# Impact of Food Source on Survival of Red Flour Beetles and Confused Flour Beetles (Coleoptera: Tenebrionidae) Exposed to Diatomaceous Earth

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J. Econ. Entomol. 93(4): 1347–1356 (2000)

**ABSTRACT** A series of experiments was conducted to determine the effect of a flour food source on survival of red flour beetle, *Tribolium castaneum* (Herbst), and confused flour beetle, *Tribolium confusum* (DuVal), exposed to the labeled rate (0.5 mg/cm$^2$) of Protect-It, a marine formulation of diatomaceous earth. Beetles were exposed at 27°C, and 40, 57, and 75% RH in 62-cm$^2$ petri dishes. When beetles were exposed for 1 or 2 d in dishes with the labeled rate (0.5 mg/cm$^2$, or 31 mg per dish) of diatomaceous earth or in dishes containing flour at varying levels from 0 to 200 mg mixed with the labeled rate of diatomaceous earth, survival of both species increased as the amount of flour increased, and quickly plateaued at levels approaching 100%. In a second set of experiments, beetles were transferred to dishes containing flour at varying levels from 0 to 200 mg after they were exposed for 1 or 2 d in dishes with the labeled rate of diatomaceous earth alone. There were no significant differences in beetle survival among the levels of flour, however, survival in dishes with flour was usually greater than survival in dishes with diatomaceous earth alone. In a third test, beetles were exposed for 1, 2, and 3 d in dishes with either the labeled rate of diatomaceous earth alone (clean dishes), dishes with diatomaceous earth and empty straws, or dishes with diatomaceous earth and ≈300 mg of flour packed in the straws. Survival was not significantly different between clean dishes or dishes with straws, but survival in dishes containing the straws with flour was usually 100%, regardless of exposure interval. In all experiments, confused flour beetles were less susceptible to diatomaceous earth than red flour beetles. In addition, survival was negatively related to exposure interval and positively related to relative humidity.

**KEY WORDS** red flour beetle, confused flour beetle, diatomaceous earth, exposure, survival

SEVERAL NEW FORMULATIONS of diatomaceous earth have been registered in recent years to control insects in bulk grain and inside processing and storage facilities (Quarles and Winn 1996). Although these products are reportedly more effective than previous commercial formulations (Subramanyam et al. 1998), insecticidal efficacy varies among these new formulations of diatomaceous earth. The origin of the material and the physical characteristics of the formulation can affect insecticidal efficacy (Korunic 1997). Increases in environmental humidity usually decrease the efficacy of inert dusts that cause dessication (Alexander et al. 1944, David and Gardner 1950, Golob 1997, Korunic 1998). In some reports, efficacy increased with temperature, whereas other studies have reported negative effects or no effects of temperature. (Golob 1997). Stored-product insects are differentially susceptible to most diatomaceous earth products; *Tribolium* spp. in particular appear to be more tolerant of diatomaceous earth compared with other stored-product beetles (Korunic 1998). Also, diatomaceous earth products and inert dusts in general may require longer exposure intervals or higher concentrations, compared with conventional insecticides, to eliminate an infestation.

When insecticides are used as spot and targeted treatments to control insect pests in food storage facilities, the presence of untreated refuge areas within the larger environment can affect survival of mobile insects exposed on treated surfaces (Barson 1991, Cox et al. 1997). If the insects are not knocked down while they are exposed, the untreated areas may offer opportunities for insects to escape exposure. In addition, the presence of food may increase survival if insects can access the food either during the time they are exposed or after they leave the treated area (Cox and Parish 1991). Survival of red flour beetles, *Tribolium castaneum* (Herbst), exposed for short intervals on concrete treated with cyfluthrin wettable powder was greatly enhanced when beetles were given flour after they were exposed (Arthur 1998c). Similar results were obtained in a study where red flour beetles were provided with flour, whole-kernel wheat, or sawdust after they were exposed on concrete treated with cyfluthrin (Arthur 2000a). Cleaning and sanitation is a recommended management practice for warehouse facilities, and elimination of extraneous food sources

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may increase the efficacy of insecticidal surface treatments.

One of the new formulations of marine diatomaceous earth is Protect-It (Korunic and Fields 1995) labeled at 0.5 mg/cm$^2$ as a surface application. Exposure intervals of 2 or 3 d are required to give 100% mortality of red flour beetles and confused flour beetles, *Tribolium confusum* (DuVal), when not provided with food after they are exposed at the labeled rate (Arthur 2000b). Also, confused flour beetle appear to be less susceptible to this formulation than red flour beetle, and longer exposure intervals are required to give complete control of confused flour beetle compared with the red flour beetle (Arthur 2000b). The objectives of the current study were as follows: (1) to determine whether flour mixed with diatomaceous earth affects survival of red flour beetles and confused flour beetles, (2) the presence of food affects survival after beetles are exposed to diatomaceous earth, and (3) whether the presence of refuge areas, with or without food, enhances beetle survival.

