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Impact of high temperatures on efficacy of cyfluthrin and hydroprene applied to concrete to control *Tribolium castaneum* (Herbst) ☆

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Abstract

In laboratory trials, concrete was treated with cyfluthrin wettable powder (WP) at 40 mg active ingredient [AI] cyfluthrin WP/m^2, then heated for 4, 8, or 16 h at either 45°C or 55°C, or treated but not heated (seven treatment combinations). Bioassays were conducted by exposing adult *Tribolium castaneum* (Herbst) for 0.5, 1, and 2 h. Survival of *T. castaneum* was generally greater on unheated concrete compared with the heating treatments, and survival appeared to decrease as heating time increased at both 45°C and 55°C. In a second laboratory trial, concrete was treated with hydroprene (Gentrol) at the label rate of 1.9×10^{-3} mg [AI]/cm^2, and bioassayed by exposing late-instar *T. castaneum* larvae on the treated surface. There were significant differences between untreated controls and the heat treatment regimes ($P < 0.05$) with respect to the percentage of live emerged adults, the percentage of those adults with deformities, and the percentage of dead adults, but heating did not reduce efficacy of hydroprene. In a field trial, concrete was treated with cyfluthrin at 2 mg [AI] cyfluthrin WP/m^2, and placed in a flour mill undergoing an experimental heat treatment and in an unheated office. Treated concrete was bioassayed by continually exposing adult *T. castaneum* for 0.5–120 h. The effect of heating time on insect mortality was not significant ($P \geqslant 0.05$). Except for *T. castaneum* exposed for 0.5 h, the percentage of beetle survival on unheated concrete was greater ($P < 0.05$) than survival on concrete that had been heated in the mill, indicating a possible beneficial effect on cyfluthrin toxicity due to heating. Results of these studies show that short-term exposures to high temperatures may have no appreciable effect on efficacy of either cyfluthrin WP or hydroprene, and combination treatments of heat plus either of these insecticides

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1. Introduction

The use of heat to disinfest structures was first reported in the entomological literature in the early 1900s (Dean, 1911, 1913), but the technique was not extensively used for most of the 20th century because conventional fumigant chemicals were the preferred option. However, with the impending loss of the fumigant methyl bromide, there is renewed interest in using heat treatments to control stored-product insects inside milling and processing facilities (Batchelor, 1998). Most of the guidelines for using heat treatments are listed in technical reports and training manuals (Hayman, 1985; Mills and Pederson, 1990; Imholte and Imholte-Tauscher, 1999), and not in the scientific literature. Several food processing companies in the United States use heat treatments on a calendar basis as a regular control measure, but results of these programs are rarely made public.

Within the United States, organophosphate chemicals such as malathion were commonly used as either surface treatments or crack and crevice applications to control insects inside milling facilities. The implementation of the 1996 *Food Quality Protection Act* may lead to the cancellation of many of these organophosphates, and few new reduced-risk low toxicity replacement insecticides are available for use inside mills and processing plants. Recent studies have demonstrated the efficacy of new diatomaceous earth (DE) formulations toward *Tribolium castaneum* (Herbst) and *Tribolium confusum* (du Val), common pests inside storage facilities, but efficacy toward most stored-product insects is severely compromised when relative humidity is high (Golob, 1997; Korunic, 1998; Arthur, 2000a; Fields and Korunic, 2000) or when food is available for exposed insects (Arthur, 2000b). The pyrethroid insecticide cyfluthrin can be used indoors on surfaces and in cracks and crevices for residual control; it is effective against both *T. castaneum* and *T. confusum* (Arthur, 1998a, b). The insect growth regulator (IGR) hydroprene (Gentrol) is labeled for indoor use as an aerosol and a general surface treatment, and is effective against late-instar *Tribolium* larvae (Arthur, 2001).

Combining or integrating heat with other control strategies would be beneficial for modern pest management approaches for controlling insects inside processing facilities. Laboratory and field studies (Dowdy, 1999; Fields et al., 1997) have shown that either the lethal temperatures or the amounts of DE required to kill certain insect species are reduced in combination treatments of heat and DE. Although cyfluthrin can be used inside mills and processing facilities, there are concerns regarding degradation of residues and loss of efficacy when using heat treatments in areas which have been previously sprayed with residual insecticides. The objective of this project was to conduct a laboratory study to determine if different heating regimes at 45°C and 55°C, temperatures that are attained during a typical heat treatment, will affect the efficacy of cyfluthrin wettable powder (WP) or hydroprene (Gentrol EC) applied to concrete. A separate field trial was conducted by exposing concrete treated with cyfluthrin WP inside a flour mill undergoing a heat treatment to determine effects of extreme temperatures on insecticide degradation and efficacy.

