Development and validation of a simple heat-accumulation model for predicting mortality of first instars of Tribolium castaneum (Herbst) exposed to elevated temperatures

Bhadriraju Subramanyam\* A. Paul W. Fine? Rizwan Malik

Abstract

First instars of the flour beetle, Tribolium castaneum (Herbst), are more resistant than eggs, adult, and 4th instars to static and variable temperatures (30-40°C) slightly used during heat treatments. Therefore, heat treatments aimed at controlling flour beetles should eliminate all life stages. Fifth instars of T. castaneum were exposed to four different constant temperatures of 50, 54, 58, and 60°C to generate time-mortality relationships. Almost 157 of the 255 observations of 7°C cooler at the study temperatures were used for model development, and the remaining 98 observations (39%) were used for model validation. The temperature-time data were expressed in degree minutes above a base temperature. Freezing mortality at corresponding degree minutes above a certain base temperature was fitted to normal, logistic, and exponential equations. The effect of constant temperatures was very similar. The equations that gave the narrowest 95% confidence limits (Chi-square/df, T, and Td values) were chosen for assessing degree minute units. The CLD model of a base temperature of 41.1°C, i.e., the maximum 95% CL, 99.9% CL, and 99% of observed first instars were 218.0 (124.8-180.4) and 345.2 (347.4-348.2), respectively. The CLD-based degree minute model underestimated mortality by 29%, but explained about 95% of the variation in observed mortality data. The ability of the degree-minute model in accurately predicting mortalities of T. castaneum first instars during heat treatments of a food-processing facility still remains to be verified.

Keywords: Heat treatments; Basic temperature; Degree minutes; Modeling

Introduction

The phase-out of methyl bromide, an atmospheric ozone- depleting gas, in the United States and Europe (Makdisi and Carys 1995) has resurrected interest in using heat- processing facilities. The use of high temperatures for disinfection purposes is termed "heat sterilization" or "heat treatment." During heat treatments, the ambient air of the entire food-processing facility or a part of it is raised to temperatures lethal to insects. Typically, temperatures in the range 50-60°C are used for disinfecting food-processing facilities, and it is important to hold these high temperatures for a period of 24-36 h to ensure proper heat penetration into critical and reservoirs, and non-entrapment of insects gener- ally reside or hide. The target temperature for effective dis- infestation should be at least 50°C (Malouf et al. 2005; Rowell et al. 2003; Wright et al. 2003). Elimination and removal of all grain and grain products are important during heat treatments, because these materials are poor conductors of heat, and insects present in them could escape the lethal effects of high temperatures.

Heat accumulation or degree day models have been developed for several stored-product insects to predict completion of development under field conditions (Sheehan et al. 1991; Ahamed and Xu 1995; Sepulveda et al. 1995), but their use in stored product processing has been rather limited. Heat accumulation methods are simple to use and predict times when insects development will be completed. A novel proposal would be to use a similar concept such as a degree hour or degree minute model for predicting mortality of insects exposed to high temperatures.

In order to develop a degree hour or degree minute model, it is essential to determine time-mortality relationships for insects at constant temperatures (Bhatia and Fields 1995; Wright et al. 2002). A true temperature to accumulate degree hours or minutes is also essential for such a model- ing approach. A portion of the present investigation (10, 20, 30, 35, 40, 45, 49.9, and 50°C) is assumed dead when a certain number of degree hours or minutes is accumulated. Hence, the applicability of this approach will depend on how well mortality can be predicted under field conditions. Therefore, validation of such models is central to making them practicable. Wright et al. (2002) developed a degree minute model using constant temperature data of 50, 52, 54, or 55°C for predicting mortality of large larvae of Tribolium confusum. The lower temperatures for accumulating degree minutes, and the intermediate antilog values of the lower regression of mortality expressed as the inverse of the standard normal deviates against degree minutes were different as each of the four temperatures.
Despite these differences, Wright et al. pooled data across 52, 54 and 56°C to describe the relationship between mortality and degree minutes. No statistical or biological link was given for pooling data across the three temperatures. In addition, the study by Wright et al. involved a series of transformations and densities that are time consuming.

