Thermal Death Kinetics of Egg and Third Instar Mediterranean Fruit Fly (Diptera: Tephritidae)

Y. GAZIT,[1] Y. ROSSLER,[1] S. WANG,[2] J. TANG,[2] AND S. LURIE[3]

J. Econ. Entomol. 97(5): 1540–1546 (2004)

ABSTRACT Two developmental stages of *Ceratitis capitata* (Wiedemann), 24-h-old eggs and third instars, 8 d after oviposition, were subjected to thermal exposures in a heating block system, at various temperatures of 46, 48, 50, and 52°C to determine the thermal death kinetics of the insects. At these temperatures, 100% mortality was achieved by exposure of 300 *C. capitata* larvae for 60, 15, 4, and 1 min, respectively. The 0.5 order kinetic model had the best fit to the survival ratio for all the treatment temperatures, hence it was used for the prediction of the lethal times. The thermal death time (TDT) curves showed that the third instars were more heat-resistant than eggs, especially at the two low temperatures (46 and 48°C). Under temperature–time combinations that did not result in complete kill, the thermal mortality for eggs was also significantly higher than that for third instars. The activation energy values calculated from the TDT curves were 490.6 and 551.9 kJ/mol, respectively, for thermal death of eggs and third instars.

KEY WORDS Mediterranean fruit fly, thermal death kinetics, heat treatment, heating block, radio frequency

FRUIT FLY INFESTATION IS a major problem in the production, storage, marketing, and export of citrus. Many countries require inspection certificates for absence of targeted live pests in a shipment after a preapproved postharvest "sanitation" treatment. Currently cold storage is the only treatment used commercially in Israel to disinfest citrus from Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae). This treatment requires that the fruit be stored for up to 16 d at 1.5°C. Unfortunately, the safety margin for fruit quality for this treatment is rather narrow, with many varieties developing chilling injury from the treatment (Wardowski et al. 1973, Schiffman-Nadel et al. 1975). Therefore, alternative heat treatments are of interest.

Citrus is of subtropical origin and thus relatively tolerant to heat. The efficacy of heat as a method for disinfesting fresh commodities from fruit flies has been demonstrated over the last decade by the success of forced hot air facilities for mangoes, grapefruits, and oranges in commercial applications (Mangan and Ingle 1992, 1994; Mangan et al. 1998). These treatments require that the fruit center reaches a target temperature within a given time and that this temperature is held for a required period to ensure a complete kill of targeted insects in fruit. Heating methods such as hot water immersion, forced hot air or vapor heat rely on heat conduction to transfer heat from the fruit surface to the fruit interior. The temperature gradient established inside the fruit is directly influenced by fruit size, with larger fruit heating at a slower rate (Wang et al. 2001b).

The lengthy conventional heating required to control insect pests for large fruit such as citrus may result in thermal injury to the product (Lurie 1998). To minimize thermal damage to fruit, a more uniform and fast heating method by using electromagnetic energy at radio frequencies (RFs) may be used. RF heating is commonly used in commercial applications in the food, textile, and other industries and has indeed shown promises as a postharvest measure to control insect pests in selected commodities (Andreuccetti et al. 1994; Hallman and Sharp 1994; Nelson 1996; Tang et al. 2000; Wang et al. 2001a, 2002c).

Information on the thermal resistance of different life stages and thermal death kinetics for targeted insects such as the Mediterranean fruit fly is needed in developing effective thermal treatments for citrus. The temperatures used in the commercial heat treatments have been determined first by testing in vitro the thermal exposure necessary to kill different stages of fruit flies, then by testing of fruits infested with a sufficient population of fruit fly to determine probit 9 values (99.9968% mortality) required by most countries to meet quarantine security levels. Temperatures above 40°C are needed against larvae of a number of commercially important fruit fly pests (Heard et al. 1991; Mangan and Ingle 1992, 1994; Nascimento et al. 1992; Sharp and Hallman 1992; Sharp and Gould 1994).

[1] The Israel Cohen Institute for Biological Control, Citrus Marketing Board of Israel, P.O. Box 80, Bet-Dagan 50250, Israel.
[2] Department of Biological Systems Engineering, Washington State University, 213 L. J. Smith Hall, Pullman, WA 99164–6120.
[3] Department of Postharvest Science, ARO, The Volcani Center, P.O. Box 6, Bet-Dagan 50250, Israel.

0022-0493/04/1540–1546$04.00/0

Two different approaches were used to determine the tolerant stage in fruit flies. The direct hot water immersion of the insects (Jang 1986, Waddell et al. 1997, Jang et al. 1999) showed that the egg is the most tolerant stage and the third instar is more susceptible to heat. Nevertheless, when fruit infested with fruit flies was subjected to forced hot air treatments (Heather et al. 1997), the opposite result was obtained where the egg was the most susceptible to the heat. Hansen and Sharp (1998) suggested that the water immersion restricted aerobic oxygen from the heated fruit fly larvae thus synergized its mortality. It is desirable to determine thermal resistance of fruit flies subjected to a heating system that will provide the heated insect free access to air.