2. Materials and methods

2.1. Experiment 1—tests with cyfluthrin WP

Concrete treatment arenas were constructed by pouring a liquid slurry of ready-mix concrete to a thickness of about 5 mm into 84 standard $100 \times 15\,mm^2$ plastic Petri dishes. After 3 days, the hardened circular concrete disks were broken out of the Petri dishes. Label directions for the high rate of cyfluthrin WP specify mixing 19 g of 20% active ingredient [AI] WP into 3784 ml of water to cover an area of $94\,m^2$ (40 mg [AI] cyfluthrin WP/m^2). The area of the concrete disk was $62\,cm^2$, therefore the spray volume that was equivalent to the label rate was 0.25 ml. Solutions of cyfluthrin were formulated in a 5 ml and a 10 ml flask, and aliquots of 0.25 ml were dispensed to each of 63 disks by using a Badger 100 artists airbrush (Franklin Park, IL, USA) to mist the solution directly onto the disk. The remaining 21 untreated dishes served as controls.

The cyfluthrin-treated disks were allowed to dry for 1 day before they were heated. A set of nine treated and three untreated disks were heated for either 4, 8, or 16 h at 45°C or 55°C to simulate conditions in a food processing plant during a heat treatment, or not heated (ambient temperature treatment). Cyfluthrin-treated and untreated disks were heated in separate programmable ovens. After the heat treatments were concluded, the disks were removed from the ovens and placed on a laboratory counter top, and a plexiglass ring (75 mm diameter by 25 mm high) was set in the center of each disk, thus forming an arena on the treated surface. Bioassays were conducted with *T. castaneum*, the red flour beetle, obtained from pesticide-susceptible colonies reared at 27°C, 60% relative humidity (r.h.). Ten mixed-sex 1–2-week-old adults were exposed inside the container for 0.5, 1, or 2 h. At each exposure interval there were three treated disks (sub-samples) and one untreated control. Upon completion of the exposure interval, beetles on each disk were classified as knocked down (on their backs) or active (upright and running), then transferred to new Petri dishes lined with filter paper. The beetles were held without food for 1 week, classified as live (actively moving) or dead (no movement when prodded or capable of reflex movement only), then discarded. The disks on which the beetles were exposed were also held in the laboratory, and residual bioassays were conducted with new insects at 2 and 4 weeks post-treatment.

This process described above was repeated on three successive weeks until four separate replicates had been conducted. Residual bioassays were conducted at 2 and 4 weeks on each replicate. All treated and untreated disks for each replicate were stored in the same location in the laboratory where the bioassays were conducted. Temperature and relative humidity at that spot, as recorded on a thermograph, ranged from 24°C to 27°C and 40% to 55% r.h.

The test was analyzed as a randomized complete block, with replicates as blocks, and heat treatment and exposure interval as main effects. Knockdown after exposure and survival was a repeated measure because observations were made on the same group of insects. Because residual bioassays were conducted on the same set of treated dishes, this was also a repeated measure. Analyses were conducted using the GLM and ANOVA procedures of the Statistical Analysis System (SAS Institute, 1987). Means for treatments were separated using the Waller–Duncan k-ratio t-test where appropriate.

2.2. Experiment 2—tests with hydroprene

A second series of tests was conducted with a commercial formulation of hydroprene (Gentrol EC, 9.0% [AI] or ≈ 90 mg/ml), an IGR labeled in the USA as an indoor surface treatment. The label rate is 29.6 ml in 3.784 l of water to cover 141 m^2, therefore the equivalent amount needed for the area of the concrete arena was 0.0013 ml of Gentrol in a spray volume of 0.17 ml water (0.0013×90 mg/ml = 0.117 mg [AI]/62 cm^2 or 1.9×10^{-3} mg [AI]/cm^2). Some of the procedures for this study were similar to those described for the cyfluthrin study, but different testing techniques were necessary because last instar *T. castaneum* larvae were exposed on the concrete treated with hydroprene. These larvae had to be provided with food because cannibalization and predation would occur when adults began to emerge.

