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Initial and delayed mortality of late-instar larvae, pupae, and adults of *Tribolium castaneum* and *Tribolium confusum* (Coleoptera: Tenebrionidae) exposed at variable temperatures and time intervals ☆

Frank H. Arthur*

USDA–ARS, Grain Marketing and Production Research Center, 1515 College Avenue, Manhattan, KS 66502, USA

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Abstract

Late-instar larvae, pupae, and adults of *Tribolium castaneum* (Herbst), the red flour beetle, or *Tribolium confusum* (DuVal), the confused flour beetle, were exposed for variable durations at 36–54 °C. Beetles were placed in laboratory ovens set at a baseline of 27 °C, the temperature was increased by 0.1 °C per minute until the target temperature was achieved, and beetles were then held for specified exposure durations. There was no mortality after initial exposure or after a 1-week holding period of any life stage of *T. castaneum* or *T. confusum* exposed for 32 h to 36, 39, or 42 °C. At 45 °C, there was no initial mortality of either species exposed for different time intervals except for those exposed for 28 h. However, there was a significant increase in mortality after the 1-week holding period of those beetles exposed initially for at least 16 h to 45 °C. There was a sharp increase in mortality after the initial exposures of 4 h at 48 °C; mortality of *T. confusum* larvae was 90.0 ± 5.7% but was only 10.0 ± 10.0% for larvae of *T. castaneum*, and no pupae of either species were dead. All life stages of both species were killed after the initial exposure of 12 h, and 1-week mortality of beetles exposed for 4 and 8 h was generally greater than initial mortality. At 51 and

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*Corresponding author. Tel.: +1 785 776 2783; fax: +1 785 537 5584.
E-mail address: arthur@gmprc.ksu.edu (F.H. Arthur).

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54 °C, 2- and 1-h exposures, respectively, killed all life stages of each species. Mortality in conditions of gradual temperature increase was less than previous studies with sudden temperature increases.
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1. Introduction

Heat treatments to disinfest milling, processing, and some storage facilities have received increased interest in recent years (Mahroof et al., 2003a, b; Roesli et al., 2003), and with the impending removal and restrictions on the usage of methyl bromide, this trend is expected to continue. As the efficiency of heat treatments increases, the total time the mill must be shut down and be out of operation will also be reduced, which could lead to even greater adoption of heat treatments by the milling industry. However, there are still some unanswered questions regarding penetration of heat into milling equipment and the temperatures and exposure intervals required to kill insects inside equipment or other inaccessible areas.

Tribolium castaneum (Herbst), the red flour beetle, and *Tribolium confusum* (DuVal), the confused flour beetle, are major pests of stored products, and when insecticides have been tested against these species, the order of susceptibility often depends on the specific insecticide and formulation that is being evaluated (Arthur, 1998a, b, 2000). Most stored-product beetles are killed within hours after they are exposed to temperatures of 50 °C or more (Fields,1992), and at lower temperatures, mortality is often related to the duration that the insects are exposed (Dowdy, 1999; Dowdy and Fields, 2002; Mahroof et al., 2003b). However, during actual heat treatments, temperatures profiles within the structural facility can be variable, and the time insects are exposed to the lethal temperatures can differ depending on their location within the facility (Roesli et al., 2003). Species variability could also be important in conferring susceptibility to lethal temperatures.

Mahroof et al. (2003b) conducted detailed modeling studies of the response of different life stages of *T. castaneum* to temperatures ranging from 44 to 60 °C. In these tests, insects were taken from laboratory rearing conditions of 27 °C and immediately exposed to higher temperatures. Upon completion of the exposure interval, the insects were held at 27 °C for an additional 24 h for mortality assessments. Previous studies with contact insecticides have shown a delayed effect when *T. castaneum* and *T. confusum* were exposed to either the pyrethroid cyfluthrin or to diatomaceous earth (Arthur, 1998a, b, 2000). Also, in actual heat treatments, it can take several hours for facilities to reach target temperatures, and short-term acclimation of insects could occur during this time. The objectives of this test were to determine: (1) mortality under conditions of gradual temperature increases to desired target temperatures, (2) if delayed mortality would result after exposure for different time intervals to particular temperatures, and (3) differential responses of species or life stages at each target temperature.

