Thermal conditioning in *Bactrocera tryoni* eggs (Diptera: Tephritidae) following hot-water immersion

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Abstract

*Bactrocera tryoni* Froggatt eggs were immersed in hot water to determine egg mortality. Eggs were either immersed in water at a constant temperature, or experienced changing temperature at a specified rate of increase and from a specified start temperature. Comparison of the estimated lethal time for 99% kill (LT99) of eggs experiencing different treatments allowed thermal conditioning to be identified and quantified. Conditioning depended on the temperature and duration of treatment, being a maximum near 38°C. Most conditioning appeared to occur during the early part of the exposure to a given temperature. Exposure to lethal temperatures (≥42°C) as a target temperature of 46 or 48°C was approached, contributed significantly to the mortality if the rate of heating was relatively slow. Calculations of egg survival in a mathematical model of the conditioning and lethal thermal responses, correlated well with experimental values in terms of both trends and magnitudes of LT99 values. The thermal conditions, prior to disinfestation treatment, influence the response to subsequent heat treatment and thus have implications for the specification of postharvest quarantine treatments which are often expressed in terms of a fruit centre target temperature. This does not take into account of the influence of temperatures and exposure times in the range 32–42°C which can have a very significant effect on the time required to reach high levels of mortality. An efficacious treatment may be the combination of a lethal stress and a particular heating rate which falls within a band, bounded by rates of heating that are too slow or too fast. The use of models will assist in the identification of promising treatments while avoiding extensive in-fruit testing. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Tephritidae; Bactrocera tryoni; Thermal conditioning; Disinfestation

1. Introduction

Postharvest treatments are being developed in New Caledonia for a range of horticultural crops
including eggplant, capsicum and mango. The presence in New Caledonia of Queensland fruit fly, *Bactrocera tryoni* Froggatt, and other species of economic significance (Sales et al., 1996), necessitates the development of postharvest treatments to allow the export of produce to overseas markets where these species do not occur. Heating as a treatment for disinfesting fresh horticultural produce of fruit flies is a proven technology (Armstrong, 1994; Mangan and Hallman, 1998) and is being researched for application to New Caledonia crops.

Frequently postharvest treatment protocols are developed for a particular product and are specified in terms of a target temperature for the product centre. This criterion for specifying disinfestation treatment compliance may be applied to other commodities because of its efficacy in one crop based on the argument that the treatment targets the insect irrespective of host crop. The use of a ‘generic heat treatment’ where one treatment protocol is applied to all commodities potentially infested by fruit flies, assumes that heating rate influences (and any other factors affecting treatment efficacy) are compensated by the use of a suitably robust treatment. This could result in the mistaken rejection of heat as a potential postharvest treatment due to host intolerance when applying such a low-risk generic treatment protocol.

Long exposure to elevated but non-lethal temperatures has been shown to condition insects such that subsequent treatment at lethal temperatures is less effective. Enhanced thermotolerance following exposure to non-lethal temperatures has been reported in fruit flies (Laidlaw et al., 1996; Osman et al., 1996; Beckett and Evans, 1997) and other dipterans such as *Sarcophaga crassipalpis* (Yocum and Denlinger, 1992, 1993). Lepidopteran species also show this response (Lester and Greenwood, 1997; Alderson et al., 1998; Neven, 1998). Survival of fruit flies can also be found following disinfestation treatment, where the rate of fruit heating is very rapid (Armstrong et al., 1995) indicating that ramp rates can be too fast as well as too slow. Consequently it is important to determine how thermotolerance is altered when insects have experienced different thermal histories.

The temperature experienced by individual insects heated within whole fruit, when immersed in a controlled temperature bath, changes with time and as such is effectively that of ‘ramped heating’. The temperature of the bath itself may be maintained at a constant target temperature or incremented following a set heating profile. Insects within the fruit will heat at a specified rate, the details depending on the bath (water or air) temperature, the heat-transfer coefficient at the fruit-bath interface, thermal conductivity of the fruit, and the location in the fruit of the pest (Armstrong, 1994). For example, fruit fly eggs are typically laid no more than 5 mm from the fruit surface while late instars can be located at the fruit centre and as a consequence the thermal experience of the different life stages will be quite different.

In-fruit experiments required to evaluate various candidate crops for heat treatment would become even more time consuming and costly if besides host type, various rates of heating required investigation. Although models of the kinetics of the response of fruit flies to heating at lethal temperatures have been developed for constant temperatures by several authors (Jang, 1986; Laidlaw and Hayes, 1990; Thomas and Mangan, 1997), no models which account for changing temperatures through the lethal temperature range have been presented. Further, no models are available which take account of the conditioning effect of non-lethal temperatures on subsequent treatment at lethal temperatures. Both non-lethal and lethal temperatures occur during ramped heating and contribute to the overall mortality response.

The aim of this research was twofold: to quantify, experimentally, the mortality of *B. tryoni* eggs following different heat treatments and to develop a mathematical model to describe and predict the contributions of conditioning and mortality in ramped heating situations. A kinetic model was developed which uses the experimental data obtained from the constant temperature treatment results. The model is developed in terms of (1) the extra time that conditioning in the temperature range 30–42°C increases the estimated lethal time required to achieve 99% mortal-
ity (LT$_{99}$) at a target temperature of 46°C, and (2) the decrease in LT$_{99}$ due to lethality in the range 42–46°C which results in insect mortality as measured at 46°C. The mortality of fruit fly eggs associated with any temperature ramp from 30 to 46°C can be described by this model.

2. Material and methods

All experiments were carried out at the Pocquereux Research Station, New Caledonia.

