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

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Abstract

First instars of the red flour beetle, *Tribolium castaneum* (Herbst), are more tolerant than eggs, older larval instars, pupae and adults to elevated temperatures (50–60°C) typically used during heat treatments. Therefore, heat treatments aimed at controlling first instars should eliminate all other stages. First instars of *T. castaneum* were exposed to four constant temperatures of 50, 54, 58 and 60°C to generate time–mortality relationships. About 157 of the 255 observations (62%) collected at the four temperatures were used for model development, and the remaining 98 observations (38%) were used for model validation. The temperature–time data were expressed in degree minutes above a base temperature. First-instar mortality at corresponding degree minutes above a certain base temperature was fitted to probit, logistic and complementary log–log (CLL) regression models. The base temperature for accumulating degree minutes was solved iteratively, and the temperature that gave the narrowest 95% confidence limits (CLs) around LT_{50} and LT_{99} values was chosen for accumulating degree minutes. The CLL model at a base temperature of 49.1°C gave the narrowest 95% CL around LT_{50} and LT_{99} values. At 49.1°C, the degree minutes (and associated 95% CLs) required to kill 50% and 99% of exposed first instars were 165.8 (148.8–180.4) and 395.2 (343.7–487.2), respectively. The CLL-based degree minute model underestimated mortality by 25%, but explained about 70% of the variation in observed mortality data. The ability of the degree minute model to accurately predict mortality of *T. castaneum* first instars during heat treatment of a food-processing facility still remains to be verified.

Keywords: Heat treatment; Base temperature; Degree minutes; Modelling

Introduction

The phase-out of methyl bromide, an atmospheric ozone-depleting space fumigant, in the United States and Europe (Makhijani and Gurney, 1995) has resurrected interest in using high temperatures for managing insects in food-processing facilities. The use of high temperatures for disinfestation purposes is termed "heat sterilisation" or "heat treatment". During heat treatment, 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 disinfesting 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 cracks and crevices, and into equipment where insects generally reside or hide. The target temperature for effective disinfestation should be at least 50°C (Mahroof et al., 2003; Roesli et al., 2003; Wright et al., 2002). Sanitation and removal of all grain and grain products are important during heat treatment, 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 (Subramanyam et al., 1991; Ahmed and Ali, 1995; Johnson et al., 1995), but their use in stored-product pest management has been rather limited. Heat accumulation models are simple to use and predict times when insect 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 (Banks and Fields, 1995; Wright et al., 2002). A base temperature to accumulate degree hours or minutes is also essential for such a modelling approach. A portion of the insect population (10, 20, 30, ... 99%) is assumed dead when a certain number of degree hours or minutes is accumulated. However, the applicability of this approach will depend on how well insect 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 at 50, 52, 54 or 56°C for predicting mortality of large larval instars of the warehouse beetle, *Trogoderma variabile* Ballion. The base temperature for accumulating degree minutes, and the intercept and slope values of the linear regression of mortality (expressed as the inverse of the standard normal deviate) against degree minutes were different at each of the four temperatures.

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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 basis was given for pooling data across the three temperatures. In addition, the model by Wright et al. involves a series of transformations and iterations that are time consuming.

In this paper, we present a simpler approach for developing a degree minute model for predicting mortality of first instars of the red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae), a pest 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 used, because this is the most heat-tolerant stage when compared with eggs, older larval instars, pupae and adults (Fig. 1), especially at temperatures ≥46°C (Mahroof, R., Subramanyam, Bh., Throne, J., unpublished data). Therefore, heat treatments aimed at controlling first instars should be able to eliminate all other stages of *T. castaneum*.

Model development

First instars of *T. castaneum* (mean ± SE wt., 0.12 ± 0.01 mg; n = 15) were separated from the bleached wheat flour + 5% (by weight) brewer's yeast diet using a 250-μm sieve, and counted under a stereomicroscope. First instars were transferred to separate square plastic boxes (4.5 cm^2) with perforated lids covered with 600-μm wire mesh screens for air diffusion. Each box held a mean ± SEM (n = 20) of 305 ± 3 mg of bleached wheat flour and 20 first instars. Boxes with insects were placed in growth chambers (Model I-36 VL, Percival Scientific, Perry, Iowa, USA) set at 50, 54, 58 and 60°C for establishing time–mortality relationships. The control treatment consisted of boxes with first instars that were kept in a chamber set at 28.2 ± 0.2°C and 44.5 ± 0.3% r.h. There was a separate control treatment for each temperature treatment.

Boxes with first instars, removed at different time periods at each temperature, were transferred to 150-mL plastic containers, each containing 40 g of whole-wheat flour plus yeast (5% 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 treatment were removed at the time intervals used for those in temperature treatments, to measure natural mortality of insects.