In this paper, we present a simpler approach for developing a degree minute model for predicting mortality of first instar of the red flour beetle, Tribolium castaneum (Herbst) (Coleoptera: Tenebrionidae). A post commonly associated with food-processing facilities worldwide (Sinha and Watters, 1985). The model was also validated using independent data. First instars of T. castaneum were held for 10 days at 30°C and then exposed to 45°C, 50°C, 55°C, and 60°C for 10 minutes. The data were analyzed using a chi-square test to determine if there was a significant difference in mortality between the different temperatures. The results showed that the mortality increased significantly with increasing temperature.

Model development

First instars of T. castaneum (mean ± SE wt: 0.12 ± 0.01 mg; n = 15) were isolated from the bleached wheat flour − 3% (by weight) breeder's yeast diet using a 250-μm screen, and counted under a stereomicroscope. First instars were transferred to separate square plastic boxes (4 cm × 4 cm × 4 cm) with perforated lids covered with 600-μm wire mesh screens for air diffusion. Each box held a mean ± SEM (n = 20) of 300 ± 3 mg of bleached wheat flour and 20 first instars. Boxes with instars were placed in growth chambers (Model 1-36 VL; Percival Scientific, Perry, Iowa, USA) at 30, 34, 38, and 40°C for establishing time-mortality relationships. The control mortality consisted of boxes with first instars that were kept in a chamber at 28.2 ± 0.2°C and 44.5 ± 3.3% rh. There was a separate control treatment for each temperature.

Boxes with first instars remained at different time periods at each temperature, were transferred to 150-ml plastic containers, each containing 80 g of whole-wheat flour plus yeast (3% by weight). Mortality of first instars was calculated from those that failed to emerge into adults. At each temperature−time combination, five boxes were removed. Five boxes in the control treatments were removed at the same time intervals used for those in temperature treatments, to maintain natural mortality of instars.

Natural mortality of first instar in the control treatments was 9%. Therefore, live-mortality data at the four constant temperatures were not corrected for natural mortality. There was a total of 255 time−mortality observations across all four temperatures. About 62% of the observations (n = 157) were used to develop the degree minute model, and the remaining 38% of the observations (n = 98) were used for model validation. Data at different temperature and time combinations were converted to degree minutes (D) above a base temperature (B) using equation 1.

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D = (T - B) \times M
\]

where, T is temperature in °C and M is time in minutes. For the first instar, B was chosen as 40°C to compute degree minutes. Degree minutes and mortality data were fitted to probit, log-logistic and polynomial log-log (CLL) regression models (Kafatos and Proctor, 1992) to estimate degree minutes and associated 95% CLs required to kill 50% (LT50) and 90% (LT90) of the exposed first instars using the Proc Probit procedure (SAS Institute, 1988). The value of B was increased in increments of 1 or 2°C for subsequent iterations. The regression model (probit, logistic or CLL) and base temperature that gave the narrowest 95% CL, around the LT50 and LT90 (LT50 and LT90, respectively) were chosen to describe the relationship between degree minutes and final instar mortality.

**Fig. 1.** Time required to kill 99% of eggs (2-4 days) for first instars (n = 4 instars; wt. 0.12 mg); white (24-h-old; n = 234); gray (2-h-old; n = 20) and adult (48-h-old) of T. castaneum at 20–60°C. A polynomial regression equation (y = a + bx + cx^2) was fitted to LT50 temperature data of each stage to describe heat tolerance. Regression equations for each stage were: 

- Eggs: y = 13.06 + 0.060x^2
- First instars: y = 13.284 + 0.000x^2
- Second instars: y = 14.84 + 0.000x^2
- Adults: y = 18.28 + 0.000x^2

**Fig. 2.** Mortality of T. castaneum (mean ± SE) at different temperatures (°C). Each point represents the mean ± SE of 150-ml plastic containers, each containing 80 g of whole-wheat flour plus yeast (3% by weight). Mortality of first instars was calculated from those that failed to emerge into adults. At each temperature−time combination, five boxes were removed. Five boxes in the control treatments were removed at the same time intervals used for those in temperature treatments, to maintain natural mortality of instars.
Model validation

Temperature-time combinations of the validation data set were converted to degree minutes above B, using Equation 1. Monthly of T. cruzi was forward at these degree minutes, was predicted using the best regression model of CCL1. The mortality predicted in the model was compared with corresponding observed mortality of the validation data set by linear regression using the Proc Reg procedure (SAS Institute, 1985).