A unique experimental heating block system (HBS) has been developed for testing responses of insects to high temperatures (Ikediala et al. 2000; Wang et al. 2002a,b; Johnson et al. 2003). The HBS is able to heat insects directly and uniformly at a given temperature–time combination. The HBS can be programmed to simulate the heating rate of the interior of fruit when subjected to different heating methods such as hot air, hot water, and RFs.

The objectives of this study were 1) to determine the thermal mortality of egg and third instars of Mediterranean fruit fly at different temperature–time combinations by using the HBS, 2) to develop thermal death kinetic models both for egg and third instars based on the experimental data, and 3) to compare the heat resistance between eggs and third instars.

Materials and Methods

Test Insects. Mediterranean fruit flies were derived from the laboratory colony held in the Israel Cohen Institute for Biological Control, Citrus Marketing Board of Israel (Rössler 1975). This colony is being replenished every 2 to 3 yr by field-collected male flies to maintain genetic diversity as close as possible to the field population. The rearing conditions were 24 ± 1°C and 60–80% RH. Two developmental stages of the flies were used: eggs, 24 h after oviposition, and fully grown third instars. To prepare the insects for the tests, ≈3,000 adult flies, supplied with hydrolyzed yeast and sugar [1:2 (wt:wt)], were placed in screen cages above trays with water. After 1 h, the newly oviposited eggs were collected from the water. Part of the eggs were placed over wet filter paper and incubated for 24 h (eggs 24 h). The rest were subjected for further development on larval media, consisting of sugar, brewer yeast, wheat bran, and water [12:8:27:50 (wt:wt/wt:wt)], added with hydrochloric acid 37% (1.6%) and nipagin (0.4%) to prevent bacterial and fungal contamination. Fully grown larvae were collected from the diet 8 d after the oviposition (1 d before pupation).

Heating Block System. The HBS consisted of two aluminum blocks, 25.4 by 25.4 cm, with the bottom and top thickness of 2.5 and 2.0 cm, respectively. When the two blocks were assembled one on top of the other, a 3-mm gap was left in between, forming a closed chamber for the insects. Electric heating pads (250 W, 120 V, Heat-Con., Inc., Seattle, WA) attached to the other sides of the blocks provided the desired heat fluxes. The HBS was capable of directly and uniformly heating insects at any rates between 0.2 and 15°C/min. Calibrated type-T thermocouples, inserted through sensor holes near the center of each block, were used to monitor the temperatures of the top and bottom blocks. The block temperature was controlled via PID controllers (I32, Omega Engineering, Inc., Stamford, CT) to increase linearly to within ±0.3°C of the desired set point.

Procedures. Insects treated in the HBS were placed in flat envelopes made of thin aluminum foil (10–20 μm in thick). In preliminary tests, we found that the envelopes readily transferred the heat to the insects without significant affect on their overall temperature profiles in the HBS. The envelopes kept the insects in closed and humid environment with enough air supply to allow a quick removal of all the insects from the HBS in <5 s. This enabled a precise termination of the thermal exposure, which was a critical advantage, especially when testing under high temperature–short time combinations that simulate RF treatments.

Handling Eggs. One hundred eggs were lined with thin paintbrush on a piece of black filter paper, 2 by 2 cm, soaked with nipagin solution (0.01% nipagin in water), which was centered on a piece of flat aluminum foil, 15 by 10 cm. The eggs were then covered with another piece of foil, 7 by 10 cm with a 3-cm-diameter round bulge, ≈1 mm in height, formed against a plastic cap. After ensuring that the eggs were not in direct contact with the top foil, the rims of the bottom foil were folded up over the edges of the covering foil forming a closed envelope. After the treatment, the filter paper carrying the eggs was removed from the envelope and soaked again with nipagin solution and flattened over larval diet in a petri dish. A few drops of the solution were dripped on the margins and on the diet to ensure sufficient wetness of the eggs. The petri dish was then covered and incubated in ambient conditions (25°C) for egg hatch and larval development. As the larvae matured, the petri dishes were uncovered and placed for pupation in 270-ml plastic caps, by using fine vermiculite as a pupation medium. After pupation, the vermiculite was sifted and the number of pupae was recorded. For each replication, one envelope with eggs or larvae was left untreated in room temperature (25 ± 1°C) for a period longer than the treatments and was served as control.