2. Materials and methods

Tests were conducted using four Salvis® programmable ovens (Cole-Palmer Instrument Company, Vernon Hills, IL, USA) that would increase temperatures by 0.1 °C per minute from a starting set point of 27 °C. In individual trials, one oven would be used as the control and remained at 27 °C, and the other three were used for the individual replicates. With each successive trial, a different oven was used as the control. Target temperatures for the exposure studies were 36, 39, 42, 45, 48, 51, and 54 °C, and the durations required to reach these temperatures were 90, 120, 150, 180, 210, 240, 270 min, respectively. These heating rates are typical of what could be encountered in an actual heat treatment of a milling facility.

Life stages evaluated in the test were 4-week-old larvae, pupae, and 1–2-week-old adults. Twenty individuals of each life stage and species were exposed in separate vials (6 total) containing about 100 mg of wheat flour. After exposure the vials were removed from the oven, the beetle stages were sifted from the flour, mortality was assessed, and the beetles and the flour were returned to the vials. The vials were in turn placed on a waffle-type grid inside a 26 × 36.5 × 15 cm plastic box, which contained saturated NaBr to maintain an approximate relative humidity of 57–60% (Greenspan, 1977). The humidity box was put inside an incubator set at 27 °C. After 1 week, the vials were removed from the incubator and the insects were again sifted from the flour. Mortality was assessed by examining each individual for movement. Most of the dead larvae and pupae were blackened and shriveled, while dead adults were on their backs and immobile. Some of the live exposed larvae and pupae remained in that stage, while the remaining live immatures had transitioned to the next life stage. After mortality was assessed and recorded the beetles and the flour were discarded.

Three replicates of initial trials at 36, 39, and 42 °C were conducted at 32 h of exposure; if no insects were dead immediately after exposure or after the 1-week holding period, no further tests were done. Similarly, at the higher temperatures of 51 and 54 °C, three replicates of trials were conducted at exposures of 1–4 h; if all insects were dead after initial exposures, no further tests were conducted. At the midrange temperatures of 45 and 48 °C, final hours set for the exposure studies were based on results of preliminary observations. For each temperature, separate sets of insects were used for the different exposure intervals, i.e. the number of vials of insects of each species and life stage that were put inside the oven was based on the number of exposure intervals. Control mortality at 27 °C was negligible throughout the test, and no corrections were necessary for treatment comparisons.

Data were analyzed separately for each temperature, with life stage, species, and exposure interval as main effects for the variables initial mortality and mortality after the 1-week holding period. The General Linear Models procedure (GLM, SAS Institute 2002) was used for data analysis, and to separate means when main effects were significant. When mean separation tests were performed, values were transformed by square root to normalize variance. Initial mortality and 1-week mortality were compared when appropriate using the *t*-test procedure of SAS.

3. Results and discussion

There was no initial mortality or mortality after the 1-week post-treatment holding period of larval, pupal, or adult stages of *T. castaneum* or *T. confusum* exposed for 32 h to 36, 39, or 42 °C. These results for mortality of *T. castaneum* exposed at 42 °C were different from those of Mahroof et al. (2003b), who reported lethal times to kill 50% of the population (LT_{50}s) as 2645, 2186, and 2371 min (44.1, 36.4, and 39.5 h), for adults, pupae and late-instar larvae, respectively. One possible reason for these discrepant results is that Mahroof et al. (2003b) exposed *T. castaneum* in incubators already set at 42 °C and other test temperatures, while in the current study the temperature was increased to the target temperature by 0.1 °C per minute. This gradual increase could be analogous to what normally occurs at the start of a commercial heat treatment, so in the current study the test insects could have become slightly acclimated to the temperature increase, in contrast to the methods used to expose *T. castaneum* in Mahroof et al. (2003b).