Natural mortality of first instars in the control treatments was <5%. Therefore, time–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.

$$D = (T - B) \times M \qquad (1)$$

where, T is temperature in °C and M is time in minutes. For the first iteration, B was chosen arbitrarily (42°C) to compute degree minutes. Degree minutes and mortality data were fitted to probit, logistic and complementary log–log (CLL) regression models (Robertson and Preisler, 1992) to estimate degree minutes and associated 95% CLs required to kill 50% (LT_{50}) and 99% (LT_{99}) of the exposed first instars using the Proc Probit procedure (SAS Institute, 1988). The value of B was increased in increments of 1 or 0.1°C for subsequent iterations. The regression model (probit, logistic or CLL) and base temperature that gave the narrowest 95% CL around the LT_{50} and LT_{99} (B_0) were chosen to describe the relationship between degree minutes and first instar mortality.

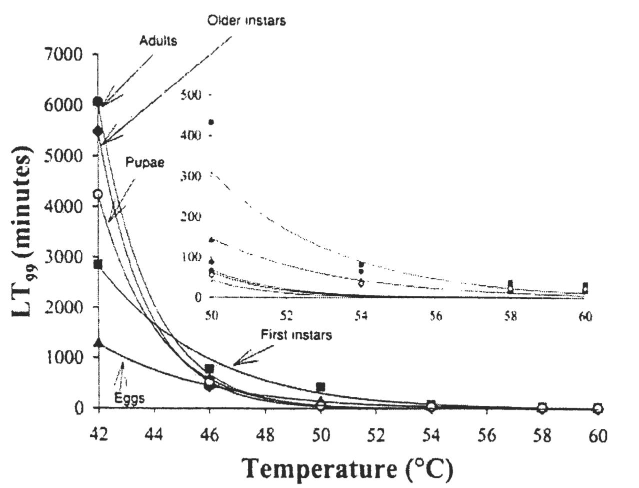

Fig. 1. Time required to kill 99% of eggs (2-d old), first instars (6-d old; wt, 0.12 mg), older instars (22-d old; wt, 3.59 mg), pupae (26-d old), and adults (≤2-wk old) of *T. castaneum* at 42–60°C. A nonlinear regression equation ($\ln y = a + bx^2$) was fitted to LT_{99}–temperature data of each stage to describe heat tolerance. Regression equations for each stage were: eggs, $\ln y = 12.346 + (-0.002)x^2$; first instars, $\ln y = 13.244 + (-0.003)x^2$; older instars, $\ln y = 19.682 + (-0.006)x^2$; pupae, $\ln y = 20.280 + (-0.006)x^2$; and adults, $\ln y = 18.267 + (-0.005)x^2$.

Model validation

Temperature–time combinations of the validation data set were converted to degree minutes above B_0 using Equation 1. Mortality of *T castaneum* first instars at these degree minutes was predicted using the best regression model (CLL). The mortality predicted by the model was compared with corresponding observed mortality of the validation data set by linear regression using the Proc Reg procedure (SAS Institute, 1988).

Results and discussion

The LT_{50} and LT_{99} values in degree minutes based on probit, logistic, and CLL models were inversely related to the base temperature (Fig. 2). The probit and logistic models gave similar LT_{50} values at base temperatures of 42–49.3°C, and these values were lower than those of the CLL model. However, the CLL model gave lower LT_{99} values than probit or logistic models. Robertson and Preisler (1992) reported that LD_{50} or LT_{50} estimates produced by the probit and logistic models are essentially similar. However, at the extreme ends of probability distribution (LD_{99} or LT_{99}), estimates produced by the logistic model are consistently higher than those produced by the probit model. Robertson and Preisler 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% CL (upper 95% CL – lower 95% CL) at the LT_{50} and LT_{99} levels generally decreased with an increase in base temperature (Fig. 3). The 95% CL width was higher for the CLL model at the LT_{50} level between 42 and 48.5°C, and at temperatures >48.5°C the 95% CL width was lower than widths for probit and logistic models. At the LT_{99} level, the CLL model consistently gave the narrowest 95% CL widths at all base temperatures when compared with the other two models. The minimum 95% CL width for LT_{50} or LT_{99} was obtained with the CLL model at a base temperature of