2.1. Insects

Adult *B. tryoni* were field collected and subsequently maintained in the laboratory on yeast, fruit fly bacteria (*Enterobacter cloacae* Jordon), water and sugar, under natural light at 26±1°C. Fruit fly colony rearing methods were very similar to those described by Clare (1997). Eggs were collected from adult females which oviposited through holes punched in the side of plastic cylinders (30 mm internal diameter by 46 mm long by 1 mm wide). The inside surface of the cylinders were smeared with diet consisting of banana fruit pulp, and were then exposed to the adults for 2 h. Eggs were washed from the cylinders with water, placed on dampened filter paper in a closed petri dish and incubated for 24 h in a photoperiod of 12:12 L:D at 26±1°C for treatment as mature eggs (26–28 h old).

2.2. Heat treatment

Eggs were heat-treated in plexiglass tubes (34 mm internal diam. by 50 mm length). Fine material gauze (5.5 strands per mm, 35% open area) inserts were placed into the end of each tube to keep the eggs in the tube while facilitating rapid water movement through the tube. Treatments were applied in a water bath system (Purbeck Limited, New Lynn, Auckland, NZ) consisting of four 25 l water baths. Heater units (Grant, Cambridge, UK Model ZD, temperature accuracy ±0.01°C) on each bath were independently controlled by a laptop computer running Workbench PC for Windows data acquisition and control software (Strawberry Tree, Sunnyvale, CA 94086). The target temperatures were verified for each test using an electronic reference thermometer (Model RT200, Measurements Standards Laboratory of New Zealand, Industrial Research Limited, Wellington, NZ) certified for accuracy under the Measurements Standards Act 1992 by the Measurements Standards Laboratory.

Immediately before treatment, approximately 200 eggs were washed into each treatment tube with between eight and 14 tubes prepared for each test. Treatment tubes were simultaneously immersed at the start of each test. Immediately following the selected immersion time (1 min to 13 h depending on treatment), individual tubes were removed from the hot water bath, drained and immersed in 25.0±1.0°C water for 2 min (hydro-cooling cycle). Control insects for each treatment were hydro-cooled for a period that exceeded the longest hot water immersion of that treatment by 2 min.

Following immersion in 25.0±1.0°C water, the treated and control eggs were washed from each tube onto gauze material and placed on moist filter paper inside petri dishes (9 cm diameter). Each petri dish was sealed with a strip of Parafilm® to prevent egg desiccation.

2.3. Static temperature lethal heat treatments

To assess mortality response to constant (static) temperature treatments in the lethal range (>42°C), *B. tryoni* eggs were immersed in static temperature water baths at 42, 43, 44, 45, 46, 47 and 48°C for various times (1.5–380 min, depending on temperature). The mortality observed for each temperature, $T_i$, at intervals $t_i$ gave a direct value for mortality as a function of static temperature treatment time. After heat treatment the eggs were hydro-cooled as described above. Three to six replicates were completed at each immersion temperature.

2.4. Static temperature conditioning heat treatments

In order to determine the effects of exposure to temperatures in the non-lethal range (30–42°C)
on the mortality response, \textit{B. tryoni} eggs were first immersed in a water bath at 32, 34, 36, 38, 40 or 42°C for times ranging from 15 min to 12 h (depending on the temperature bath employed). The tubes containing eggs were removed from the pretreatment bath, drained of hot water and within 2 min simultaneously immersed in a second water bath operating at 46°C. Samples were removed after various exposures (0–70 min) until the lethal time for 99% mortality (LT$_{99}$) could be established. The samples were then hydro-cooled. Comparison of LT$_{99}$ for conditioned and non-conditioned eggs provided a quantitative measure of the conditioning as a function of temperature and time of the treatment. The temperatures and exposure durations of the pretreatment were chosen so that conditioning and not mortality would result. This was verified in each test with a second control, which experienced the pretreatment component only. Three to seven replicates were completed at each pretreatment time and temperature combination. Fig. 1 illustrates the procedure schematically for a case where the first group of samples was immersed directly in the 46°C target bath, the second group of samples was pretreated at 38°C for 30 min and then immersed in the target 46°C bath, and a final group of samples was pretreated at 38°C for 60 min before being immersed in the target 46°C bath. Samples were removed at intervals from the 46°C bath, the number of non-hatched eggs determined for each sample and the LT$_{99}$ times evaluated.

2.5. Ramped heating

Although the static temperature treatment results provide an assessment of mortality due to exposure to particular temperatures in the range 30–48°C, it is the effect of the continuously changing temperature during ramped heating that comes closer to addressing the insect mortality response as it occurs in whole fruit. To mimic this, \textit{B. tryoni} eggs were immersed in a water bath which changed temperature linearly with time from 30 to 46°C in 1, 3, 6, or 9 h (heating rates of 16, 5.3, 2.7 and 1.8°C h$^{-1}$ respectively). The eggs so treated were then maintained at 46°C until the LT$_{99}$ value could be established. A comparison of the LT$_{99}$ for eggs undergoing ramped heating and the LT$_{99}$s for eggs immersed in 46°C without prior treatment then provided a direct measure of the conditioning due to temperatures and times encountered for a particular ramp. The effect of ramped heating from 25 to 48°C in 1 h (23°C h$^{-1}$) was also determined. Longer ramp times to a target of 48°C were of limited value because of the high levels of kill at 48°C and temperatures near this target. Three replicate tests were conducted at each ramp. Fig. 2 contains a schematic of the procedure for the smooth linear ramp used in experiments as well as a step-wise ramp which will be used later to illustrate the connection between static and ramped treatments.