For each replicate, 28 concrete disks were made as previously described. The heat treatments were the same (seven combinations), and there were three sub-samples and an untreated control for each combination. Four replicate solutions were formulated by mixing 0.38 ml of Gentrol in 50 ml of tap water, and the airbrush was used to spray each of four dishes in each replicate with aliquots of 0.17 ml. After the concrete was heated, the disks were set on a laboratory counter, and a plexiglass ring was placed on each disk. Approximately 250 mg of flour medium were put in each ring along with 10 last instar mixed-sex red flour beetles. Food was provided for the larvae to ensure that adult emergence was not delayed or reduced in untreated controls, and that the first adults that emerged would not cannibalize the remaining larvae and pupae. After 2 weeks, adults were removed at weekly intervals for up to 4 weeks and classified as live or dead. Morphological deformities (incomplete wings and elytra, unsclerotized patches on the abdomen) were noted for live adults. Temperature and relative humidity in the treatment room where beetles were exposed fluctuated between 22°C and 28°C and 40% and 60% r.h. during the test.

Each replicate was treated separately. No residual tests were conducted because the larvae were continuously exposed for 4–6 weeks on the treated surface, depending on how long it took for adult emergence to be completed. The test was analyzed as a randomized complete block, with replicates as blocks, and heat treatment as the main effect. Analysis variables were the percentage of live adults that emerged from the exposed larvae, the percentage of those live adults with morphological deformities, and the percentage of adults that died either during or shortly after emergence.

2.3. Field trial

The field trial was conducted in a small experimental flour mill which has five floors and is part of the Department of Grain Science at Kansas State University. The mill had been heated on numerous occasions during the past few years to disinfest the facility and to conduct experimental trials. In this trial, natural gas heaters, with ducting equipment to distribute the heat within the facility, provided the heat source. Specific information about the types and location of heating equipment used in this trial is proprietary and cannot be disclosed.

Seventy concrete disks were prepared as described for Experiment 1, and treated with 2 mg [AI] cyfluthrin WP/m^2, which was 10% of the low label rate. This rate was used to simulate natural residual degradation and inactivation on a treated flour mill surface. There were five replicates of 14 dishes, each treated with a separate formulated solution. Half of the dishes in each replicate were placed in an unheated room and the other half at the middle of the third floor of the mill undergoing the heat treatment. A HOBO recording sensor (Onset Computers, Pocassett, MA, USA) was placed in the center of each group of disks to record temperatures attained during the heat treatment. The heaters were turned on at 1800 h on 25 June, turned down at about 2300 h on 26 June, and turned off at about 0800 h on 27 June.

Disks from each replicate were removed from the mill and the unheated control room at intervals of 0, 8, 16, 24, 34, 40, and 52 h after initiation of the heat treatment, and returned to the laboratory. Plexiglass containers described in Experiment 1 were placed on each disk, and bioassays were conducted by exposing 10 mixed-sex 1–2-week-old adult *T. castaneum* inside each ring. At intervals of 0.5, 1, 1.5, 2, 2.5, 3, 5, 8, 18, 24, 48, 72, 96, and 120 h, the number of beetles that were either active or knocked down on each dish were assessed. Analyses were conducted on the percentage of active beetles, because the beetles that were knocked down on the treated surface would likely die if they could not escape exposure. Results were analyzed using the GLM and *t*-Test Procedures of SAS (SAS Institute, 1987) to determine significant differences with exposure duration and between disks held in the unheated room versus the heated mill.

3. Results

3.1. Experiment 1—exposure studies with cyfluthrin WP

The main effects exposure interval ($F = 361.5$, df = 2, 60), heat treatment regime ($F = 27.9$, df = 6, 60), the repeated measures initial knockdown and survival ($F = 10.3$, df = 1, 63), and residual bioassays ($F = 102.4$, df = 2, 126) were all significant ($P < 0.01$). All interactions except for exposure interval × heat treatment and exposure interval × heat treatment × residual bioassay were also significant ($P < 0.05$). Results were separated by exposure interval and residual bioassay, and analyzed for differences between treatments.

