Reaction Orders for Thermal Mortality of Third Instars of Mexican Fruit Fly (Diptera: Tephritidae)

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ABSTRACT Mexican fruit fly, *Anastrepha ludens* (Loew), is a quarantine pest of several fruit, including citrus, avocados, and mangoes, from extreme southern Texas to Costa Rica. To provide information for modeling heat phytosanitary treatments, third instars were heated with an aluminum heating block between 44 and 50°C for time intervals up to those causing 100% mortality. At 44 and 50°C, 100% mortality was achieved at 100 and 2 min, respectively. Each 2°C increase in temperature resulted in a three-fourths reduction in the amount of time required to achieve 100% mortality. Mortality was modeled using thermal death kinetics, and the most suitable reaction order was the 0.5th. The thermal death activation energy was 560.7 kJ/mol, which is very similar to the value found for Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann), in a previous study, indicating similar modes of action for heat mortality. However, the Mexican fruit fly had a lower threshold for heat-induced mortality, resulting in less time at all temperatures studied to achieve 100% mortality compared with the Mediterranean fruit fly. This type of information being gathered for fruit flies could lead to the development of generic phytosanitary heat treatments, which are available for other major phytosanitary treatments, such as cold storage, methyl bromide fumigation, and ionizing irradiation.

KEY WORDS *Anastrepha ludens*, heat treatment, phytosanitary, quarantine, commodity treatment

PHYTOSANITARY TREATMENTS ARE USED on quarantined commodities to control regulated pests that might be present so that the commodities may be marketed across quarantine barriers. Most quarantined fresh agricultural commodities are treated with one of four major methods: cold air storage (approximately −0.5–2°C for 7–40 d), heated water or air (≈43–50°C for 0.3–14 h), fumigation with methyl bromide (8–64 g/m^3), or ionizing irradiation (150–400 Gy) (Hallman 2002). Research to develop and certify these treatments must demonstrate that they are efficacious at levels of security near 100%. This requires the treatment of large numbers (up to 100,000, in some cases) of organisms with no failures.

All of these major treatment methods except heat currently have some generic, broad application. Cold requires considerable time, and the time count is usually not started until the entire load reaches a certain temperature as ascertained by temperature probes strategically located in what are considered the hardest-to-cool areas. Time to reach that critical temperature is usually not a regulatory consideration, so size of commodity is seldom an issue. For example, a cold treatment at 1.1°C against Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann), requires 14 d for several types of fruit ranging in size from grape to grapefruit. Methyl bromide fumigation is often used in the same manner. For example, the same methyl bromide fumigation parameters are used against *Anastrepha* spp. on citrus fruit from Mexico whether the host is tangerine or grapefruit although the former may be one-eight the weight of the latter. With irradiation, the same absorbed dose per pest is used regardless of the commodity.

Heat treatments have not been used generically. Heat treatments are relatively short, and fresh commodities generally do not tolerate longer heat treatments designed to work on any size commodity. Fruit size, density, and shape; orientation in the fluid stream; fluid speed; makeup of the fluid; and other factors may affect heating rate. Therefore, developing a heat treatment to the stage that it can be used commercially has typically been specific to pest and commodity, even shape and size of commodity. For example, the mango hot water immersion treatment at 46.1°C consists of five different treatment times (between 65 and 110 min), depending on the shape and weight of the mangoes, and is only valid for mangoes from specific geographical regions of the New World (APHIS 2003). Mangoes >0.9 kg are not approved for hot water treatment. Commercial development of heat treatments has required considerably more work to achieve less progress compared with other major treatments (Hallman 2006). The research done to allow the import of hot water-treated mangoes into the United States involved the treatment of millions of fruit fly immatures and thousands of mangoes (Sharp 1988). Even then, the treatment experienced efficacy

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problems, and further research was required to correct the problems (APHIS 2003).

One promising avenue to reduce the amount of data needed to accelerate the development of heat phytosanitary treatments is through modeling thermal death kinetics of quarantine pests coupled with heating of commodities (Jang 1996). The 0.5th order thermal death kinetic model fit heat mortality of Mediterranean fruit fly third instars (Gazit et al. 2004). Wang et al. (2001) developed a simulation model to study heat transfer in spherical fruit. The combination of thermal death models for quarantine pests with simulation models for heat transfer in fruits could provide for models of heat phytosanitary treatments.