2.6. Mortality assessment

Egg hatch normally begins about 40 h after laying. Treated and control eggs were held at 26±1.0°C for 3 days to ensure all hatching was complete and then examined for mortality using a binocular microscope (10–40× magnification). Egg mortality was based on the presence or absence of an exit rupture in the chorion.
Ramped heating treatments, where *B. tryoni* eggs were immersed in water which was then heated from 30°C to the lethal target (46 or 48°C), and maintained at target until the LT$_{99}$ could be determined, were conducted over 1 (depicted), 3, 6 and 9 h intervals. Smooth linear ramps were achieved in the water bath trials while the step-wise ramp was used in the mathematical model to estimate the magnitude of conditioning and lethality over the ramping period.

Fig. 2. Ramped heating treatments, where *B. tryoni* eggs were immersed in water which was then heated from 30°C to the lethal target (46 or 48°C), and maintained at target until the LT$_{99}$ could be determined, were conducted over 1 (depicted), 3, 6 and 9 h intervals. Smooth linear ramps were achieved in the water bath trials while the step-wise ramp was used in the mathematical model to estimate the magnitude of conditioning and lethality over the ramping period.

2.7. Statistical methods for assessing LT$_{99}$

Analyses of observed mortality due to treatment at individual temperatures from 42 to 48°C used the model:

\[ \log(-\log(1 - p)) = a + bt, \]  

where \( p \) was the expected observed mortality, and \( t \) was the treatment time in minutes (Preisler and Robertson, 1989). This gave approximate linearity in time. The estimated LT$_{99}$, which relates to mortality after there has been allowance for control mortality, was calculated to give an expected mortality of \( c + (1-c)0.99 \), where \( c \) was the control mortality as determined in the 25°C immersion treatments. Eq. (1) was fitted using a robust version of the generalized linear model analysis available in S-Plus (Chambers and Hastie, 1991) that assumed that any variance was proportional to that of a binomial distribution. The robust version reduces the weight given to points lying away from the main body of the data. This method handles the variability in the response associated with occasional large outliers (Maindonald, 1992).

Confidence intervals were calculated so that non-overlap was equivalent to a statistically significant difference in a Student’s \( t \)-test at the 1% level.

Although the log–log analysis of Eq. (1) can be used successfully to determine LT$_{99}$ times we have utilized a kinetic expression for mortality to model the response of *B. tryoni* eggs over the entire treatment history. Rather than use percentage mortality we shall use its complement, percentage survivors,

\[ S(T, t) = 100 \frac{1 - \text{mortality}}{1 - \text{control mortality}} \]

which incorporates Abbott’s method (Abbott, 1925) to correct for control mortality. The treatment time, \( t \), is the independent variable and the equation used for \( S \) at each temperature is

\[ S(T, t) = 100e^{-(k_L \cdot t)^2} \]  

This form is obtained by simplifying a form, \( S = 100e^{-(c + k_Lt)^1/a} \), suggested by earlier workers (King et al., 1979) and used by several authors to model lethal response of fruit flies (Jang, 1986; Laidlaw and Hayes, 1990). The constant ‘\( c \)’ is normally small and was omitted, ‘\( 1/a \)’ ranges from 1.5 to 2.5 and was set near the mid-point of the range so that only the ‘rate’ constant, \( k_L \), was retained as a temperature dependent parameter. To simplify notation we drop the explicit reference to the independent variables and write \( S(T, t) = S \).

2.8. Model curve fitting

The curve fits shown were initially obtained using the defined-fit option of the Kaleidagraph suite of programs (Kaleidagraph, 1997) and were confirmed using the non-linear platform of the JMPIN suite of programs (JMPIN, 1997). The latter program also provided the confidence limits for the parameters obtained in the curve fitting functions.
3. Results

3.1. Static temperature lethal treatment

The LT$_{99}$ values obtained from the complementary log–log analysis for trials where the water temperature remained constant, decreased from 253 to 5.5 min as the exposure temperature was increased from 42 to 48°C (Table 1). The long LT$_{99}$ at 42°C (253 min) suggests this was approaching the lower limit for mortality due to heat stress.

3.2. Static temperature conditioning treatments

Table 2 contains the results for eggs treated at sublethal temperatures (32–42°C) and then exposed to lethal conditions (46°C). The LT$_{99}$ values shown indicate the LT$_{99}$ times are longer for pretreated (or conditioned) eggs than for eggs which did not receive the pretreatment. The magnitude of the conditioning response ($\Delta t_{46}$) was determined as the extra time at 46°C required to achieve LT$_{99}$ compared to non-pretreated eggs. The amount of conditioning was positively correlated with duration of exposure for short times for all temperatures. For the lower static conditioning temperatures, e.g. 34°C there was an increase in $\Delta t_{46}$ for treatment times up to 12 h. At higher temperatures like 38°C the positive correlation response peaked at about 9 h and $\Delta t_{46}$ declined at 12 h. At its maximum the LT$_{99}$ was increased from 18.6 min at 46°C in the absence of pretreatment to 63.1 min at 46°C following pretreatment for 9 h at 38°C. In general the maximum $\Delta t_{46}$ at each temperatures moved to shorter times as the temperature increased. The static temperature conditioning at 42°C had a more complex dependence on time, rising rapidly to a maximum and then becoming negative. This suggests that 42°C is approaching the upper temperature limit for the conditioning response.

3.3. Ramped conditioning treatments

Conditioning was also observed in the ramped trials where the temperature was continuously changed from 30°C to a maximum of 46°C at different rates of increase. The resulting LT$_{99}$ values, shown in the right hand two columns of Table 3, for the shorter three ramp durations (1, 3, 6 h) were significantly longer than the non-ramped value (18.6 min). The LT$_{99}$ peaked at 50.4 min when the ramp duration was 6 h and declined to 27 min when the ramp duration was increased to 9 h. Trials for the 1 h ramp to 48°C resulted in conditioning which shifted the LT$_{99}$ from 5.5 (4.4–6.8) to 18 min (6–36) i.e. 12.5 min longer than the non-ramped value.