Knockdown after 0.5 h of exposure on the cyfluthrin-treated concrete ranged from 34.7 ± 9.1% to 58.0 ± 8.9% at week 0, with no difference ($P \geqslant 0.05$) between heat treatment regimes (Fig. 1A). However, survival was greater on unheated concrete than on concrete heated at 45°C and 55°C after being treated with cyfluthrin WP (Fig. 1B). As the exposure interval was increased to 1 and 2 h, knockdown increased and survival decreased, with no difference ($P \geqslant 0.05$) among heat treatments (Fig. 1C and D). At the 2 h exposure interval, knockdown was greatest on concrete heated at 55°C compared to unheated concrete and concrete heated at 45°C (Fig. 1E). Survival of these beetles did not exceed 25.4 ± 10.3%, and there was no difference in survival ($P \geqslant 0.05$) among treatments (Fig. 1F).

At the 2-week residual bioassays, knockdown of beetles exposed for 0.5 h was greater on concrete heated for 8 and 16 h at 45°C and 55°C compared with the other three treatments

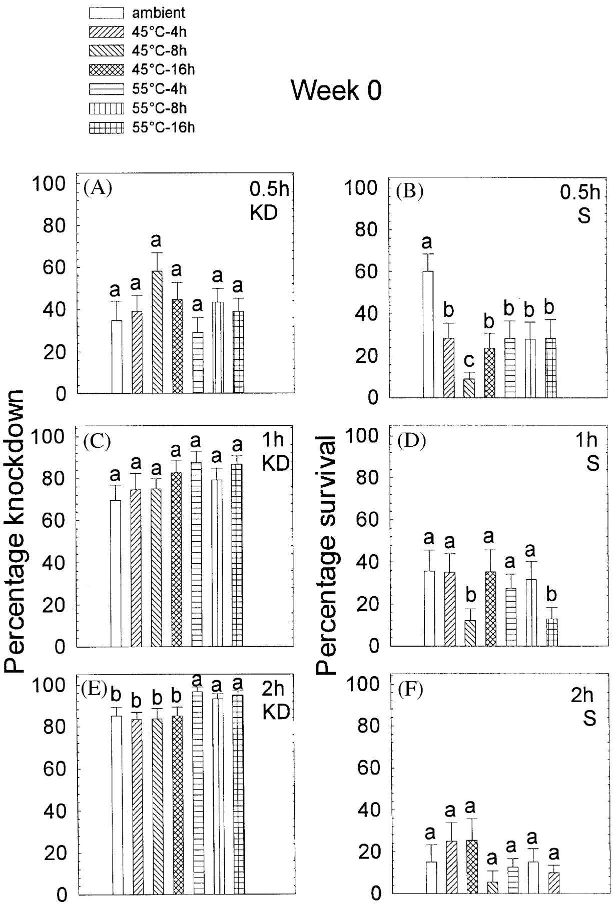

Fig. 1. Percentage knockdown (KD; Fig. 1A, C and E; mean ± SEM) of adult *T. castaneum* exposed for 0.5, 1, or 2 h on concrete that had been heated for 4, 8, or 16 h at either 45°C or 55°C, or left unheated after being treated with cyfluthrin WP at the rate of 40 mg [AI]/m^2. After exposure, beetles were removed from the treated concrete and survival was assessed after 1 week (S; Fig. 1B, D and F; percentage mean ± SEM). Means for knockdown and survival that are denoted with the same letter are not significantly different ($P \geqslant 0.05$, Waller–Duncan k-ratio t-test).

(Fig. 2A), but survival was not significantly different ($P \geqslant 0.05$) among treatments (Fig. 2B). Knockdown after 1 h of exposure was not significantly different ($P \geqslant 0.05$) among treatments (Fig. 2C), but survival was greatest on unheated concrete and least on concrete that was heated for 16 h at 45°C and 55°C (Fig. 2E). Knockdown of beetles exposed for 2 h was 82.7 ± 4.9–96.5 ± 2.4% (Fig. 2E), survival did not exceed 26.7 ± 8.6% (Fig. 2D), and there were no differences in knockdown or survival among treatments ($P \geqslant 0.05$).