The Mexican fruit fly, *Anastrepha ludens* (Loew), is a quarantine pest of several fruit, including citrus, avocados, and mangoes, from extreme south Texas to Costa Rica (Thomas 2000). This pest has resulted in quarantines and market disruptions in parts of California. Norrbom and Kim (1988) list 50 specific hosts of *A. ludens.* Thomas (2004) documents the host plasticity of the species by finding it to be a quarantine pest of a previously unknown host, manzano chile peppers, *Capsicum pubescens* Ruis & Pavon. These hosts vary greatly in size, weight, and shape, complicating the development of broadly applicable quarantine heat treatments.

A quarantine treatment should be effective against the most tolerant stage that may be present in the marketed commodity. Mangan et al. (1998) review several studies and concluded that third-stage *A. ludens* was the most heat tolerant stage based on tests to eggs and larvae inside mangoes and in vitro hot water immersion tests.

The objectives of this study were to determine thermal mortality of third instars of *A. ludens* at different temperature–time combinations within the range of temperatures used for heat treatments and to develop thermal death kinetic models as a step in modeling phytosanitary heat treatments against the insect and fruit flies in general by using rapid heating technologies such as radiofrequency and ohmic heating (Hallman 2002).

Materials and Methods

Mexican Fruit Fly. *A. ludens* used in this research was from a colony held at the Weslaco ARS laboratory for 2 yr. The colony originated from feral flies collected near Montemorelos, Nuevo Leon, Mexico. The larval rearing medium is described by Spishakoff and Hernandez-Davila (1968). Rearing conditions were ≈26°C, 80% RH, and a photoperiod of 12:12 (L:D) h. Fully grown third instars were separated from the diet by using water and a steel mesh sieve.

Heating System. A heating block system developed by Ikediala et al. (2000) was used to heat the third instars. It consisted of two aluminum blocks (25.4 cm^2) with bottom and top thickness 2.5 and 2 cm, respectively. The blocks fit together, leaving a 3-mm gap between them where the larvae are placed. Electric heating pads were attached to the back of each plate. Type T thermocouples inserted through holes near the center of each block monitored temperatures. Heating rate, set-point temperature, and exposure time were controlled by a customized Visual Basic program and PID controllers (Omega Engineering Inc., Stanford, CT) via a solid state relay.

Procedures. One hundred third instars freshly removed from rearing medium were placed along with ≈7 ml of the rearing medium on the bottom of the heating block at 24°C, and the top was immediately put in place. The temperature was raised at the rate of 15°C per minute until it reached 44, 46, 48, or 50°C and held at that temperature for different times to obtain a range of mortalities up to 100%. Immediately upon completion of the holding time, the block was opened and held at an elevated angle to allow larvae to roll into plastic containers with ≈100 ml of moist vermiculite. Any larvae that did not fall off were immediately removed by delicate brushing. Each temperature–time combination was replicated six times. Control insects were placed on the heating block at 24°C for 100 min, the time of the longest heated exposure.

Because dead larvae are not always obvious, mortality was determined 24 h after treatment. If the larva had turned dark gray, had not initiated pupariation, or would not respond to gentle prodding, it was considered dead.

Data Analyses. Mean survival ratios as a function of exposure times at each of the four treatment temperatures were used to develop the thermal death kinetic model. It follows a model used for Mediterranean fruit fly (Gazit et al. 2004) and is based on the following equation:

$$\frac{d(N/N_0)}{dt} = -k(N/N_0)^n \qquad [1]$$

where N_0 and N are the initial and surviving numbers of larvae, t is exposure time (minutes), k is the thermal death rate constant (1/min) when temperature is constant, and n is the kinetic order of the reaction. Where survival was 0%, a value of 0.6 (the addition of 0.1 for each of six replicates) was used for N as a hypothetical value too low to be measured by the sample size to avoid zero in the numerator. An equal value for each replicate avoids adding artificial experimental error. The integration form of equation 1 can be obtained for different reaction orders as follows:

$$\begin{aligned} \ln(N/N_0) &= -kt + c \quad (n = 1) \\ (N/N_0)^{1-n} &= -kt + c \quad (n \neq 1) \end{aligned} \qquad [2]$$

where c is the Y intercept of the regression line. For each temperature, survival (N/N_0) was regressed against exposure time (t) according to equation 2. Previous studies used reaction orders in increments of 0.5 (Wang et al. 2002a, b, Johnson et al. 2004, Gazit et al. 2004). In all of these previous four studies the 0.5h reaction order resulted in the largest mean coefficient of determination (R^2), which thus was considered the most suitable. Results from some of those studies indicate that further refinements in the selection of reaction order might give better fit. For example, the