The experimental data from the static temperature treatment results outlined above can be used to develop a model to account for the different effects of heat treatments, i.e. conditioning and lethality of fruit flies. The first part of the model is developed in terms of the extra time that static temperature conditioning (in the range 30–42°C)

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Complementary log–log model (Eq. (1))</th>
<th>Kinetic model (Eq. (2))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LT$_{99}$ (min)</td>
<td>95% CI</td>
</tr>
<tr>
<td>42</td>
<td>253.9</td>
<td>209.3–308</td>
</tr>
<tr>
<td>43</td>
<td>108.2</td>
<td>87.2–134.3</td>
</tr>
<tr>
<td>44</td>
<td>65.9</td>
<td>53.1–81.8</td>
</tr>
<tr>
<td>45</td>
<td>34.2</td>
<td>27.6–42.5</td>
</tr>
<tr>
<td>46</td>
<td>18.6</td>
<td>15.6–22.2</td>
</tr>
<tr>
<td>47</td>
<td>8.4</td>
<td>6.8–10.4</td>
</tr>
<tr>
<td>48</td>
<td>5.5</td>
<td>4.4–6.8</td>
</tr>
</tbody>
</table>
Table 2
Estimated lethal times for 99% kill (LT_{99}) in min for *B. tryoni* eggs immersed in 46°C water after an initial conditioning treatment at sub-lethal temperatures for various specified immersion intervals. Results were analysed using complementary log-log analyses.

<table>
<thead>
<tr>
<th>Time at conditioning temperature</th>
<th>Conditioning temperature (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>32</td>
</tr>
<tr>
<td></td>
<td>LT_{99}</td>
</tr>
<tr>
<td>0 min</td>
<td>18.6</td>
</tr>
<tr>
<td>15 min</td>
<td>29.5</td>
</tr>
<tr>
<td>30 min</td>
<td>31.8</td>
</tr>
<tr>
<td>45 min</td>
<td>27.3</td>
</tr>
<tr>
<td>1 h</td>
<td>34.1</td>
</tr>
<tr>
<td>2 h</td>
<td>37.4</td>
</tr>
<tr>
<td>3 h</td>
<td>40.3</td>
</tr>
<tr>
<td>6 h</td>
<td>42.7</td>
</tr>
<tr>
<td>9 h</td>
<td>49.3</td>
</tr>
<tr>
<td>12 h</td>
<td>53.3</td>
</tr>
</tbody>
</table>

\textsuperscript{a} The increase in thermal tolerance is termed ‘extra time’ at 46°C and is calculated as the tabulated LT_{99} minus 18.6 min, the non-pretreated LT_{99} at 46°C.

\textsuperscript{b} 95% confidence interval.
Table 3
Comparison between model (calculated using smoothed ramp) and experimental times for 99% mortality (LT99 Total time) of *B. tryoni* eggs when immersed in water and ramped from 30 to 46°C over various specified times. Calculated times are $\Delta t_{46,C(E)E} + \Delta t_{46,LE} + 18.8 \text{ min}$.

<table>
<thead>
<tr>
<th>Ramp time, h</th>
<th>Calculated times (min)</th>
<th>Experimental times (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\Delta t_{46,C(E)E}^a$</td>
<td>$\Delta t_{46,LE}^b$</td>
</tr>
<tr>
<td>0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>1</td>
<td>24.0</td>
<td>−3.3</td>
</tr>
<tr>
<td>3</td>
<td>35.0</td>
<td>−11.3</td>
</tr>
<tr>
<td>6</td>
<td>43.6</td>
<td>−21.6</td>
</tr>
<tr>
<td>9</td>
<td>46.9</td>
<td>−32.2</td>
</tr>
</tbody>
</table>

* $\Delta t_{46,C(E)E}$: Conditioning Equivalent component of model estimated LT99 at 46°C.
* $\Delta t_{46,LE}$: Lethal Equivalent component of model estimated LT99 at 46°C.

Increases the LT99 at 46°C. The second addresses how mortality in the range (42–46°C) decreases the total LT99 at the target temperature. The model describes and estimates the mortality associated with any ramp from 30 to 46°C and can be used to analyze the experimental results of the linear ramped heating trials.

### 3.4. A kinetic model for static temperature treatments in the lethal range 42–48°C

The experimental values of % survivors, $S$ were fitted to Eq. (2) for all times at each temperature in the range 42–48°C. Fig. 3 illustrates the data for six replications at 46°C and the fit of Eq. (2) to the pooled data of the six replications. At 46°C the fit to the pooled data yielded a value of $k_0^L = 0.114$ and the upper and lower 95% confidence limits are 0.102 and 0.129 (here the superscript 0 is used to denote the rate constant $k_L$ at the reference temperature of 46°C). Fits to the individual replications were also carried out and the average $k_0$ obtained. Both methods gave essentially the same value of $k_0$ and that for the pooled data is reported, for each temperature, in column 4 of Table 1. LT99 values for the kinetic model were extracted by solving Eq. (2) for $t$ when $S = 1$. For example, the derived LT99 for 46°C is 18.8 min and the derived lower and upper confidence limits (CL) on LT99 are 16.2 and 21.0 min, respectively. Thus the kinetic model gives LT99 values and lower and upper CL which are essentially the same as those determined using the complementary log–log form of analysis. Further the experimental results are within the 95% confidence intervals of the values determined using either the kinetic or the complementary log–log form of analysis (Table 1) for LT99.