## Materials and Methods

**Experiment 1: Effects of Food at Time of Exposure.** A standard plastic disposable petri dish bottom (100 by 15 mm, with an area of 62 cm$^2$) served as the exposure arena. The labeled rate for the Protect-It formulation of diatomaceous earth is 0.5 mg/cm$^2$, therefore, the equivalent rate for the area of the petri dish was 31 mg. Humidity chambers were created by pouring 750 ml of saturated solutions of either $K_2CO_3$, NaBr, or NaCl in each of three plastic boxes (26 by 36.5 by 15 cm) containing a waffle-type insulating grid cut to fit the bottom. These salt solutions maintained humidities at ≈40, 57, and 75%, respectively (Greenspan 1977). Three humidity chambers were placed in a temperature incubator set at 27°C. HOBO data loggers (Onset Computer, Pocasset, MA) were put in each chamber to monitor temperature and relative humidity.

Separate tests with adult red flour beetles and adult confused flour beetles were conducted at each humidity in random order. For each humidity, 10 unsexed adults (1–2 wk old) of each species were exposed for either 1 or 2 d in separate petri dishes lined with filter paper and containing either 0, 40, 80, 120, 160, or 200 mg of wheat flour mixed with 31 mg of diatomaceous earth. The diatomaceous earth was placed in a dish, spread around on the filter paper, and flour was added. There were five treated replicates at each exposure interval plus an untreated control replicate with no diatomaceous earth (36 dishes per species and exposure interval). The dishes were covered with lids and stacked on top of the waffle grid in the humidity chamber. These procedures were similar to those used in a previous test documenting the effects of relative humidity on efficacy of diatomaceous earth (Arthur 2000b). Upon completion of the exposure interval, the dishes were removed from the humidity chamber, beetles were classified as live or dead, and all were transferred to new petri dishes lined with filter paper. These dishes did not contain flour or diatomaceous earth. These new dishes were returned to the humidity chamber, put back in the temperature incubator, and held for an additional week. Mortality was reassessed after this 1-wk holding period, and afterward the beetles and the dishes were discarded.

The data were analyzed using analysis of variance (ANOVA, SAS Institute 1987), with beetle survival as the response variable and species, relative humidity, exposure interval, and the amount of food as main effects. Survival after exposure (initial survival) and survival after a 1-wk holding period were repeated measures. Survival of untreated controls of both species was virtually 100% and no corrections were necessary. Survival data for treatments were summarized using the means procedure (SAS Institute 1987). The general linear models procedure (SAS Institute 1987) was used to determine significance of regressions with amount of flour as the independent variable and beetle survival as the dependent variable. When regressions were significant, lack-of-fit tests (Draper and Smith 1981) were conducted using Table Curve 2D software (Jandel Scientific 1996) to determine appropriate regression equations, the $R^2$ of the regression equation, the amount of variation that could be explained by any model fit to the data given the variation in the data (maximum $R^2$), and the amount of variation explained by the regression equation ($R^2$ values adjusted as a percentage of the maximum).

**Experiment 2: Effects of Food after Exposure.** All exposure conditions were the same as in experiment 1 except that each series of treated replicates consisted of six dishes lined with filter paper and containing 31 mg of diatomaceous earth only (36 dishes per exposure interval). Ten 1- to 2-wk-old unsexed adults of both species were exposed in separate sets of dishes. Upon completion of the exposure interval, beetles from each successive dish in the five treated replicates and the untreated replicate with no diatomaceous earth were classified as live or dead; transferred to new petri dishes that contained 0, 40, 80, 120, 160, or 200 mg of flour; and returned to the chamber for 1 wk. After the 1-wk holding period, beetles were classified as live or dead and discarded. Trials were conducted at 40, 57, and 75% RH in random order, and data were analyzed as described for experiment 1.

**Experiment 3: Effects of Refuge Sites and Food on Efficacy of Diatomaceous Earth.** Exposure arenas were the same standard petri dishes lined with filter paper, and tests were conducted at 27°C and 40, 57, and 75% RH. Humidity chambers with saturated salt solutions were constructed as described for experiment 1. Ten 1- to 2-wk-old unsexed adults of both species were put in separate sets of three sets of petri dishes: petri dishes with filter paper only, petri dishes that contained two drinking straws (5.1 cm in length by 0.5 cm in diameter) taped to the filter paper, or dishes that contained two straws filled with ≈300 mg of flour. For each species, there were five treated replicates plus an untreated control at each temperature humidity combination (six total replicates × five treated + one untreated × three sets of dishes × three treatments = 54 total dishes).