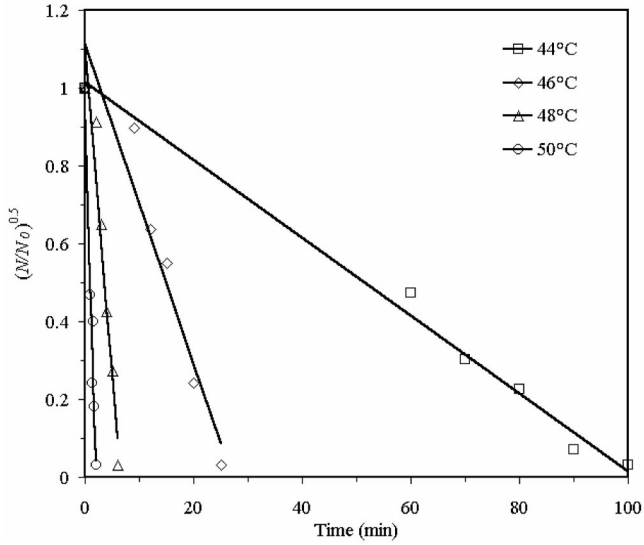

Fig. 1. Thermal mortality curves of third-instar Mexican fruit fly at four temperatures by using a 0.5th order kinetic model.

0.5th order yielded the highest R^2 for Mediterranean fruit fly eggs at the lowest temperatures used, whereas the 1.0st order gave the highest R^2 for the highest temperatures (Gazit et al. 2004). Perhaps an intermediate order, say 0.75, would yield a better fit. In this study, therefore, we tested the 0, 0.25, 0.5, 0.75,1, and 1.5th reaction orders. The reaction order with the largest mean coefficient of determination (R^2) across all four temperatures was selected as the most suitable for further calculations. Upon selection of the reaction order, values of k and c were derived by regression.

Activation Energy. Two independent methods were used to calculate the thermal death activation energy (E_a in Joules per mole) of third instars of Mexican fruit fly. E_a is used to determine the sensitivity of organisms to changes in temperature; higher activation energy denotes higher sensitivity. In one method, E_a was calculated by the following equation:

$$E_a = \frac{2.303RT_{\min}T_{\max}}{z} \qquad [3]$$

where R is the universal gas constant (8.314 J/mol K), T_{min} and T_{max} are the minimum and maximum absolute temperatures (°K), respectively, of a test range, and z is the negative inverse of the slope of the thermal death time curve expressed in degrees Celsius.

The second method for calculating E_a was through the slope of an Arrhenius plot of log k versus the reciprocal of the absolute temperature ($1/T$) as follows:

$$log\ k = log\ k_0 - \frac{E_a}{2.303RT} \qquad [4]$$

where k_0 is the reference thermal death rate constant (1/min).

Results and Discussion

Mexican Fruit Fly Mortality. Mortality of control third instars of *A. ludens* was very low (<1%) because the time involved was short (100 min at 24°C). Abbotts formula for removing natural mortality from the treatments (Finney 1971) was applied to the high levels of mortality observed in the heat treatments and rounded to the nearest percentage; however, the values did not change because the control (natural) mortality was so low. Mortality of third instars of *A. ludens* subjected to 44–50°C in the heating block is presented in Fig. 1. For every 2°C increase in temperature, the time to reach 100% mortality decreased by approximately three-fourths of that time. This result is similar to mortality estimates presented by previous studies with Mexican fruit fly eggs and third instars heated in water without host material (Mangan et al. 1998, Thomas and Mangan 1997), estimates of Mediterranean fruit fly third instars in the heating block (Gazit et al. 2004), and data for older larvae of the red flour beetle, *Tribolium castaneum* (Herbst), treated in the heating block (Johnson et al. 2004). Agreement is mixed for estimates of Mexican fruit fly first and second instars heated in water without host material (Mangan et al. 1998) and estimates of Mediterranean

Table 1. Coefficients of determination (R^2) from kinetic order (n) models for thermal mortality of third instars of Mexican fruit fly at four temperatures

Temp (°C)	$n = 0$	$n = 0.25$	$n = 0.5$	$n = 0.75$	$n = 1$	$n = 1.5$
44	0.950	0.981	0.990	0.925	0.755	0.424
46	0.930	0.944	0.935	0.871	0.735	0.490
48	0.938	0.953	0.945	0.870	0.705	0.435
50	0.868	0.905	0.928	0.878	0.701	0.370
Mean	0.922	0.946	0.950	0.886	0.724	0.430

