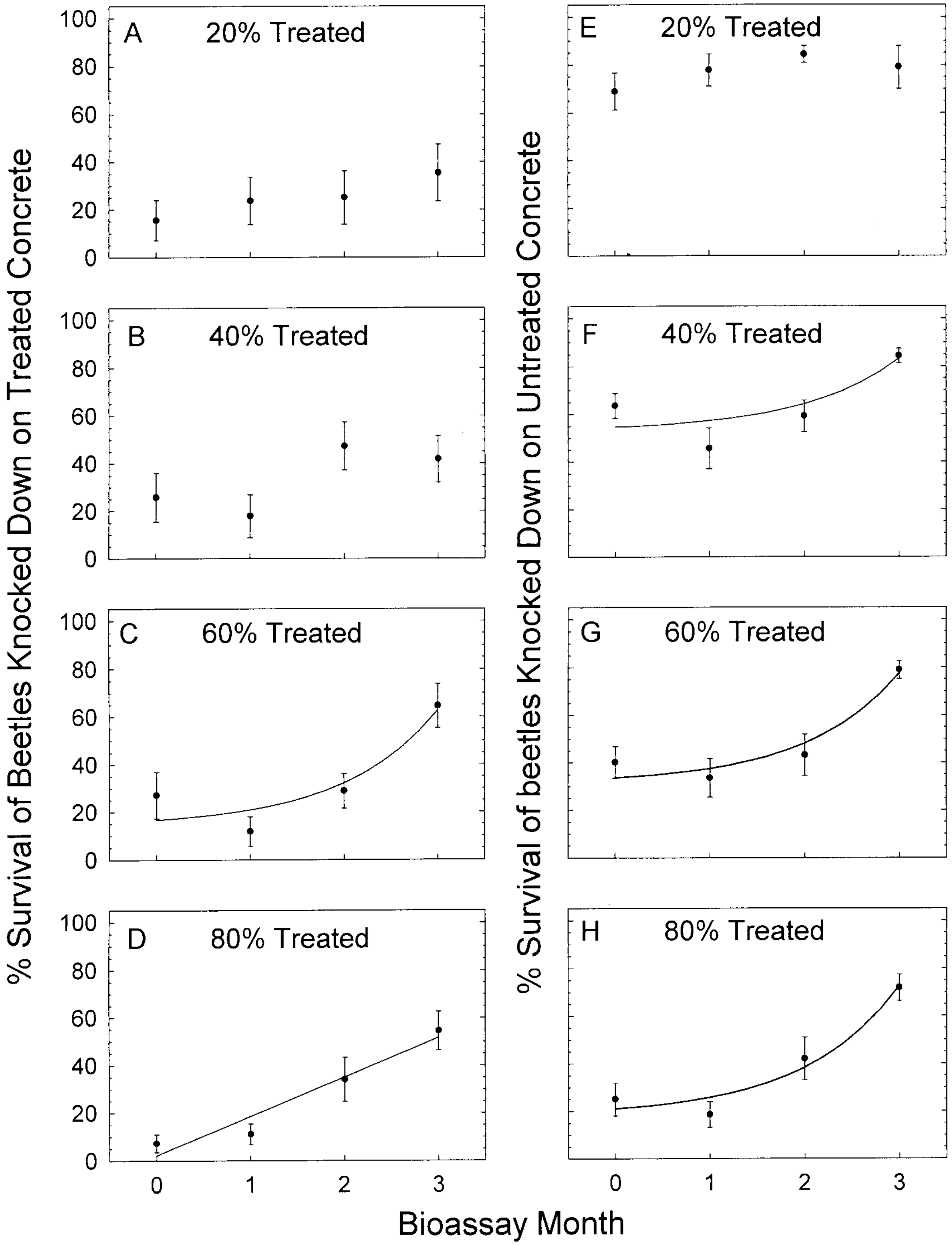

Fig. 3. Percent survival (means ± SEM) of red flour beetles that were knocked down either on the treated portion of concrete with (A) 20%, (B) 40%, (C) 60%, or (D) 80% of the total area treated with 200 mg/m^2 of 20% (AI) cyfluthrin wettable powder, or on the untreated portion of concrete with (E) 20%, (F) 40%, (G) 60%, or (H) 80% of the total area treated with cyfluthrin. Curve-fit lines are from the equations in Table 3.

Table 3. Probability of a significant difference in survival between red flour beetles knocked down on treated concrete compared with concrete

% treated area	Month			
	0	1	2	3
20	0.01	0.01	0.01	0.04
40	0.01	0.02	0.42	0.01
60	0.09	0.01	0.21	0.52
80	0.04	0.42	0.63	0.14

treated area (Table 3). However, as the proportion of treated area increased to 60 and 80%, there was no significant difference $P > 0.05$ in beetle survival for 6 of the 8 comparisons (Table 3).

Discussion

Survival of beetles that were still mobile after they were exposed for 1 h to insecticide decreased as the proportion of treated area increased, indicating that the beetles could have been absorbing more insecticide because they were spending comparatively less time on the untreated portions of the concrete arenas. Cyfluthrin does have a delayed toxic effect because some of the beetles did not survive although they were still mobile after they were exposed. However, this delayed toxic effect was more dramatic in previous studies in which the entire surface of the experimental arena was treated and the beetles were continually exposed to the residues (Arthur 1998a, b). The presence of these untreated areas also led to increased recovery and survival of beetles that were knocked down upon completion of the exposure interval. Beetles knocked down on the treated concrete were still in contact with the residues and could continue to absorb the insecticide until they were removed from the treated arena, as opposed to beetles knocked down on untreated concrete. This effect is demonstrated by comparing survival of beetles knocked down on treated compared with untreated areas (Fig. 3).

The results of this test also indicate that the efficacy of cyfluthrin WP is not dependent on the density of the insect population. Cox et al. (1990) showed that refuge-seeking behavior in rusty grain beetle, *Cryptolestes ferrugineus* (Stephens), varied with density, but they did not evaluate the effects of density on pesticide efficacy. In a similar study, strains of rusty grain beetles that exhibited the highest degree of refuge-seeking behavior had higher survival rates when exposed to fenitrothion, but there was no evidence that population density affected toxicity (Cox et al. 1997). However, when infestations within storage structures are severe, the high label rate of cyfluthrin may be necessary to eliminate the population because the insects will not be continually exposed to the same level of residues.

Stored-product insects can escape exposure to insecticidal treatments either by passively moving to areas that have not been treated or by actively seeking refuge sites that cannot be treated. Trapping and monitoring programs should be used to determine where insect pests are located within a storage facility before insecticides are applied to reduce the opportunities for insects to escape exposure. Traps have been developed that can effectively capture and monitor red flour beetles (Mullen 1992, Stejskal 1995), and traps have been used to determine the distribution of red flour beetles in a rice warehouse (Ho and Boone 1995). Precision targeting strategies developed from geostatistical analysis of trapping data have been used to identify focal points of cockroach infestations, and populations have been reduced by applying insecticidal treatments to selected areas (Brenner and Pierce 1991, Brenner et al. 1998). Similar approaches could be used to manage red flour beetles and other stored-product pests in food storage facilities.

Acknowledgments

I thank C. A. Trotter for excellent technical assistance with the research, Gustafson, Incorporated, for supplying the cyfluthrin WP used in the test, and A. B. Brocé and P. A Weston for reviewing the manuscript.

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Received for publication 21 December 1998; accepted 12 April 1999.