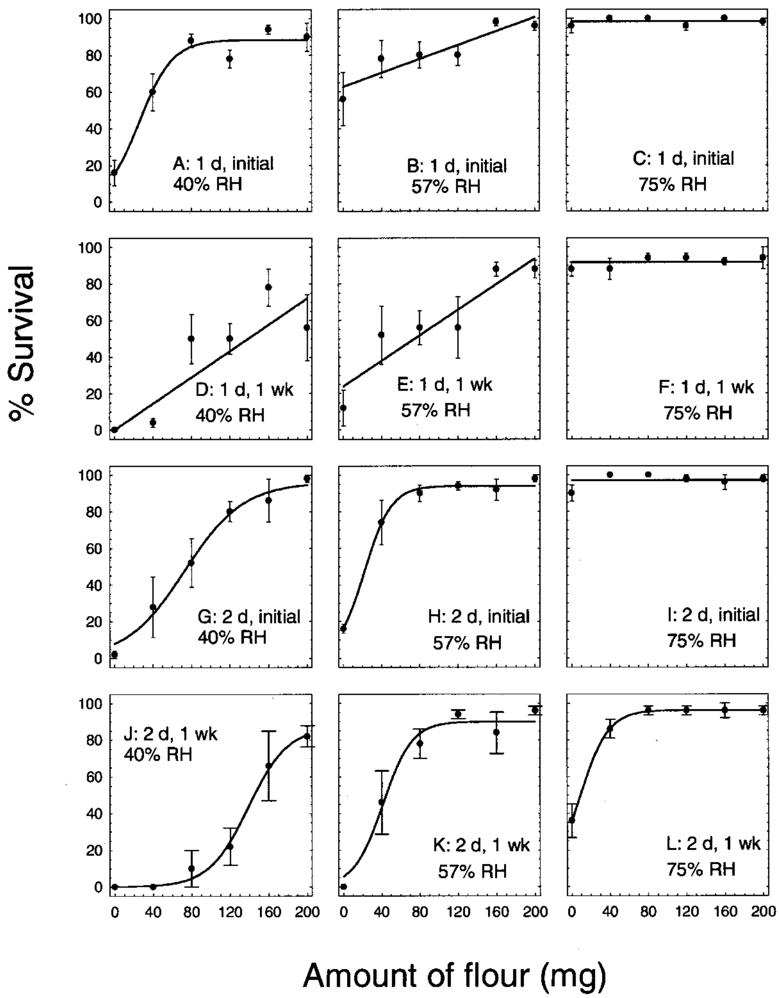


**Fig. 1.** Survival of red flour beetles (mean ± SE) exposed for 1 or 2 d at 27°C and 40, 57, or 75% RH in petri dishes to diatomaceous earth at the rate 0. 5 mg/cm$^2$ or in dishes with 40, 80, 120, 160, or 200 mg of flour mixed in with diatomaceous earth, then transferred to new dishes without flour for 1 wk. Beetles were held at the humidity as they were exposed. Curve-fit lines are from equations in Table 1.

Beetles of each species were placed in the exposure arenas for 3 d, after which 31 mg of diatomaceous earth was added to the 45 dishes comprising the five treated replicates. Beetles in one set of dishes containing all three treatments were exposed for 1 d, beetles in the second set were exposed for 2 d, and beetles in the third set were exposed for 3 d. Upon completion of the exposure interval, beetles were removed from the humidity chamber, classified as live or dead, transferred to new dishes lined with filter paper, and returned to the humidity chambers and put back in the incubator. These new dishes did not contain food or diatomaceous earth. After 1 wk, beetles were again classified as live or dead, then discarded.

This experiment was analyzed with ANOVA, with beetle survival as the response variable. Species, humidity, treatment (exposure on dishes with filter paper only [clean dishes], dishes with straws, or dishes with straws containing flour), and exposure interval were main effects and initial versus 1-wk survival were repeated measures. The GLM procedure and the Waller–Duncan *k*-ratio *t*-test were used to determine the effect of humidity on survival of each species at each of the three exposure intervals.

## Results

**Experiment 1: Effects of Food at Time of Exposure.** The main effects species ($F = 190.7$; df = 1, 303), time of exposure ($F = 18.3$; df = 1, 303), relative humidity ($F = 136.6$, df = 2, 303), and the presence of flour during exposure ($F = 98.2$; df = 5, 303), and the repeated measure exposure ($F = 328.8$; df = 1, 323) were all significant at the 0.01 level. All associated interactions except species × day ($P = 0.06$) and day × exposure interval ($P = 0.50$) were significant at

**Table 1. Equations describing survival of red flour beetle and confused flour beetles after initial exposure (1 or 2 d) to diatomaceous earth and after a 1-wk holding period**

| Exposure | Survival | Equation parameters ± SE[a] | | | c | $R^2$ | Max $R^2$ | % of max |
|---|---|---|---|---|---|---|---|---|
| | | RH[b] | a | b | | | | |
| Red flour beetle | | | | | | | | |
| 1 d | Initial | 40 | 88.4 ± 3.6 | 26.5 ± 5.2 | 17.2 ± 4.4 | 0.79 | 0.81 | 98.7 |
| | | 57 | 62.8 ± 0.2 | 0.40 ± 0.09[c] | | 0.82 | 0.83 | 98.8 |
| | 1 wk | 40 | — | 0.3 ± 0.07[d] | | 0.48 | 0.63 | 76.2 |
| | | 57 | 23.8 ± 8.2 | 0.35 ± 0.07[c] | | 0.49 | 0.57 | 86.0 |
| 2 d | Initial | 40 | 95.8 ± 9.9 | 73.3 ± 12.4 | 30.4 ± 10.3 | 0.73 | 0.74 | 98.6 |
| | | 57 | 94.0 ± 3.0 | 22.0 ± 4.1 | 14.1 ± 2.8 | 0.84 | 0.85 | 98.8 |
| | 1 wk | 40 | 86.9 ± 14.9 | 138.7 ± 13.0 | 20.4 ± 9.3 | 0.72 | 0.73 | 98.6 |
| | | 57 | 90.1 ± 5.1 | 41.2 ± 6.2 | 15.2 ± 7.0 | 0.76 | 0.78 | 97.4 |
| | | 75 | 96.2 ± 2.4 | 7.7 ± 3.1 | 15.1 ± 3.3 | 0.83 | 0.83 | 100 |
| Confused flour beetle | | | | | | | | |
| 1 d | 1 wk | 40 | 93.9 ± 2.9 | 12.9 ± 4.4 | 20.7 ± 4.3 | 0.80 | 0.82 | 97.5 |
| | | 57 | 91.6 ± 2.6 | 35.6 ± 6.3[e] | | 0.53 | 0.62 | 85.5 |
| 2 d | Initial | 40 | 100.1 ± 2.4 | 34.1 ± 5.0 | 0.05 ± 0.03[f] | 0.64 | 0.64 | 100 |
| | | 57 | 100.9 ± 4.7 | 24.0 ± 5.8 | 0.01 ± 0.01[f] | 0.42 | 0.49 | 85.7 |
| | 1 wk | 40 | 95.4 ± 4.6 | 39.7 ± 4.8 | 13.5 ± 6.4 | 0.42 | 0.49 | 85.7 |
| | | 57 | 92.9 ± 7.1 | 59.2 ± 9.5 | 23.9 ± 8.1 | 0.72 | 0.72 | 100 |