### 3.5. A kinetic model for surviability following treatment in the conditioning range 30–42°C

The extension of this analysis to a treatment at a lethal temperature following conditioning at non-lethal temperatures, presents conceptual challenges to the mortality model. Insect tolerance to
what would have been a lethal subsequent treatment at 46°C is induced and as a result extra time at 46°C is required to generate the same level of mortality observed in non-conditioned insects. Consequently an indirect measure of the role which temperatures in the 30–42°C regime have on mortality is required, and the ‘extra time at 46°C’, denoted by $\Delta t_{46}$, is utilized. Rewriting Eq. (2) to give the % survivor curve for treatment time, $t$, at some target temperature, when there has been some prior treatment we have:

$$S = 100e^{-k^*t^2}$$

where $k^*$ is the rate constant for treatment at the temperature of interest when conditioning has occurred. For any chosen value of $S$ an expression for $k^*$ in terms of $\Delta t_{46}$ and the equivalent values of $k^0_1$, $t_0$ when no treatment has occurred, is readily found. For example if $S = 1$ (the LT$_{99}$ limit) and the target temperature is 46°C then Eq. (3) is $S = 100e^{-(k^*t)^2} - 1$ where $k^*$ is now the rate constant for treatment at 46°C when conditioning has occurred and $t^*$ is the treatment time required for LT$_{99}$ at 46°C when the fruit fly eggs have undergone conditioning. But Eq. (2) can also be written as $S = 100e^{-(k^0_1t_0)^2} = 1$ where again $k^0_1$ is the rate constant at 46°C when there is no conditioning and $t_0$ is the time required to achieve LT$_{99}$ at 46°C when there is no conditioning. Equating the two expressions for $S$ gives $k^* = k^0_1t_0/t^*$. The explicit dependence of $k^*_{46}$ on $\Delta t_{46}$ is evident if one notes that $t^* = t_0 + \Delta t_{46}$ so that $k^* = k^0_1t_0/(t_0 + \Delta t_{46})$. Eq. (3) becomes

$$S = 100e^{-(k^0_1t_0 + \Delta t_{46})t^2}$$

Consequently to get a mortality curve with $t$ as the independent variable one need only find the value of $\Delta t_{46}$ due to prior treatment. The observed extra time, $\Delta t_{46}$, required for treatment at 46°C for selected static conditioning treatments applied prior to exposure to 46°C can be derived from data in Table 2.

3.6. A kinetic model for conditioning

The amount of conditioning observed at 46°C as extra LT$_{99}$ time due to prior conditioning for time $t_c$ at some temperature $T_c$ can be thought of as a result of conditioning time multiplied by an effective conditioning rate, $K$, where $K$ depends on conditioning temperature $T_c$ and time of conditioning $t_c$. Hence

$$\Delta t_{46}(T_c, t_c) = t_c K(T_c, t_c).$$

Again, to simplify notation, we drop the explicit reference to the independent variables and rewrite this expression to give the conditioning rate as

$$K = \frac{\Delta t}{t_c}$$

The values of $K$ given directly by Eq. (5b) and the experimental values of $\Delta t_{46}$ are shown in Fig. 4 for static conditioning temperatures $T_c = 34, 38, 40$ and 42°C and for conditioning times, $t_c$, from 15 to 720 min. There is a rapid initial decay in the effective conditioning rate, $K$, at all temperatures. For temperatures in the range 34–40°C the initial decay is followed by a slower decay rate. For a conditioning temperature of 42°C at the upper boundary of the conditioning regime, the rapid initial decay is followed by a strong decay through zero and the transition to a lethal effect.

Fig. 4. The relationship between the effective conditioning rate $K(T_c, t_c)$ for B. tryoni eggs immersed in heated water (34–42°C) and the duration of the conditioning treatment. The values of the rate, $K(T_c, t_c)$ were obtained by dividing the experimental values of the extra time required at 46°C to achieve 99% mortality by the conditioning time $t_c$ and these values are shown as the open circles. The solid line is the fit of (Eq. (6)), $K(T_c, t_c) = m_1e^{-m_2t_c} + m_3e^{-m_4t_c}$, to this data.
The data in Fig. 4 suggest that for each temperature, $K$ is a sum of two logarithmic decay terms as in

$$K = m_1e^{-m_2t} + m_3e^{-m_4t}$$

where, in principle, $m_1, ..., m_4$ are functions of $T_C$.

The fits of Eq. (6) to $K$ for $T_C = 34, 38, 40^\circ$C for $t_C$ from 15 to 720 min are shown in Fig. 4 and appear quite good.

Analysis indicates there is some correlation between parameters, for example, at $38^\circ$C the two ‘rates’ $m_2 = 0.036, m_4 = 0.0024$ have a correlation coefficient of 0.49; with lower and upper 95% CL values of 0.023 and 0.063 for $m_2$ and lower and upper 95% CL values of 0.0012 and 0.0037 for $m_4$. This may reflect, in part, the limited number of data points or that the functional form of Eq. (6) is inappropriate.