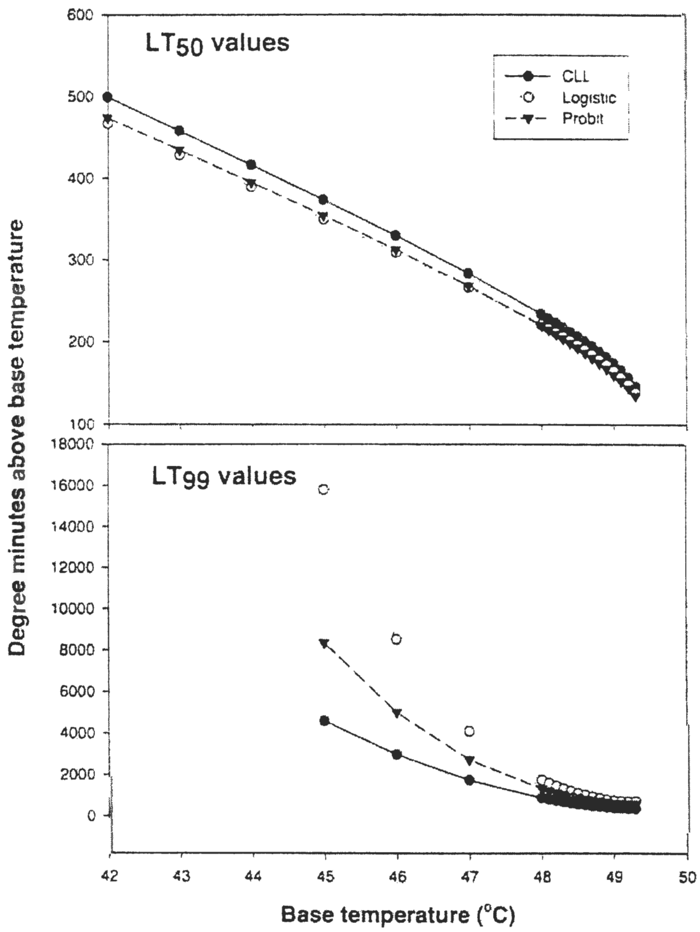

Fig. 2. Plots showing the inverse relationship between LT_{50} and LT_{99} values generated by probit, logistic and complementary log–log (CLL) regression models and base temperature.

49.1°C (Fig. 3, inset). Therefore, the CLL model was chosen for describing the relationship between degree minutes above 49.1°C and first instar mortality (Fig. 4). Robertson and Preisler (1992) reported that the CLL model is best suited for describing time-mortality responses of insects.

The LT_{50} and LT_{99} values and associated 95% CLs produced by the CLL model using a base temperature of 49.1°C were 165.8 (148.8–180.4) and 395.2 (343.7–487.2) degree minutes, respectively. The χ^2 value for the goodness-of-fit of data to the CLL model was highly significant ($\chi^2 = 2325$; df = 155; $P < 0.001$), suggesting that the responses of first instars at the temperatures tested were heterogeneous. Heterogeneity in responses may be attributed to age or sex-related differences in susceptibility to high temperatures. In our tests, age variation among first instars was ≤2 d. Furthermore, thermal acclimation of certain individuals at each of the temperatures tested, especially at longer exposure times, may have contributed to the heterogeneity observed.

The linear regression of mortality predicted by the degree minute model against observed mortality was highly significant (regression slope, $b = 0.75$; df = 96; $P < 0.001$). The degree minute model predicted about 70% of the variation in the observed mortality data (Fig. 5). The slope value of 0.75 indicated that the degree minute model underestimated first instar mortality by 25%. 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 χ^2 value. An improved fit of data to the model (i.e. a non-significant χ^2 value) may have improved model predictions. A model that underestimates mortality is better than one that overestimates mortality under field conditions. In the former case, actual mortality would be higher than mortality predicted by the model, and insects would be exposed to more degree minutes than necessary, resulting in complete disinfestation and possibly an increase in treatment cost. In the latter case, insects would be exposed to less than the required degree minutes, resulting in poor insect control.