Beetles were exposed to diatomaceous earth in either clean petri dishes at the rate of 31 mg/62 $cm^2$ (0.5 mg/$cm^2$) or in dishes with 40, 80, 120, 160, or 200 mg of flour mixed in with the diatomaceous earth, then transferred to new dishes for 1 wk. Beetles were exposed and held at 27°C and 40, 57, or 75% RH. For all equations, Y = percent survival and x = amount of flour.

[a] Sigmoid equations, $y = a/[1 + \exp(-[x - b]/c)]$ unless otherwise noted.

[b] Regressions for red flour beetle at 75% RH, 1-d exposure and 1-wk holding period, 2-d exposures, and 1-wk holding period were not significant ($P \geq 0.05$); survival at each combination was 98.3 ± 0.8, 88.7 ± 3.1, 97.0 ± 1.2, and 84.3 ± 4.5%, respectively. Regressions for confused flour beetle at 75%, 1-wk holding period for the 1-d exposures, and 2-d exposures and holding period were not significant ($P \geq 0.05$). Survival at each combinations was 97.0 ± 1.2, 99.0 ± 0.7, and 97.0 ± 0.1%, respectively. Survival of confused flour beetle after the initial 1-d exposures was 100% at all relative humidities.

[c] Linear equation, $y = \mathrm{ax} + b$.

[d] Linear equation, $y = \mathrm{bx}$, intercept not significantly different ($P \geq 0.05$) from zero.

[e] Two-parameter nonlinear equation $y = a + \mathrm{be}^{(-x)}$.

[f] Three-parameter nonlinear equation $y = a + \mathrm{be}^{(-\mathrm{cx})}$.

the 0.02 level. For each species, survival after 1 and 2 d of exposure and after the 1-wk holding period for each exposure was analyzed at each of the three relative humidities, with the amount of flour (0–200 g) as the independent variable.

When red flour beetles were exposed for 1 d, survival after exposure and after the 1-wk holding period was directly related to the amount of flour mixed with the diatomaceous earth and the relative humidity (Fig. 1 A–F). Survival of beetles exposed at 40 and 57% RH was lower after 1 wk than after the initial exposures; however, at 75% RH survival was nearly 100%. As relative humidity increased from 57 to 75%, there was a dramatic increase in survival; for example, survival after the 1-wk holding period of beetles exposed on filter paper that contained diatomaceous earth with no flour was 0, 10, and 100% for beetles exposed at 40, 57, and 75% RH, respectively. Survival after exposure and after 1 wk of beetles exposed at 40 and 57% RH generally increased relative to the amount of diatomaceous earth mixed in with the flour and is described by linear and nonlinear regression (Table 1).

Survival after the initial exposures and after the 1-wk holding period of beetles exposed at 40 and 57% RH generally decreased when beetles were exposed for 2 d compared with 1 d, but was still greatly reduced at 75% RH (Fig. 1 G–L). When beetles were exposed on filter paper with no flour, survival after the 1-wk holding period was zero for beetles exposed and held at 40 and 57% RH versus 36 ± 9.3% for beetles exposed and held at 75% RH. Survival after exposure and after holding of beetles exposed at 40 and 57% RH increased in proportion to the amount of diatomaceous earth mixed in with the flour, and survival is described by nonlinear regression (Table 1).

Confused flour beetles were less susceptible than red flour beetles when exposed to diatomaceous earth alone or in combination with flour. Regressions for survival after the initial exposures at 1 d were not significant with respect to the amount of flour mixed with the diatomaceous earth ($P \geq 0.05$), and survival was 100% at 40 and 75% RH and averaged 96 ± 2.4% at 57% RH. Survival after 1 wk for beetles exposed and held at 40 and 57% RH on diatomaceous earth alone was 32 ± 3.7 and 56 ± 9.3%; however, survival greatly increased when flour was added to the diatomaceous earth (Fig. 2 A and B). Initial survival after the 2-d exposures at 40 and 57% RH ranged from 60–100% (Fig. 2 D and E), but survival after the 1-wk holding period was greatly reduced for beetles exposed to diatomaceous earth with no flour or to diatomaceous earth mixed in with 40 mg of flour (Fig. 2 G and H). Data for survival at 40 and 57% RH were described by nonlinear regression (Table 1). Survival after the initial 2-d exposures and after the 1-wk holding period of beetles exposed at 75% RH did not vary with the amount of flour mixed with the diatomaceous earth