Handling Third Instars. One hundred larvae, 8 d after oviposition (1 d before pupation), were collected from the diet with forceps and placed in the center of 15 by 20-cm aluminum foil, over a 3 by 3-cm filter paper soaked with nipagin solution. The foil was then gently folded along its center and the free margins were folded tightly (to prevent the escape of the larvae) to form a closed envelope. After the treatment, the envelope was open and the larvae were transferred with a thin paintbrush to a petri dish with larval

diet at room temperature. A few drops of the nipagin solution were added to moisten the larvae, which were then mixed gently with forceps into the diet to ensure wetness and air. The dish then was covered and maintained at 25°C for 1 d, allowing the surviving larvae to complete their development. Larvae were allowed to continue development to pupation as described for eggs.

Treatment Design and Statistical Analysis. For the two developmental stages, four temperature set points were chosen: 46, 48, 50, and 52°C. At each temperature, four to six exposure times were selected to provide a wide range of mortality levels, including 100%. Before each treatment, the HBS was preheated to 25°C, bringing the insects to a constant initial temperature. Based on our preliminary results, the heating rate of oranges in RF systems can easily reach 15°C/min. This heating rate was selected for all treatments to reduce the effect of ramping time and simulate the rapid heating of citrus when subjected to RF energy. After each run, the HBS was chilled back to room temperature (25°C) by placing plastic bags containing ice water on the blocks. For the lower temperature sets, we placed more than one envelope (up to four) in the HBS. Because removing one envelope was relatively fast and caused no effect on the overall temperature of the others, removing them one at a time enabled serial exposures in a single run at a fixed temperature.

Mortality rates both for eggs and for third instars were determined by subtracting the number of pupae from the initial number of insects, with respect to the rate of the control nontreated insects, by using Abbott's formula (Abbott 1925). The mean and standard deviation values of the corrected data were calculated over three replicates for a total of 300 insects. These data were used to plot the mortality–exposure curves for each temperature. The data were then further used to establish thermal death kinetic models and to compare the heat resistance between eggs and third instars. The thermal resistance between eggs and third instars was compared at four different temperature–time combinations: 46°C for 20 min, 48°C for 2 min, 50°C for 2 min, and 52°C for 0.17 min. Corrected treatment mortality for eggs and third instars was compared for each temperature–time combination using the SAS *t*-test (TTEST) procedure (SAS Institute 1989). We used an arcsine transformation to normalize the data before analysis.

Thermal Death Kinetic Model. The mean survival ratios as a function of exposure times at each temperature were used to develop the thermal death kinetic models. A value of 0.001 was used for the survival ratio (N/N_0) where the actual survival was 0%. This value corresponded to 0.3 insect survival among 300 insects. The kinetic model was similar to that previously used for codling moth, *Cydia pomonella* (L.) (Wang et al. 2002a), Indianmeal moth, *Plodia interpunctella* (Hübner) (Johnson et al. 2003), and navel orangeworm, *Amyelois transitella* (Walker) (Wang et al. 2002b). It was based on the following equation:

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

where k is the thermal death rate constant (1/min), n is the kinetic order of the reaction, N and N_0 are the surviving and initial numbers of insects, and t is the exposure time (minutes). The integration form of equation 1 can be obtained for different reaction orders as follows:

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

For each temperature, survival (N/N_0) was regressed against exposure time (t) according to equation 2 for the 0, 0.5, 1, 1.5, and 2 reaction orders. The most suitable reaction order was determined by comparing the mean coefficients of determination (r^2) for all treatment temperatures. After the reaction order was fixed, the values of k and c were derived from the regression equation. The developed kinetic models were reversibly used to estimate the LT_{95}, LT_{99}, $LT_{99.67}$ and $LT_{99.9968}$ (probit-9) for each treatment temperature by inputting 0.05, 0.01, 0.0033, and 0.000032 for N/N_0, respectively. The estimated $LT_{99.67}$, which was one survivor out of 300 treated insects, closely corresponded to the observed exposures that produced a complete kill of 300 insects.

A thermal death time (TDT) curve for eggs and third instars was developed by plotting each temperature against the minimum exposure time required to achieve 100% mortality of 300 tested insects on a semilog scale (Wang et al. 2002a,b, Johnson et al. 2003). The z value, the temperature difference required for a 10-fold change in the mortality rate, was obtained from the negative inverse of the slope of the TDT curve (Celcius). It was used to calculate the activation

Table 1. Coefficients of determination (r^2) from kinetic order (n, 0, 0.5, 1, 1.5, and 2) models for thermal mortality of Mediterranean fruit fly eggs and third instars at four temperatures

Temp. (°C)	Eggs					Third instars				
	0	0.5	1	1.5	2	0	0.5	1	1.5	2
46	0.911	0.974	0.952	0.766	0.600	0.945	0.992	0.842	0.562	0.472
48	0.915	0.974	0.896	0.731	0.606	0.924	0.990	0.831	0.608	0.557
50	0.885	0.932	0.947	0.917	0.819	0.889	0.981	0.932	0.663	0.545
52	0.740	0.913	0.958	0.723	0.592	0.789	0.893	0.957	0.954	0.869
Mean	0.863	0.948	0.938	0.784	0.634	0.870	0.964	0.891	0.697	0.611