Table 2. Comparison of predicted lethal times (minutes) obtained by the 0.25th kinetic model and observed exposure to achieve 100% mortality for third instars of Mexican fruit fly at four temperatures

Temp (°C)	Observed 100% mortality	0.25th order kinetic model							
		LT_{95}	95% CI	LT_{99}	95% CI	$LT_{99.83}$	95% CI	$LT_{99.9968}$ (probit 9)	95% CI
44	100	82.1	75.2–89.0	89.2	81.6–96.8	91.4	83.5–99.3	92.2	84.2–100.2
46	25	22.1	18.3–25.9	23.8	19.6–28.0	24.4	20.1–28.7	24.5	20.1–28.9
48	6	5.3	4.5–6.1	5.8	4.9–6.7	5.9	4.9–6.9	5.9	4.9–6.9
50	2	1.6	1.3–1.9	1.8	1.5–2.2	1.8	1.4–2.2	1.9	1.5–2.3

Table 3. Comparison of lethal time (minutes) predictions obtained by the 0.5th kinetic model and observed exposure to achieve 100% mortality for third instars of Mexican fruit fly at four temperatures

Temp (°C)	Observed 100% mortality	0.5th order kinetic model predictions							
		LT_{95}	95% CI	LT_{99}	95% CI	$LT_{99.83}$	95% CI	$LT_{99.9968}$ (probit 9)	95% CI
44	100	79.2	74.4–84.0	91.5	85.9–97.2	97.4	91.3–103.6	101.0	94.4–107.5
46	25	21.7	17.7–25.6	24.7	19.9–29.4	26.1	21.0–31.2	27.0	21.6–32.3
48	6	5.3	4.4–6.1	6.0	4.9–7.0	6.3	5.2–7.5	6.6	5.3–7.8
50	2	1.6	1.3–1.9	1.9	1.5–2.2	2.0	1.6–2.4	2.1	1.7–2.5

fruit fly eggs in the heating block (Gazit et al. 2004). Our data do not agree with mortality estimates for oriental fruit fly, *Bactrocera dorsalis* (Hendel), third instars heated in water without host material (Jang 1991).

Thermal Death Kinetic Orders. R^2 values for kinetic reaction orders 0.25 and 0.5 were very similar and higher than R^2 values for the other orders studied (Table 1). Lethality time estimates at the high levels of mortality required of phytosanitary treatments (near $LT_{99.9968}$) for the 0.5th order model were ≈10% greater than those for the 0.25th order model for all temperatures (Tables 2 and 3). Because regulatory agencies may be more likely to accept models that yield higher levels of quarantine security, all other factors being equal, we have chosen the 0.5th kinetic reaction order for all further calculations. Thermal death mortality curves for the 0.5th order are shown in Fig. 1. As expected, the slope increased sharply as temperature increased, resulting in much lower times to achieve 100% kill.

Model constants fitted by the 0.5th kinetic order are given in Table 4. The thermal death rate constant k increased with temperature, whereas the constant c was nearly within the margin of error of the ideal of one for all four temperatures. A value of one for c means that at the initiation of the time interval (after the temperature increase phase was completed) no mortality had occurred compared with unheated controls. The results show that mortality during the temperature increase phase was minimal.

Thermal Death Time Curve and Activation Energy. Fig. 2 plots the minimum times to achieve 100% mortality of 600 third instars on a semilog scale versus temperature. The z value derived from the negative inverse of the slope of the thermal death time curve was 3.5°C. This means that every 3.5°C increase in temperature leads to a 10-fold decrease in time required to reach the same level of mortality. The thermal death activation energy value (E_a) was 560.7 kJ/mol from equation 3 and similarly, 550.0 kJ/mol from equation 4 (Fig. 3). The thermal death activation energy for Mediterranean fruit fly third instars was similar (551.9 kJ/mol) (Gazit et al. 2004), leading to speculation that heat-induced mortality may have the same mode of action for third instars of both species. If true, this could aid the modeling of generic heat treatments for tephritid fruit flies within the same or similar commodities.