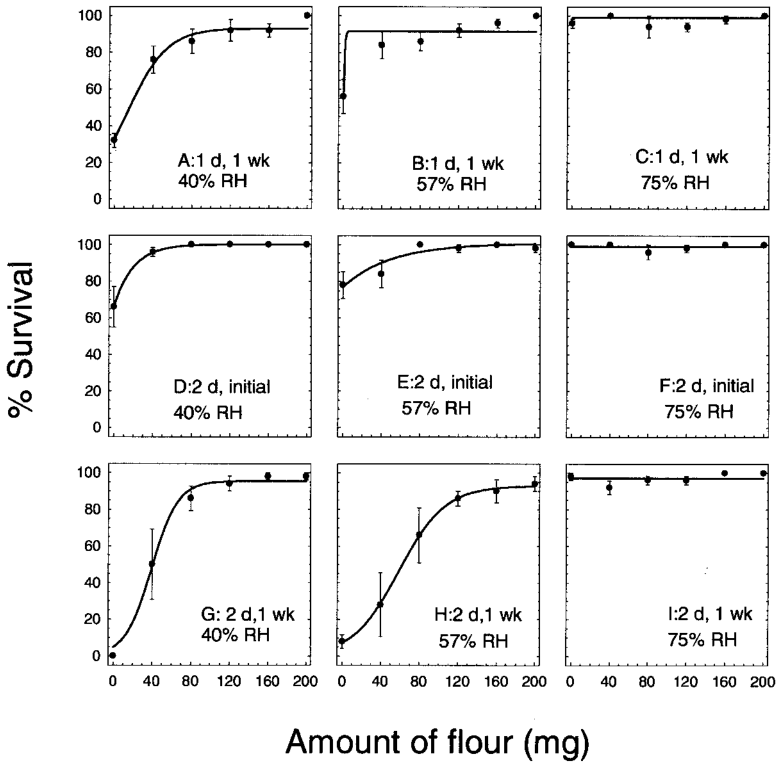


**Fig. 2.** Survival of confused flour beetles (mean ± SE) exposed for 1 or 2 d at 27°C and 40, 57, or 75% RH in petri dishes to diatomaceous earth at the rate 0. 5 mg/cm$^2$ or in dishes with 40, 80, 120, 160, or 200 mg of flour mixed in with diatomaceous earth, then transferred to new dishes without flour for 1 wk. Beetles were held at the same humidity as they were exposed. Curve-fit lines are from equations in Table 1.

($P \geq 0.05$), and averaged nearly 100% (Fig. 2 C, F, and I).

**Experiment 2: Effects of Food after Exposure.** The main effects species ($F = 875.6$; df = 1, 303), time of exposure ($F = 634.2$; df = 1, 303), relative humidity ($F = 177.4$; df = 2, 303), and the presence of flour after exposure ($F = 12.6$; df = 5, 303), and the repeated measure exposure ($F = 363.5$; df = 1, 323) were all significant at the 0.01 level. All associated interactions except species × flour ($P = 0.15$), species × exposure ($P = 0.07$), relative humidity × flour × exposure ($P = 0.99$), species × flour × exposure ($P = 0.67$) and species × relative humidity × flour × exposure ($P = 0.59$) were significant at $P < 0.03$. Although the presence of flour after exposure was significant, regressions were not significant with respect to the amount of flour ($P \geq 0.05$) and all data for flour were combined. For each species, significance in survival at each relative humidity was determined between beetles provided with flour after exposure versus beetles exposed to filter paper with no flour.

Survival of red flour beetles exposed for 1 d at 40, 57, and 75% RH averaged 70.3 ± 2.8, 85.0 ± 2.3, and 91.3 ± 1.9%, respectively. Most of the starved beetles died during the 1-wk holding period, however, survival increased three- to fourfold when beetles were given flour (Fig. 3C). When red flour beetles were exposed for 2 d, survival exceeded 20% only in beetles exposed at 75% RH (Fig. 3B). None of the beetles exposed to diatomaceous earth for 2 d survived at any relative humidity when starved during the holding period, and the only appreciable survival in the flour treatments occurred at 75% RH (Fig. 3D).

Survival of confused flour beetle was generally greater than survival of red flour beetles under all treatment conditions. Survival after 1 d of exposure was at least 97%, and survival after the 1-wk holding period between starved beetles and beetles given flour was significantly different only for beetles exposed and held at 57% RH (Fig. 3 E and G). Initial survival decreased slightly when confused flour beetles were exposed for 2 d, and few survived at 40 and 57% RH when not given flour (Fig 3F and H). Survival at all relative humidities was greater when beetles were given flour compared with when they were starved. Survival of both species generally increased when relative humidity increased, similar to the results for experiment 1. However, confused flour beetles appeared to be less susceptible than red flour beetles.