At 45 °C, initial mortality was significant for main effects exposure ($F = 10.6$, df = 6, 84, $P<0.01$) and life stage ($F = 5.5$, df = 2, 84, $P<0.01$), and the exposure × life stage interaction ($F = 5.5$, df = 12, 84, $P<0.01$), but not for main effect species ($F = 2.4$, df = 1, 84, $P = 0.12$), or any of the other interactions ($P \geqslant 0.05$). However, the significant ANOVA was because there was no initial mortality of any life stage of either species except for the exposures at 28 h. These data for life stage were combined, and initial mortality for *T. castaneum* and *T. confusum* exposed for 28 h at 45 °C was 11.1±11.1% and 21.1±10.8%, respectively. In the analysis for 1-week mortality, main effects exposure ($F = 35.7$, df = 6, 84, $P<0.01$) and species ($F = 4.6$, df = 1, 84, $P = 0.04$) were significant, but not life stage ($F = 3.0$, df = 2, 84, $P = 0.06$), or any interaction ($P \geqslant 0.05$). Data for life stage were combined and analyzed for differences between initial and 1-week mortality (Table 1). There was a significant increase in mortality after the 1-week holding period of *T. castaneum* and *T. confusum* exposed for 16 h or more (Table 1), indicating a latent effect from the exposure. Comparisons by species indicate lower mortality of *T. castaneum* versus *T. confusum* only at the 32-h exposures ($P<0.05$) despite the apparent difference in mean values, in part because of the standard errors associated with those means.

Results from Mahroof et al. (2003b) show LT_{50}s of 53.5–547.5 min and LT_{99}s of 430.7–780.4 min for *T. castaneum* exposed to 46 °C, depending on the life stage, which is far less than the results in this study for exposures at 45 °C. In addition, in their tests mortality was assessed after a 1-day holding period, while in this test mortality was assessed upon completion of the exposure period and after a 1-week holding period. The discrepant results could again be

Table 1
Percentage initial mortality and mortality after 1 week (mean±SEM) of *T. castaneum* and *T. confusum* exposed for 4–32 h to 45 °C[a]

Hours	*T. castaneum*		*T. confusum*	
	Initial	1-week	Initial	1-week
4	0±0.0	2.2±2.2	0±0.0	0±0.0
8	0±0.0	0±0.0	0±0.0	0±0.0
12	0±0.0	1.1±1.1	0±0.0	8.9±5.1
16[b]	0±0.0	14.7±7.3	0±0.0	33.3±11.4
24[b]	0±0.0	28.9±10.3	0±0.0	46.7±15.2
28[b]	11.1±11.1	45.6±12.3	21.1±10.8	64.4±13.7
32[b]	0±0.0	80.0±7.8	0±0.0	97.8±2.2

[a]No significant difference in initial mortality and 1-week mortality of larvae, pupae, or adults of either species ($F = 3.0$, df = 2, 84, $P = 0.06$); data for all life stages were combined for further analysis.

[b]Mortality after 1 week was significantly greater ($P<0.05$, *t*-test in SAS) than initial mortality for both *T. castaneum* and *T. confusum* at these exposure times.

related to the differing methods of exposure. These results also show a delayed effect of heat, similar to other studies in which *T. castaneum* or *T. confusum* were exposed on concrete surfaces treated with the conventional pesticides cyfluthrin (Arthur, 1998a, b, 1999) and with inert dusts (Arthur 2000; Arthur and Puterka, 2002). This delayed mortality effect could be very important when assessing the results of heat treatments and other forms of lethal temperatures used for insect control.

There was a sharp increase in mortality of all life stages of both species as the exposure temperatures were increased. At 48 °C, initial mortality was significant for main effects exposure ($F = 113.2$, df = 2, 36, $P<0.01$), life stage ($F = 36.3$, df = 2, 36, $P<0.01$), and species ($F = 14.1$, df = 1, 36, $P<0.01$), and all interactions were significant ($P<0.05$). After the initial exposures of 4 h, mortality of *T. confusum* larvae was 90.0 ± 5.7% but only 10.0 ± 10.0% for *T. castaneum*, and no pupae of either species were dead (Table 2). The difference between the two species for late-stage larvae could be an indication of a difference in susceptibility between the two species regarding their response to lethal temperatures.