At the 4-week bioassays, knockdown at all exposure intervals decreased and survival increased compared with knockdown and survival at 0 and 2 weeks (Fig. 3). Knockdown after 0.5 h of exposure varied among treatments, with no consistent pattern (Fig. 3A). Survival also varied among treatments, with the greatest survival occurring on unheated concrete and concrete that

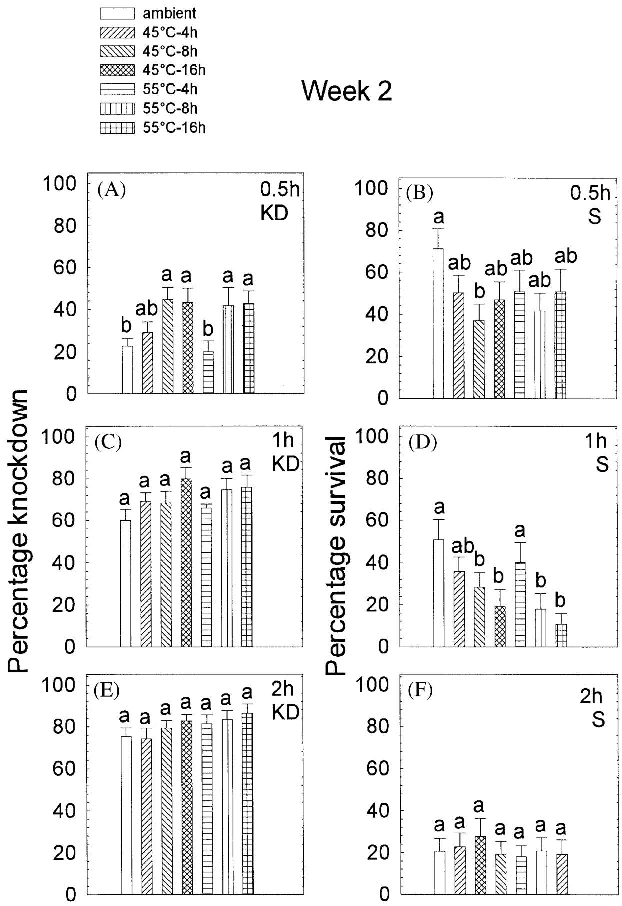

Fig. 2. Percentage knockdown (KD; Fig. 2A, C and E; mean ± SEM) of adult *T. castaneum* exposed for 0.5, 1, or 2 h on concrete 2 weeks after being treated with cyfluthrin WP at the rate of 40 mg [AI]/m^2 and heated for 4, 8, or 16 h at either 45°C or 55°C, or left unheated. After exposure, beetles were removed from the treated concrete and survival was assessed after 1 week (S; Fig. 2B, D and F; percentage mean ± SEM). Means for knockdown and survival that are denoted with the same letter are not significantly different ($P \geqslant 0.05$, Waller–Duncan k-ratio t-test).

had been heated for 4 h at 45°C and 55°C (Fig. 3B). When beetles were exposed for 1 h, knockdown was lowest on unheated concrete compared with the other treatments (Fig. 3C). Survival was also greater on unheated concrete, and there appeared to be a pattern of decreased survival as the heating time increased at both 45°C and 55°C (Fig. 3D). There was no difference in knockdown ($P \geqslant 0.05$) among treatments when beetles were exposed for 2 h (Fig. 3E), but survival was lowest on concrete heated for 16 h at 45°C and 8 and 16 h at 55°C (Fig. 3D).

3.2. Experiment 2—exposure studies with hydroprene

In this study where last instar *T. castaneum* larvae were exposed on concrete treated with hydroprene, there were significant differences between untreated controls and the heat