**Experiment 3: Effects of Refuge sites and Food on Efficacy of Diatomaceous Earth.** Main effects species ($F = 789.3$; df = 1, 232), relative humidity ($F = 365.3$; df = 2, 232), exposure interval ($F = 866.4$; df = 2, 232), and treatment ($F = 5863.0$; df = 2, 232), and the

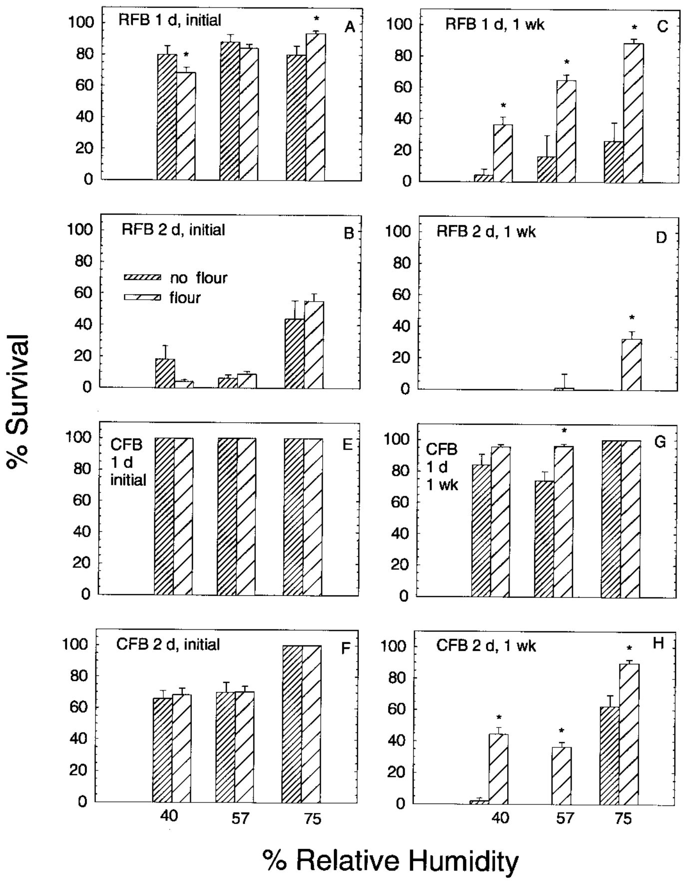


**Fig. 3.** Survival (mean ± SE) of red flour beetles (RFB) and confused flour beetles (CFB) after the initial 1- and 2-d exposures at 27°C and 40, 57, or 75% RH in petri dishes to diatomaceous earth at the rate of 0. 5 mg/cm$^2$, and survival 1 wk after being transferred and held on clean dishes versus dishes containing flour. Beetles were held at the same humidity as they were exposed. Significant differences ($P < 0.05$) in survival between beetles held in clean dishes versus dishes containing flour are noted with an asterisk.

repeated measure exposure ($F = 1006.4$; df = 1, 200) and all associated interactions were highly significant ($P < 0.01$) for the response variable survival. Data were separated by species and analyzed with respect to survival among treatments (clean dishes, dishes with straw only, and dishes with flour in the straw) after initial exposure and the 1-wk holding period at each relative humidity.

Survival of red flour beetles after the initial 1-d exposures in clean dishes or dishes with straw only increased as relative humidity increased, but survival in dishes that contained straw and flour was 100% at all three humidities (Fig. 4A). Nearly all of the beetles that were exposed in the clean dishes or dishes with straw only died during the 1-wk holding period, whereas beetle survival in dishes with straw and flour remained at 100% for beetles exposed and held at 57 and 75% RH (Fig. 4C). When the exposure interval was increased to 2 d, few beetles survived in clean dishes or dishes with straw only at 40 and 57% RH, and although some were still alive in these dishes at 75% RH (Fig. 4C), most of them died during the 1-wk holding period (Fig. 4D). With one exception, survival of beetles exposed in dishes with straw and flour was 100% both after the initial exposures and the 1-wk holding period (Fig. 4 C and D). As the exposure interval increased to 3 d, all red flour beetles exposed in clean dishes or dishes with straw only were dead after exposure, whereas all beetles survived the initial exposures in dishes with flour (data not shown). Survival after 1 wk for beetles exposed and held at 40% RH was 70.0 ± 11.4%, whereas survival was 100% at 57 and 75% RH. More beetles survived when exposed in dishes with flour than in dishes with diatomaceous earth and straws alone ($P < 0.05$), with the exception of the initial 1-d exposures at 75% RH.

Confused flour beetles were less susceptible to diatomaceous earth compared with the red flour beetle. Initial survival of confused flour beetles exposed for 1 d ranged from 91.2 ± 3.9 to 100% for all treatments and relative humidities, and there were no significant differences among treatments (Fig. 5A). Although many of the beetles exposed in clean dishes and dishes with straws only died during the 1-wk holding period,