The two decay terms in Eq. (6) could represent independent depletion of precursors $B_1$ and $B_2$ necessary for development of the heat shock proteins involved in temperature conditioning. But an alternative two term decay form results from a sequence $A \rightarrow B \rightarrow D$ where $B$ is taken to be a precursor to heat shock proteins (Laidlaw et al., 1996). This model restricts, in principle, the temperature dependence to two rate constants and allows one to write

$$K = B = A_0 + \frac{m_2}{m_4 - m_2} e^{-m_2t} + \left( B_0 - A_0 \frac{m_2}{m_4 - m_2} \right) e^{-m_4t}$$

where $A_0$ and $B_0$ are initial concentrations. When this form is fitted to $K$ for 34, 38 and $40^\circ$C the ratio $B_0/A_0 = 2.2 \pm 0.1$, i.e. approximately independent of temperature as one might expect. Both $m_2$ and $m_4$ follow the expected Arrhenius function of temperature for rate constants, however $A_0$ remains temperature dependent. Rather than using this model on the conditioning rate $K$, the simple two exponent form Eq. (6) was used. The effective rate at $42^\circ$C becomes negative (lethal) after some time indicating the transition from a conditioning to a lethal effect on insect response. To effect this transition the fit of Eq. (6) to $K$ for this temperature included a constant $k = 0.0088$, the mortality rate found for Eq. (2) at $42^\circ$C.

The extra treatment time, $\Delta t_{46}$ given in the kinetic model by the product of conditioning time and effective conditioning rate (i.e. Eq. (5a)) is shown in Fig. 5 along with the experimental values of $\Delta t_{46}$. The error bars displayed are the upper and lower 95% confidence limits obtained for the complementary log–log treatment of the experimental data. The values given by the model all lie within these experimental error bars.

3.7. A kinetic model for ramped heating treatments in the conditioning range $30–42^\circ$C

The notion of an effective rate can be used in the development of an equation which permits the calculation of the extra time, $\Delta t_{46}$, for each time interval when conditioning temperatures are changing. Thus the contribution to $\Delta t_{46}$ of treatment of duration $\Delta t_{T_C}$ at temperature $T_C$ which begins at time $t_C$ and spans the interval $t_C$ to $t_C + \Delta t_{T_C}$ in a ramp treatment is given by

$$K(T_C, t_C) \Delta t_{T_C} \text{ i.e. by a product of the appropriate effective rate times the treatment duration.}$$

For treatment at a sequence of temperatures the Conditioning Equivalent, $\Delta t_{CE}$ is calculated via:

$$\Delta t_{CE} = \sum_C \Delta t_{T_C} K(T_C, t_C)$$

where the summation over $C$ refers to the various conditioning intervals of duration $\Delta t_C$ and temperature $T_C$.

It is to be emphasized that this procedure is an approximation because it is a simplification to use a static equation such as Eq. (5b) when the temperature $T_C$ is changing as in a ramp treatment. We have chosen to retain the form of Eq. (5b) and to evaluate the effective rate $K$ in Eq. (7) at the beginning of each conditioning interval.

3.8. A kinetic model for ramped heating treatments in the lethal range $42–46^\circ$C

The rate expression in Eq. (2), for mortality due to treatment at a static temperature presents challenges in representing mortality when the temperature changes as it does during ramped heating to
some target temperature (again because the log of
the response function, \(\log S\), is non-linear in time
(Eq. (2))). A simple approximation calculates a
time at the lethal target temperature, which is
equivalent to the time at each temperature en-
countered in the ramp to target temperature. This
is easily done for the temperatures in the range
42–46°C since the rate constants are those given
in column four of Table 1. For a ramp which
changes in steps from 42 to 43 to 44°C etc. one
simply multiplies the appropriate rate constant, \(k_L\)
(cf Table 1) by the length of time at each step,
\(\Delta t_{T,L}\) divided by the target rate constant
\(k_0\) and then sums this over all lethal intervals, \(L\), in the
ramp as in:

\[
\Delta t_{LE} = \sum_{L} \frac{\Delta t_{T,L} k_L}{k_0}
\]  

(8)

Thus \(\Delta t_{LE}\) is the Lethal Equivalent of the ramp in
terms of subsequent treatment at 46°C. If the
temperature changes smoothly and continuously
from 42 to 46°C one can fit the rate constants of
Table 1 to a polynomial in time and integrate
over the appropriate range.

3.9. A kinetic model for treatment in the range
30–46°C

For a treatment in which the temperature in-
creases through the conditioning regime (30–
42°C), then moves through a lethal regime (e.g.
42–46°C) and eventually reaches the target tem-
perature (i.e. 46°C) where treatment continues for
a further period of time, the total extra time, \(\Delta t_{46}\),
is a sum of two components given by Eq. (7) and
Eq. (8).

**Fig. 5.** Relationship between static temperature conditioning of *B. tryoni* eggs at 34°C (a), 38°C (b), 40°C (c) and 42°C (expanded scale, d) and the value of “extra treatment time”, \(\Delta t_{46}\), required at 46°C to achieve 99% mortality, compared to eggs which had not
been conditioned. The data \(\Delta t_{46}\) for each treatment time are plotted as open circles with 95% confidence limits as error bars. The
solid lines are the values of \(\Delta t_{46}\) calculated from the model (cf (Eq. (5a))).
\[ \Delta_{46} = \Delta_{t_{CE}} + \Delta_{t_{LE}}. \]  

(9)

The value of the conditioning equivalent, \( \Delta_{t_{CE}} \) will be positive and will increase the total treatment time, while the value of the lethal equivalent of the ramp, \( \Delta_{t_{LE}} \) will be negative and will decrease the treatment time. The value \( \Delta_{t_{46}} \) calculated from Eq. (9), for an arbitrary process, can be substituted into Eq. (4) and the \( LT_{99} \) determined.