Results and discussion

The LT₅₀ and LT₉₀ values in degree minutes based on probit, logistic, and CCL1 models were inversely related to the base temperature (Fig. 2). The probit and logistic models gave similar LT₅₀ values at base temperatures of 42–49.3°C, and these values were lower than those of the CCL1 model. However, the CCL1 model gave lower LT₅₀ values than probit or logistic models. Robertson and Preston (1962) reported that LD₅₀ or LT₅₀ estimates produced by the probit and logistic models are essentially similar. However, in the extreme end of probit-distribution (LD₉₀ or LT₉₀) estimates produced by the logistic model are consistently higher than those produced by the probit model. Robertson and Preston recommend a thorough analysis of data by different models to select the one that best describes the dose-response or time-response data.

The width of the 95% CI upper 95% CI lower 95% CI, and lower 95% CI at the LT₅₀ and LT₉₀ levels generally increased with an increase in base temperature (Fig. 3). The 90% CI width was higher for the CCL1 model at 42.5°C, LT₅₀ level between 42 and 48.5°C, and at temperatures ≥48.5°C the 95% CI width was lower than widths for probit and logistic models. At the LT₉₀ level, the CCL1 model consistently gave the narrower 95% CI width at all base temperatures when compared with the other two models. The minimum 95% CI width for LT₅₀ or LT₉₀ was obtained with the CCL1 model at a base temperature of

![Diagram showing the relationship between LT₅₀ and LT₉₀ values predicted by probit, logistic, and complementary log-log (CCL1) regression models and base temperature.](image)

Fig. 3: Plots showing the chronic relationship between LT₅₀ and LT₉₀ values predicted by probit, logistic, and complementary log-log (CCL1) regression models and base temperature.
The linear regression of proportion predicted by the degree minute model against observed mortality was highly significant (coefficient of determination, $r^2 = 0.75$; $df = 99$; $P < 0.001$). The degree minute model predicted 70% of the variation in the observed mortality data (Fig. 5). The slope value of 0.75 indicated that the degree minute model underestimated (10% mortality by 28%) the original data used to develop the degree minute model were heterogeneous, and the goodness-of-fit of data to the model (Fig. 4) was less than satisfactory, as indicated by the large $r^2$ value. An improved fit of data to the model (i.e., a non-significant $r^2$ value) may have improved model predictability. A model that underestimated mortality, in better years, may have overestimated mortality under field conditions. In the former case, actual mortality would be higher than mortality predicted by the model, and a few would be exposed to more degree minutes than necessary, resulting in complete disturbance and possibly an increase in minimum size. In the latter case, insects would be exposed to less than the required degree minutes, resulting in poor insect control.

![Diagram](image-url)
In summary, a simple degree minute model was developed and validated for predicting mortality of *T. confusum* fruit flies exposed to heat at constant temperatures between 50°C and 55°C. The model presented here is more predictive ability than that reported in the paper. The performance of this simple degree minute model at predicting *T. confusum* fruit fly mortality during heat treatment of a food-processing facility can be improved by using more data on the relation between temperature and mortality.

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**Fig. 4**
A degree minute model based on the exponential model with the regression line for predicting mortality of *T. confusum* fruit flies exposed to a constant temperature of 45°C. The standard error for the intercept, slope, and the slope of the data were 0.12 and 0.39.

**Fig. 5**
Linear regression shows the relationship between observed and predicted mortality by the degree minute model and their observed in the validation data set.

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References


SAS Institute, Cary, North Carolina, USA.