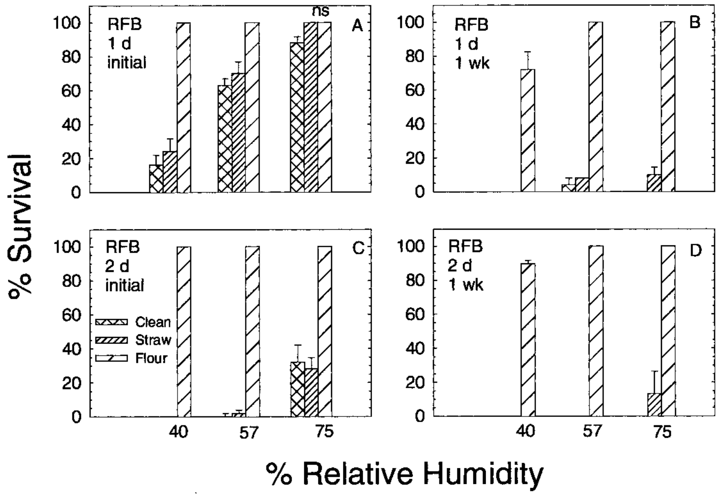


**Fig. 4.** Survival (mean ± SE) of red flour beetles after initial exposures of 1 and 2 d in petri dishes to diatomaceous earth at the rate 0. 5 mg/cm$^2$, dishes containing diatomaceous earth and straw only, and dishes containing diatomaceous earth and straw packed with 300 mg of flour, and survival (mean ± SE) after a 1-wk holding period in clean dishes. Beetles were held at the same humidity as they were exposed. Survival after initial exposures and after 1 wk was usually greater for beetles exposed with flour compared with clean dishes or dishes with straw only ($P < 0.05$), exceptions denoted by "ns."

there was a progressive increase in survival with increasing relative humidity (Fig. 5B). Survival of beetles exposed with flour was always 100% and was significantly different ($P < 0.05$) with respect to the other two treatments at 40 and 57% but not at 75% RH. Survival in clean dishes and dishes with straws only decreased at the 2-d exposures, with a positive increase in survival as relative humidity increased (Fig. 5C). There was a significant difference between survival of beetles exposed in clean dishes versus dishes with straws at 57% RH. Nearly all of the beetles exposed and held at 40 and 57% RH in clean dishes or dishes with straws only were dead after the 1-wk holding period; however, survival in these dishes at 75% was 40.6 ± 17.3 and 41.0 ± 5.7%, respectively (Fig. 5D). Survival was always 100% in dishes containing flour. As the exposure interval increased to 3 d, all beetles exposed at 40% RH on clean dishes or dishes with straws were dead, whereas a number of beetles survived exposure on these dishes at 57 and 75% RH (Fig. 5E). Again, there was a significant difference between survival of beetles exposed in clean dishes versus dishes with straws at 57% RH. None of the beetles exposed on clean dishes or dishes with straws were alive after 1 wk, except for 2.8% survival in one replicate of beetles exposed at 75% RH.

## Discussion

Diatomaceous earth and other inert dusts disrupt the epicuticle through the adsorption of epicuticular lipids to sorptive particles (Glenn et al. 1999), and once insects lose this waterproof layer, they become vulnerable to dessication (Quarles 1992). Normally, diatomaceous earth products do not decrease in effectiveness if they are kept dry, and they have residual insecticidal activity (Quarles and Winn 1996). However, accumulated food material may reduce residual efficacy of a diatomaceous earth treatment. In experiment 1, mixing flour with diatomaceous earth at the time red flour beetles and confused flour beetles were exposed led to increased survival, even though the beetles were not given food after they were removed from the exposure arenas. The presence of the flour may have reduced exposure of the beetles to diatomaceous earth, or provided nutrition so beetles could restore some of the water lost through dessication (Loschiavo 1988). Survival of both beetle species quickly plateaued at levels approaching 100% as the amount of flour mixed with diatomaceous earth was increased. In the field, residual control could be compromised if food material is accumulates on a surface treated with diatomaceous earth.

Several reports discuss increased survival of stored-product beetles when given food after exposure to inert dusts; however, the magnitude of these effects can be variable depending on the specific dust, exposure conditions, type of food material, and insect species. Vrba et al. (1983) exposed confused flour beetles in dishes containing 50 mg of silica aerogel/5 cm diameter, or 2.5 mg/cm$^2$, which is five times the label rate for the Protect-It formulation of diatomaceous earth. After exposure, beetles were then either starved or given food, however, neither test conditions nor the type and amount of food were specified. In addition, it is unclear whether beetles were held in the same dishes after they were exposed or transferred to new dishes. Mortality of starved beetles increased with increased exposure time and eventually reached 100%, whereas mortality of beetles given food was much lower at each exposure interval and ceased after 5 or 6 d. White and Loschiavo (1989) exposed confused