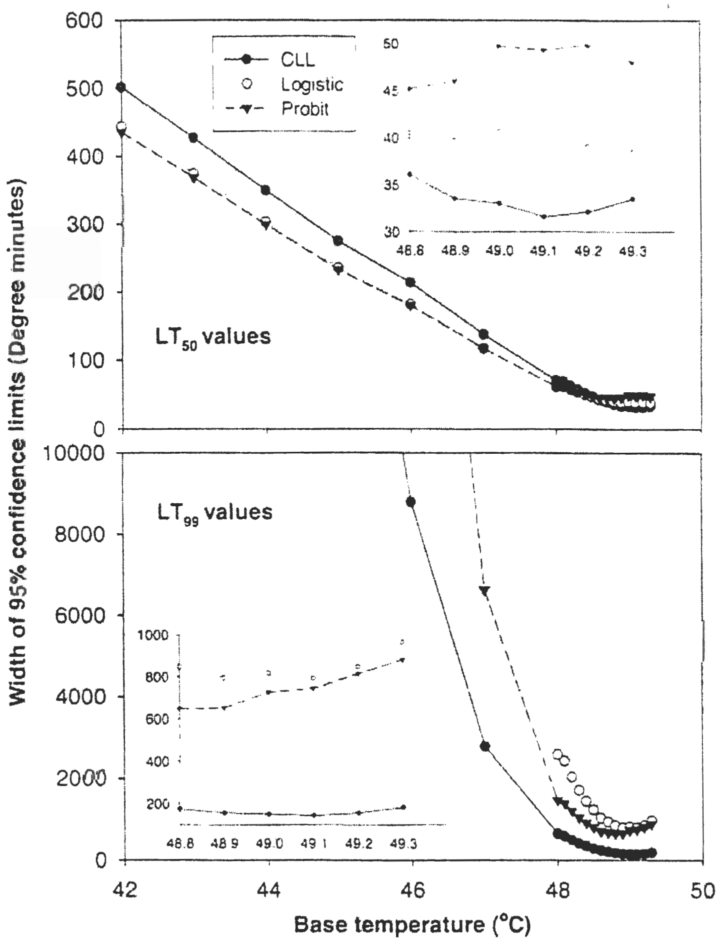

Fig. 3. The width of 95% confidence limits for LT_{50} and LT_{99} values generated by probit, logistic and complementary log-log (CLL) regression models plotted against corresponding base temperatures.

In summary, a simple degree minute model was developed and validated for predicting mortality of *T. castaneum* first instars exposed to four constant temperatures between 50°C and 60°C. The model presented here and its predictive ability may be improved by collecting more data on first instars at constant temperatures (above 49.1°C) other than those reported in this paper. The performance of this simple degree minute model in predicting *T. castaneum* first instar mortality during heat treatment of a food-processing facility remains to be tested.

Measuring temperatures continuously during heat treatment is essential for ensuring that lethal temperatures have been maintained for the required time to kill insects. The temperature–time data collected at various locations within a facility can be used in a degree minute model for gauging the effectiveness of heat treatment against insects.

Acknowledgements

Research reported here was supported by funds from Temp-Air®, Burnsville, Minnesota, USA, and partially by funds from USDA-CSREES (RAMP) under Agreement No. 00-51101-9674.

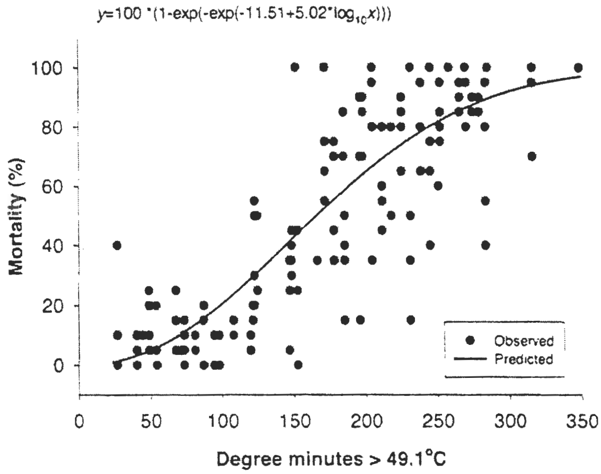

Fig. 4. A degree minute model based on complementary log–log regression for predicting mortality of *T. castaneum* first instars exposed to temperatures above 49.1°C. The standard error for the intercept value of 11.51 was 1.31 and for the slope value of 5.02 was 0.56.

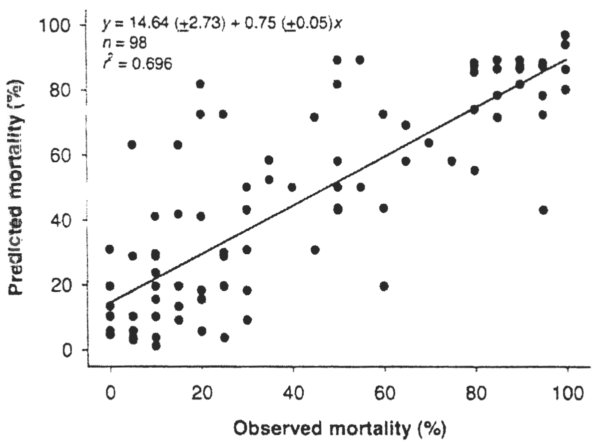

Fig. 5. Linear regression showing relationship between mortality predicted by the degree minute model and that observed in the validation data set.

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