The Mediterranean fruit fly was found to be more heat tolerant than the Mexican fruit fly when comparable methodology was used (Fig. 2; Gazit et al. 2004). This indicates that Mediterranean fruit fly has a higher threshold for heat mortality than the Mexican fruit fly, but after that threshold is reached mortality progresses in a similar manner. Thus, quarantine heat treatments for commodities that could be infested by both species, such as fruit in Mexico and parts of Central America, need only be researched with Mediterranean fruit fly, because any heat treatment to control it would control Mexican fruit fly. Indeed, data from Sharp (1988) used to summarize research to develop a hot water immersion treatment for mangoes indicate that a Mediterranean fruit fly laboratory col-

Table 4. Thermal death kinetic parameters for the 0.5th kinetic model, SEM, and determination coefficients (R^2) for third instars of Mexican fruit fly at four temperatures

Temp (°C)	Thermal death constants of $(N/N_0)^{0.5} = -kt + c$		
	$k \pm$ SE	$c \pm$ SE	R^2
44	0.0100 ± 0.0005	1.0152 ± 0.0395	0.990
46	0.0412 ± 0.0054	1.1160 ± 0.1058	0.935
48	0.1690 ± 0.0204	1.1127 ± 0.0984	0.945
50	0.4607 ± 0.0640	0.9631 ± 0.1012	0.928

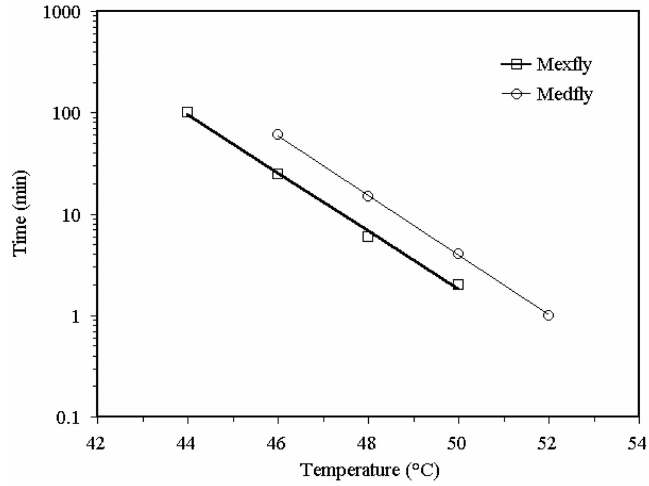

Fig. 2. Minimum time–temperature combinations for complete kill of 600 third instars of Mexican fruit fly (Mexfly) compared with those for 600 third-instar Mediterranean fruit fly (Medfly). Line for Mexican fruit fly represents linear regression equation $\log t = 14.554–0.286\ T$ ($R^2 = 0.997$) where t is time (minutes) and T is temperature (°C).

ony in Mexico may have been more heat tolerant than two laboratory colonies of Mexican fruit fly in Texas. However, a feral colony of Mexican fruit fly in Mexico may have been more tolerant than the three laboratory colonies of both flies observed. But there were differences in the techniques used in these studies, which illustrate that modeling of heat treatments can be affected by various factors, such as source of test organisms, rearing temperature regime, treatment technique, and precise definition and measurement of efficacy (Hallman 2000). These factors must be examined, and any effect on mortality must be quantified before modeling of thermal death for heat quarantine treatments can be used as a phytosanitary measure (Jang 1996). The development and adoption of a uniform heating system, as exemplified by the heating block used in this study (Ikediala et al. 2000), and its employment to quantify thermal mortality on several insect species already, as summarized in Gazit et al. (2004), is a step in that direction.

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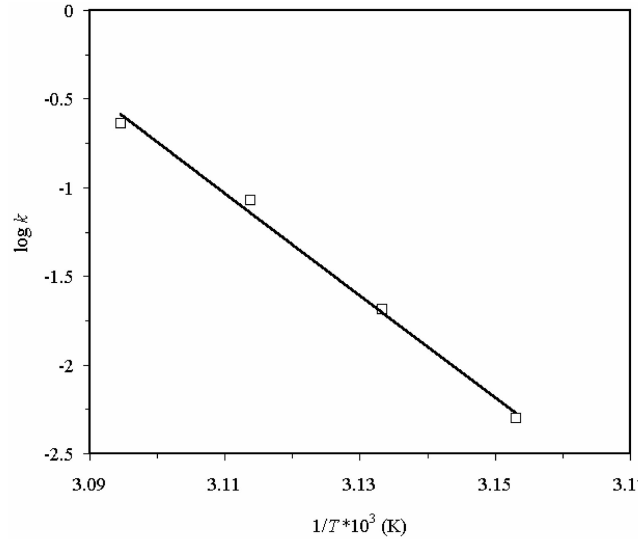

Fig. 3. Arrhenius plot for temperature effects on thermal death rate constant for third-instar Mexican fruit fly. The straight line ($\log k = 88.300 - 28.724 * 1000/T$) was obtained by linear regression ($R^2 = 0.995$).

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