### 3.10. Calculation of mortality for a step-wise ramp from 30 to 46°C

The procedure is most easily understood in terms of the 1 h stepwise ramp shown in Fig. 2 where the temperature increases by 2°C every 7.5 min between 30 and 42°C and then by 1°C every 3.75 min. The selection of the time intervals reflects the static temperature conditioning data which were obtained at two degree intervals, whereas the lethal range was measured at one degree intervals. The process, illustrated by the solid heavy line in Fig. 6, starts at 30°C and simply follows the effective rate curves, \( K(T_C, t_C) \), as they change with time and then with temperature up to 42°C. For example, the fit of the experimental data as shown in Fig. 4, yields an effective rate, \( K_C \), at 34°C for 15 min of 0.60 and at 22.5 min \( K_C \) has fallen to 0.5. The contribution to \( \Delta_{t_{46CE}} \) from this 7.5 min interval is easily approximated as \( 7.5 \times (0.6 - 0.5)/2 = 0.41 \) min. In this way all the values of \( K_C \) for all times and temperatures for the step-wise ramp can be obtained and a value for \( \Delta_{t_{46CE}} \) of 24.8 min is obtained. During the last 15 min the bath temperature moves through 42–46°C, which is in the lethal temperature range and the process follows the solid heavy line defined by the rate constants \( k_L/k_0 \) from 1 (here \( k_0 \) is \( k_1 \) at 46°C). For example at 44°C \( k_1 = 0.028 \) and \( k_0 = 0.114 \) so the contribution to \( \Delta_{t_{46CE}} \) for 3.75 min at 44°C is 3.75* \( 0.028/0.114 = 0.95 \) min. This procedure can be followed for all temperatures in the range 42–46°C and the resulting \( \Delta_{t_{46LE}} \) gives a decrease of 3.3 min.

The 1 h ramp used in the experiments varies smoothly and one can improve this simulation by fitting the data points for \( K(T_C, t_C) \) and \( k_1 \) to a polynomial. This gives the heavy broken line in Fig. 6 and on integrating yields \( \Delta_{t_{46CE}} = 24.0 \) and \( \Delta_{t_{46LE}} = -3.3 \) for 20.7 min of extra time at 46°C for a linear 1 h ramp (compared to an experimental time value of 21.2 min). The calculated total time at 46°C would then be 24.0 – 3.3 = 18.8 min compared to an experimental value of total time of 39.8 min.

A similar analysis can be performed for other ramps which raise the temperature from 30 to 46 over intervals of 3, 6 and 9 h. As the ramp time is increased the system is exposed to temperatures in the lethal range of 42–46°C for longer and longer times and the kill becomes more significant. On the other hand as conditioning time is increased, the conditioning rate \( K \) decreases substantially (cf. Fig. 4). Overall the calculated values fall within the 95% confidence limits for the experimental values at the three fastest ramps (Table 3). Fig. 7 illustrates how the generation of the extra time
can be analyzed in terms of two components which are accumulated over time for a given ramp.

4. Discussion and conclusions

The experimental results indicate that both static temperature conditioning and ramped heating affect the treatment time required to achieve 99% mortality of *B. tryoni* eggs at a target temperature of 46°C. Ramped heating also extended the treatment time when a more extreme target temperature of 48°C was used. Conditioning occurs from 30 to 42°C and increases the LT99 as a function of the temperature and duration of treatment, being a maximum near 38°C. The conditioning rate, *K*, is largest during the initial exposure to a given temperature. Exposure to lethal temperatures (≥42°C) encountered as target temperatures are approached, adds to the thermal stress and can decrease the LT99 at target temperatures. These contributions can be significant if the ramp rate is relatively slow.

Beckett and Evans (1997) describe conditioning in mid-aged (18 h) eggs of *B. tryoni* when exposed to air at 35°C for 11 h immediately prior to immersion in 46°C water. The LT99.999 for conditioned eggs was 26.0 min compared to 6.6 min for non-conditioned eggs showing an increase in tolerance by a factor of about four times. This compares with an estimated LT99 for conditioned mature (26–28 h) eggs following exposure to 34°C water for 12 h immediately prior to immersion in 46°C water of 42.7 min compared to non-conditioned eggs with an associated LT99 of 18.6 min. Conditioning in the present study increases egg tolerance by a smaller factor (2.3 × ) compared to the former study (about 4 × ). Direct comparison is made difficult between the studies because of differences in methods (water compared to air as the conditioning medium), insect age, mortality criteria (egg hatch in the present study compared to larval survival in the former study) and the mathematical models used (*c* log–log compared to probit). Most of the differences would bias upward the estimated lethal times of the present study compared to Beckett and Evans (1997), whose values are also low compared to results reported by Heard et al. (1991) for the same species. The differences between the two reported *B. tryoni* studies from Australia are attributed to strain differences. A further study investigating *B. tryoni* conditioning shows significantly enhanced survival in mature eggs (26 h) but not in young eggs (2 h) (Osman et al., 1996). Differences in the mortality response of *B. tryoni* egg with increasing age are well documented (Corcoran et al., 1993).

Meats (1987) did not observe a conditioning effect related to temperature ramps on the mortality of adult *B. tryoni* when the insects were heated from their rearing temperature (15, 25 and 35°C) to 40°C at a rate of 2°C h⁻¹, compared to insects that were more-or-less instantaneously exposed to the target temperature. While the rearing temperature had an influence on the survival at 40°C, (as has been reported by Hallman, 1994), with survival positively correlated with rearing temperature, the rate at which the target was attained had no effect. Possibly 40°C, as a temperature target, does not represent enough of a thermal stress to allow discrimination between insects that have spent time at conditioning temperatures versus those that have not. In the same study ramp effects were observed on the mortality of adult *B. tryoni* when a cold temperature target was attained at a cooling rate of 1°C h⁻¹ versus 1°C min⁻¹, with slower cooling rates being associated with higher mortality.
with greater ability to survive cold temperatures. However, this was only observed when the stress was \(-4.0^\circ\text{C}\) or below and not when the target temperature was \(1.0^\circ\text{C}\), again suggesting the intensity of the stress is important (Meats, 1987). The range of results obtained from the various studies of *B. tryoni*, highlights some of the difficulties encountered when comparing different heat treatment studies even when made using the same species.