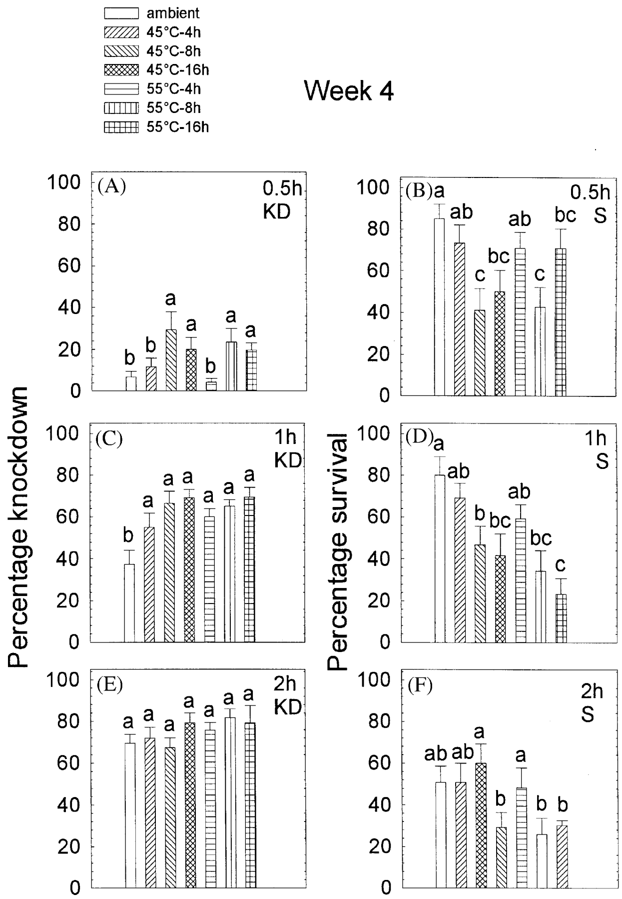

Fig. 3. Percentage knockdown (KD; Fig. 3A, C and E; mean ± SEM) of adult *T. castaneum* exposed for 0.5, 1, or 2 h on concrete 4 weeks after being treated with cyfluthrin WP at the rate of 40 mg [AI]/m^2 and heated for 4, 8, or 16 h at either 45°C or 55°C, or left unheated. After exposure, beetles were removed from the treated concrete and survival was assessed after 1 week (S; Fig. 3B, D and F; percentage mean ± SEM).

treatment regimes ($P<0.05$) with respect to the percentage of live emerged adults, the percentage of those adults with deformities, and the percentage of dead adults. However, there were few differences among the seven heat treatment regimes ($P \geqslant 0.05$, Table 1). The percentage of live adults in untreated controls was 85.4 ± 3.8%, few of which were deformed. In contrast, the percentage of live adults in the heat treatments ranged from 42.5% to 60.0%, with 25.9–50.0% of those live adults exhibiting visual deformities. There were few dead adults in the untreated controls, while the number of dead adults in the heat treatments ranged from 12.5% to 45%. Nearly all of the dead adults were grossly deformed, with either missing or incomplete body parts or exhibiting both pupal and adult characters, and were often unable to completely emerge from the puparium. Heating the concrete after it was treated appeared to have little adverse effect on the efficacy of hydroprene.

Table 1
Percentage (means ± SEM) of live adult *Tribolium castaneum*, live adults with morphological deformities, and adults that were unable to emerge or died shortly after emergence (dead adults). Insects were exposed as last-instars on untreated concrete disks or concrete disks treated with hydroprene at the rate of 1.9×10^{-3} mg [AI]/per cm^2. The treated concrete was either heated for 4, 8, and 16 h at 45°C or 55°C, or not heated (ambient)[a]

Treatment	Live adults (%)	Deformed live adults (%)	Dead adults (%)
Untreated	85.4±3.8 a	0.7±0.4 b	2.1±0.9 b
Ambient	60.0±4.9 b	29.0±12.5 a	25.0±8.0 a
45°, 4 h	69.2±11.9 ab	35.9±10.1 a	12.5±4.8 a
45°, 8 h	65.0±6.2 ab	25.9±11.1 a	18.3±4.0 a
45°, 16 h	70.0±7.9 ab	27.3±5.3 a	20.8±7.5 a
55°, 4 h	50.8±12.1 b	27.7±9.3 a	40.0±16.5 a
55°, 8 h	45.8±16.5 b	50.0±20.9 a	44.2±14.9 a
55°, 16 h	42.5±14.6 b	27.6±16.4 a	45.0±19.1 a

[a] Means within columns followed by the same letter are not significantly different ($P \geq 0.05$, Waller–Duncan k-ratio t-test).

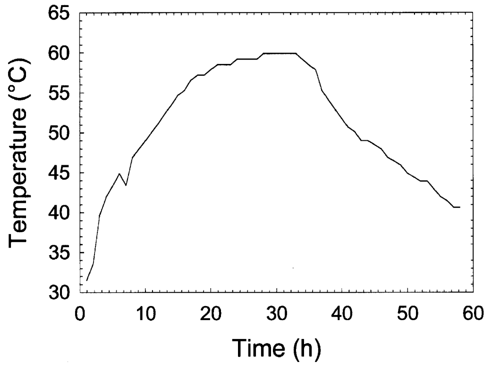

Fig. 4. Temperature during the heat treatment at the site within the flour mill where the disks treated with cyfluthrin WP at the rate of 2 mg [AI]/m^2 were placed. Natural gas heaters were turned down after about 29 h, and turned off after about 40 h.