The lack of mortality of *T. castaneum* pupae is similar to the results presented by Mahroof et al. (2003b); at 46 °C pupae were the most tolerant life stage, but at temperatures of 50 °C or more early instars were the most tolerant life stage. Exposures of eggs and early instars were not done in the current study because the intent was to provide information regarding effects of acclimation and latent heating. At temperatures of 51 and 54 °C, complete mortality of all life stages should occur in a matter of hours, and even the most tolerant stages should be killed during a normal heat treatment. This possible shift in susceptibility of life stages at different temperatures may be another effect that should be considered when examining the results of exposure studies done at extreme temperatures. Initial mortality of *T. confusum* exposed for 8 and 12 h at 48 °C was 100%, while mortality of *T. castaneum* was 100% except for the pupal stage. Mortality of *T. castaneum* larvae and pupae of both species that were exposed for 4 h increased after the 1-week holding

Table 2
Percentage initial mortality and mortality after 1 week (mean ± SEM) of *T. castaneum* and *T. confusum* late-instar larvae, pupae, and adults[a] exposed for 4, 8, and 12 h to 48 °C

	4 h[b]		8 h		12 h	
	Initial	1-week	Initial	1-week	Initial	1-week
T. castaneum						
Larvae	10 ± 10.0b	30.0 ± 20.9b	100 ± 0.0	100 ± 0.0	73.3 ± 26.7	100 ± 0.0
Pupae	0 ± 0.0b	46.7 ± 23.3b	83.3 ± 16.7	83.3 ± 23.7	100 ± 0.0	100 ± 0.0
Adults	100 ± 0.0a	100 ± 0.0a	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0
T. confusum						
Larvae	90.0 ± 5.7a	90.0 ± 5.7a	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0
Pupae	0 ± 0.0b	33.3 ± 33.3b	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0
Adults	100 ± 0.0a	100 ± 0.0a	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0

[a]Means for initial and 1-week mortality of larvae, pupae, and adults, within each column for each species, that are followed by different letters were significantly different ($P<0.05$, Waller–Duncan k-ratio t-test, SAS).

[b]For this exposure interval only, mortality of *T. castaneum* pupae 1 week after exposure was significantly different from initial mortality ($P<0.05$, t-test, SAS).

period (Table 2); however, all of the dead *T. confusum* pupae were in one replicate. Mortality of *T. castaneum* larvae exposed for 4 h increased to $46.7 \pm 23.3\%$, while mortality of pupae exposed for 8 and 12 h was $83.3 \pm 16.7\%$ and 100%, respectively.

At 51 °C, all life stages of *T. confusum* were dead after they were exposed for 1 h, while initial mortality of late-instar larvae, pupae, and adult *T. castaneum* was $83.3 \pm 8.8\%$, $96.7 \pm 3.3\%$, and 100%, respectively. Mortality of late-instars and pupae was 100% after the 1-week holding period. All *T. castaneum* life stages were dead after the initial 2-h exposure. At 54 °C, all life stages of both species were dead after they were exposed for 1 h. In general, exposure for 1 h to temperatures of 50 °C or more is sufficient to kill stored-product insects (Fields 1992), and target temperatures for most standard heat treatments are in the range of 50–60 °C (Imholte and Imholte-Tauscher, 1999). Uneven and unequal distribution of heat may occur during an actual field treatment, and pockets can remain below the target temperatures so that insects may not be exposed for a sufficient time interval for complete kill (Roesli et al., 2003). Latent mortality could result when *T. castaneum* and *T. confusum* are exposed at 45–50 °C, depending on the actual time interval. There is an obvious interaction between exposure temperature and life stage, and potential differences among life stages of *T. castaneum* and *T. confusum*, and these factors may be important in short-term exposures of a few hours or less.

In tests by Wright et al. (2002), large larvae of *Trogoderma variable* Ballion were the most heat-tolerant stage. In the current test, there was some slight difference between susceptibility of large larvae, pupae, and adults of *T. castaneum* and *T. confusum*, with pupae being the most tolerant stage. Mahroof et al. (2003b) had identified the early instars of *T. castaneum* as the most susceptible stage at temperatures exceeding 50 °C, while pupae were more tolerant at lower temperatures. The methods whereby insect species or life stages are exposed to lethal temperatures could be an important factor in determining susceptibility of individual life stages. However, all life stages of *T. castaneum* and *T. confusum* die quickly at temperatures above 50 °C, the target temperatures for most heat treatments. Any differences would be important primarily in those instances where sub-lethal or sub-optimal exposures occur during a heat treatment. Also, the results of this study show that *Tribolium* species may respond differently to gradual temperature increases than to sudden temperature increases.

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