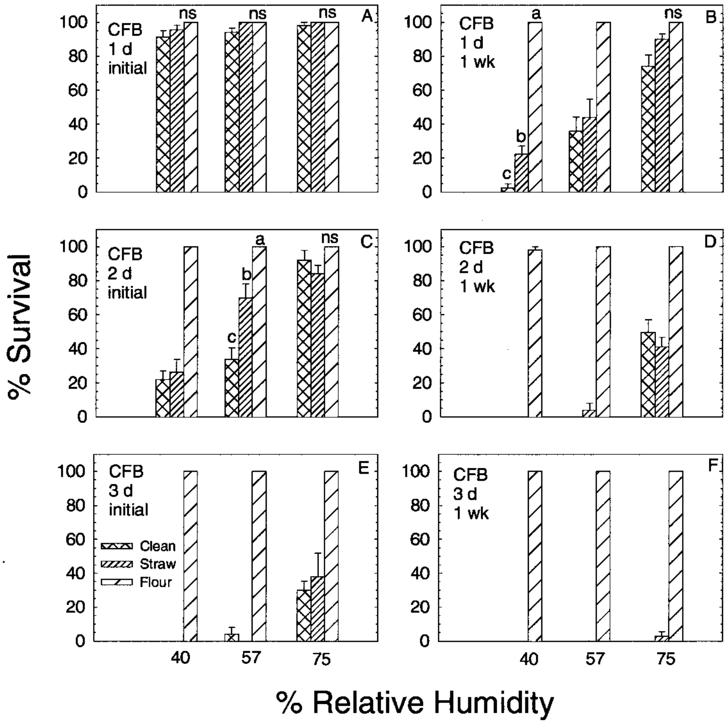


**Fig. 5.** Survival (mean ± SE) of confused flour beetles after initial exposures of 1, 2, and 3 d in petri dishes to diatomaceous earth at the rate 0. 5 mg/cm$^2$, dishes containing diatomaceous earth and straw only, and dishes containing diatomaceous earth and straw packed with 300 mg of flour, and survival (mean ± SE) after a 1-wk holding period in clean dishes. Beetles were held at the same humidity as they were exposed. Survival after initial exposures and after 1 wk was usually greater for beetles exposed with flour compared with clean dishes or dishes with straw only ($P < 0.05$). Exceptions are denoted by letters for significant differences among the three treatments ($P < 0.05$), or by "ns" for no significant difference ($P \geq 0.05$).

flour beetle and merchant grain beetle, *Oryzaephilus mercator* (Fauvel), adults on filter paper treated with silica aerogel at the rate of 0.72 mg/cm$^2$, which was similar to the labeled rate of 0.5 mg/cm$^2$ for Protect-It. Beetles were either starved after exposure or given 125 mg to 1 g of whole rolled oats, ground rolled oats, or ground bread crumbs. All starved beetles were dead within 3 d, whereas mortality of both species in the food treatments was greatly reduced. For both species, the amount of food material was not significant. These findings were similar to the results for experiment 2 in which red flour beetles and confused flour beetles were put in dishes containing no flour or 40–200 mg of flour after they were exposed. The presence of the food material greatly enhanced survival but the differing amounts of flour were not significant. The presence of the food material may have reduced dessication and provided a physical means for the exposed beetles to remove the diatomaceous earth particles from the cuticular surface.

Dowdy (1999) evaluated five different diatomaceous earth formulations, including Protect-It, by exposing red flour beetles for 15 and 30 min to 0.5 mg/cm$^2$ of each product, at temperatures of 34 and 50°C. After insects were exposed they were held for 1 wk at 34°C and 60% RH in dishes without food or dishes with 500 mg of flour. Mortality of beetles exposed to Protect-It was 100% in dishes without flour and 3.8 ± 2.4–100% in dishes with flour, depending on exposure interval and temperature. The holding temperature of 34°C may have enhanced the activity of diatomaceous earth. Previous studies with Protect-It have documented a positive increase in toxicity as temperature increases (Arthur 2000b, 2000c). Herein, a progressive increase in survival of starved beetles and beetles provided with food occurred in response to increasing humidity, and the effects of food were magnified at 75 compared with 40 and 57% RH. The effectiveness of inert dusts in general decreases with increased humidity; therefore, if insects have access to food after they are exposed to diatomaceous earth, the effects of food on survival probably will be enhanced at lower humidities.

The concept of refuge areas within stored-product environments and refuge-seeking behavior has been discussed by Cox et al. (1989, 1997). These refuges can provide food and shelter for insects, and may enable them to either escape exposure to insecticides on treated surfaces or to minimize the effects of exposure. In experiment 3 where beetles were exposed in dishes containing diatomaceous earth versus dishes with straws, there was no positive effect on survival from the addition of the straws. Beetles apparently did not avoid the diatomaceous earth by congregating in straws, and although they were observed to enter and leave straws, they could not remove diatomaceous earth particles and eventually died.

Although *Tribolium* species are reported to be more tolerant of diatomaceous earths than other stored-product beetles (Korunic 1998), there are few comparative studies of the red flour beetle versus confused flour beetle. In my experiments, confused flour beetles were much less susceptible to diatomaceous earth than red flour beetles under nearly all treatment conditions, similar to results obtained in a previous study (Arthur 2000b). Longer exposure intervals were required to produce 100% mortality of confused flour beetles compared with red flour beetles, and the effects of increased humidity on survival of confused flour beetles compared with red flour beetles were noticeable in all three experiments. When the same strains were exposed on concrete treated with the pyrethroid cyfluthrin, red flour beetle was the more tolerant species (Arthur 1998a, 1998b), whereas in studies with a dust formulation of deltamethrin, the reverse was true (Arthur 1997). There may be a difference in the way the two species adsorb insecticidal dusts or inert dusts such as diatomaceous earth.

The effects of food on insect survival were similarly noted when red flour beetles and confused flour beetles were exposed on concrete treated with cyfluthrin, then transferred to dishes that contained flour, sawdust, or wheat kernels (Arthur 2000a). These results emphasize the importance of sanitation to reduce the occurrence of food material within the storage environment and to eliminate harborage sites and refugia when possible. When insecticides are used as spot, target, or crack-and-crevice treatments in specific areas, these areas should be kept clean to maximize the effectiveness of the insecticide treatment. Failure to do so may reduce the effectiveness of insecticidal treatments.

## Acknowledgments

I thank J. E. Steele and C. K. Hoernemann for their excellent technical assistance. I also thank G. L. Puterka and J. D. Sedlacek for reviewing the manuscript, and two anonymous journal reviewers for their comments.

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*Received for publication 8 December 1999; accepted 20 March 2000.*