3.3. Field trial

The temperature at the site within the mill where the treated disks were placed rose to a maximum of about 60°C, then began to drop quickly once the heaters were turned down and eventually turned off (Fig. 4). Main effect treatment (heated versus unheated, $F = 37.1$, df = 1, 48) and the repeated measure exposure interval ($F = 143.3$, df = 13, 616) were both significant ($P < 0.01$) but the main effect time at which concrete disks were removed from the heated flour mill was not significant ($F = 1.0$, df = 45, 48, $P = 0.45$) with respect to survival, and all data for time were combined. Except for *T. castaneum* exposed for 0.5 h, more beetles survived on the unheated disks in the control room versus the heated disks in the mill, indicating a beneficial effect of heating on cyfluthrin toxicity, instead of a negative effect (Table 2).

Table 2
Percentage (mean ± SEM) of *Tribolium castaneum* surviving after 0.5–120 h of exposure on concrete disks treated with 2 mg [AI] cyfluthrin WP/m^2. The treated concrete disks were either put inside the four mill that was being heated or held in an unheated room, and had been removed from the mill and the unheated control room approximately 0, 8, 16, 24, 34, 40, and 52 h after initiation of the heat treatment[a]

Time (h)	Location	
	Mill	Room
0.5	94 ± 3.6	100 ± 0.3
1	85 ± 4.3	99 ± 0.8
1.5	65 ± 4.7	88 ± 2.9
2	45 ± 4.8	78 ± 5.6
2.5	39 ± 4.8	70 ± 5.9
3	34 ± 4.4	68 ± 5.8
5	25 ± 3.4	57 ± 7.1
8	13 ± 3.5	44 ± 6.9
18	5 ± 2.2	40 ± 6.9
24	12 ± 3.6	53 ± 6.9
48	5 ± 1.8	52 ± 6.2
72	10 ± 3.1	40 ± 4.8
96	10 ± 2.6	35 ± 5.0
120	0.3 ± 0.3	32 ± 5.1

[a] The time at which adult *T. castaneum* were removed from either the heated mill or the unheated control room was not significant ($P \geqslant 0.05$) for the percentage of active beetles, and all data for time were combined. Except for *T. castaneum* exposed for 0.5 h, the percentage of active beetles was greater on concrete disks held in the unheated room than on disks in the heated mill ($P < 0.05$), PROC *t*-test, SAS Institute, 1987.

4. Discussion

The effects of temperature on insecticidal residues have been documented in many studies where insecticides have been applied to stored grain. The degradation rates of most organophosphate insecticides generally increase very rapidly with increases in temperature and either grain moisture content or relative humidity (Snelson, 1987; Arthur et al., 1992). Although pyrethroid insecticides are generally more stable than organophosphates, increased temperatures will also enhance pyrethroid degradation (Noble et al., 1982). In one test where cyfluthrin was applied to wheat and held at 12% m.c., half-lives of different isomers ranged from 41 to 114 weeks at 25°C compared with 28–69 weeks at 35°C (Noble and Hamilton, 1985). The upper thermal heat limits that will cause death of most stored-product insects are about 50–60°C (Fields, 1992), which are much higher than the ranges normally evaluated in studies with grain protectants.

The adverse impact of temperatures attained during flour mill heat treatments may be minimized because the treated substrates are usually subjected to those temperatures for only 20–30 h (Dowdy, 1999). In fact, results of the two laboratory experiments show that short-term exposures to high temperatures may have had a positive effect on toxicity of both cyfluthrin and hydroprene when *T. castaneum* were exposed on treated concrete. Further, in the field trial, survival of *T. castaneum* was actually greater on unheated compared to heated concrete treated with 90% of the low label rate of cyfluthrin. Although the reasons for this result were not evaluated in the experiment, it is possible that the high temperatures volatilized residues from the concrete substrate that were more readily absorbed by *T. castaneum*.

Insecticides used as crack and crevice or spot treatments in combination with heat may be beneficial when the insecticides flush insects from hidden areas out into the open where they are more accessible to the heat treatment. Also, there may be some toxicological benefits of combination treatments although any such effects may depend on the specific insecticide, because organophosphates generally have a positive increase in toxicity with increased temperatures while toxicity of most pyrethroids decreases with temperature (Johnson, 1990). In addition, repellent activity from pyrethroids (Watters et al., 1983) could be beneficial, though there is little documentation of this claim in field studies. Regardless, it appears that heat used in combination with residual insecticide treatments has no negative impact on insecticidal efficacy, and combination treatments such as those reported here may be effective alternatives to methyl bromide for disinfesting milling facilities.

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