The quantification of the magnitude of the conditioning response in *B. tryoni* allows us to develop a kinetic model to describe the mortality of fruit fly eggs in response to changing temperature conditions which occur during ramped heating. The results indicate that mortality can be analyzed in terms of the kinetic expression for percentage survivors, \(S\) as given in Eq. (4) where \(\Delta t_{96} = \Delta t_{CE} + \Delta t_{LE}\). For any heating protocol, values of the conditioning equivalent, \(\Delta t_{CE}\) can be developed in terms of static conditioning experiments using Eq. (7) and values of the lethal equivalent can be obtained from static treatments at lethal temperatures using Eq. (8). Since calculations of \(S\) correlated well with experimental values both as to trends and as to magnitudes of LT\(_{99}\) estimates, one can conclude that the model adequately represents the conditioning and mortality processes. Fig. 6 illustrates how the generation of the extra time can be analyzed in terms of two components which are accumulated over time for a given ramp. Further, as Fig. 7 illustrates, it is possible to follow trends in the components of the extra time as the ramp time is increased.

The amount of conditioning, \(\Delta t_{46}\), as given by Eq. (5a), depends on conditioning time, \(t_C\), and an effective conditioning rate, \(K = m_1(e^{-m_2 t_C} + m_2 e^{-m_1 t_C})\) which changes with conditioning time and temperature \(T_C\). For temperatures 34–40\(^\circ\text{C}\) the decay constants \(m_2\) and \(m_4\) are of the order of \(3 \times 10^{-2}\) and \(2 \times 10^{-3}\) \(\text{min}^{-1}\) respectively, indicating a rapid decay with time and a slower decay with time, respectively. One of the possible uses of such a model is to provide a framework for speculation about the underlying processes associated with conditioning. One could speculate that two processes associated with \(m_2\) and \(m_4\), represent depletion of precursors \(B_1\) and \(B_2\) necessary for development of the heat shock proteins involved in conditioning. An alternative two-term decay form is that of a sequence \(A \rightarrow B \rightarrow D\) where \(B\) is a precursor for the production of heat shock proteins (Laidlaw et al., 1996). Alternatively the inability to condition to high temperatures could reflect the failure of cellular processes, for example disruption of membranes leading to their loss of function.

Another use of the model is in the development of postharvest handling systems and disinfection heat treatments for different commodities which can be a time-consuming and costly exercise. Postharvest handling procedures could add to insect heat tolerance when practices such as de-greening (Shellie et al., 1993) or fruit conditioning are employed (Woolf, 1997; APHIS, 1998). Exposure to temperatures in the conditioning regime of 30–42\(^\circ\text{C}\) which occurs during heating of any fruit from ambient to a target disinfection temperature is not so simply circumvented. Heating of whole fruit necessarily means that fruit tissue and the pests therein spend some time in the range 30–42\(^\circ\text{C}\). However, the results for *B. tryoni* indicate that there may be heating ramps which minimize conditioning. Chamber air ramps with a higher initial temperature, e.g. 42\(^\circ\text{C}\), or chamber ramps which minimize the time fruit spends at strongly conditioning temperatures (around 38\(^\circ\text{C}\)) are suggested approaches to manage the risk of treatment failure.

When in the field, commercially grown fruit and vegetables could easily experience 34\(^\circ\text{C}\) temperatures for 1 h (Ferguson et al., 1998). Such a simple occurrence could increase the required treatment time to achieve a specified level of mortality by up to 100\%. Postharvest treatments are developed using laboratory colonies reared at constant temperature where such occurrences are not present and yet rearing temperature is known to influence thermo-tolerance (Hallman, 1994). On the other hand, commercial treatments are often very robust in that they target the last fruit in a treatment batch to achieve the
disinfestation target treatment thereby ensuring that the treatment is over-delivered for all other fruit in the batch (Waddell et al., 1997a).

The most tolerant life stage of the most tolerant species associated with fruit at the time of harvest is normally the focus of quarantine treatments (MAF, 1994; Waddell et al., 1997b). However, in developing such treatments account should be taken of differences in the thermal history of the crop which is potentially infested with insect pests. Specification of a treatment in terms of a fruit centre target temperature is likely to be inadequate since temperatures and exposure times in the range 32–42°C can have a very significant effect on the time required to reach LT$_{99}$. An efficacious treatment may be the combination of a lethal stress and a particular heating rate which falls within a band, bounded by rates of heating that are too slow or too fast. The use of models will assist in the identification of promising treatments while avoiding extensive in-fruit testing. Additional effort may however be required with regards to the thermal history associated with laboratory insect colonies, the field conditions likely to be experienced by the insects and their hosts (summer versus winter temperatures) and any postharvest handling practices applied to the crops that could alter the efficacy of the treatment.

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References


Hallman, G.J., 1994. Mortality of third instar Caribbean fruit fly (Diptera: Tephritidae) reared at three temperatures and exposed to hot water immersion or cold storage. J. Econ. Entomol. 87, 405–408.


Kaleidagraph, v 5.08d, 1997. Synergy Software, 2457 Perkomen Ave., Reading, PA 19606, USA.


Waddell, B.C., Clare, G.K., Maindonald, J.H., 1997b. Comparative mortality of two Cook Islands fruit fly (Tephritidae) species to hot water immersion. J. Econ. Entomol. 90, 1352–1356.