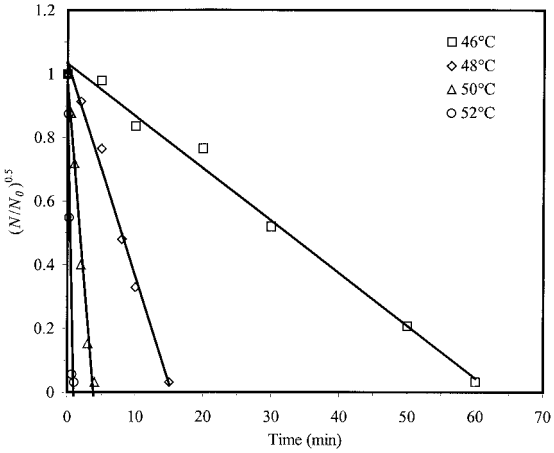

Fig. 1. Thermal mortality curves of third instars of Mediterranean fruit fly at four temperatures by using a 0.5 order kinetic model.

energy (E_A, Joules per mole) needed for thermal death of test insects according to the following relationship (Tang et al. 2000, Wang et al. 2002b, Johnson et al. 2003):

$$E_A = \frac{2.303RT_{\min}T_{\max}}{z} \quad [3]$$

where R is the universal gas constant (8.314 J/mol K), $T_{\min}$ and T_{max} are the minimum and maximum absolute temperatures (Kelvin) of a test range, respectively. Activation energy for thermal death of test insects was also calculated from the slope of an Arrhenius plot of log k versus the reciprocal of the absolute temperature ($1/T$) (Tang et al. 2000, Wang et al. 2002b) as follows:

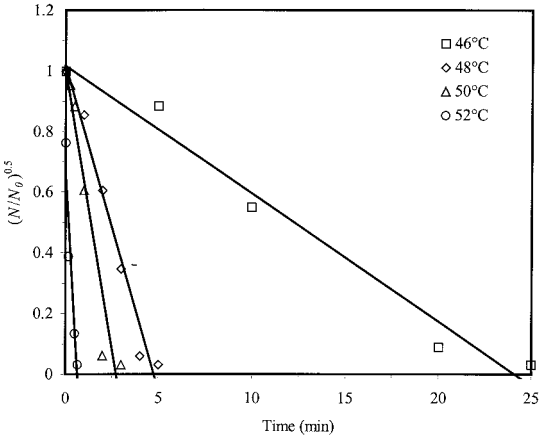

Fig. 2. Thermal mortality curves of Mediterranean fruit fly eggs at four temperatures by using a 0.5 order kinetic model.

$$k = k_{ref}e^{\frac{-E_A}{R}\left(\frac{1}{T}-\frac{1}{T_{ref}}\right)} \quad [4]$$

where T is the absolute temperature (Kelvin), and k_{ref} is the thermal death rate constant at the reference absolute temperature T_{ref} (Kelvin).

Results and Discussion

Determination of Thermal Death Kinetic Orders. The rates of pupation of the untreated control insects were 89.3 ± 0.55 and 97.3 ± 0.69% of the untreated eggs and third instars, respectively. This indicates that the handling procedure and the treatment method were appropriate for the study. But the low control mortality was still used for final mortality corrections.

Coefficients of determination (r^2) derived for each kinetic order model and treatment temperature for eggs and third instars are listed in Table 1. Although the first order model provided higher r^2 values both at 50 and 52°C for eggs, and the first and 1.5 order models provided a higher r^2 value at 52°C for third instars, the 0.5 order model (Figs. 1 and 2) produced the highest mean r^2 values over all the four treatment temperatures, both for third instars and eggs. The 0.5 order model has been found to be the most applicable for codling moth (Wang et al. 2002a), Indianmeal moth (Johnson et al. 2003), and navel orangeworm (Wang et al. 2002b). Thus, the 0.5 order model was used for further calculations in this study.

Table 2 lists the model constants fitted by the 0.5 order kinetic models at each temperature for eggs and third instars, respectively. The thermal death rate constant k increased with the temperature. Ideally, the value of constant c in equation 2 should be equal to 1 because at the time zero the survival number should be the same as the initial number of insects (i.e., $(N/N_0)^{0.5} = 1$ at $t = 0$). In reality, however, the best-fitted curve may not pass this point as shown in Table 2, because of some initial mortality at $t = 0$, especially for 52°C. The fitted regression lines based on the experimental data at each temperature were shown in Figs. 1 and 2 for third instars and eggs, respectively. The r^2 values in Table 1 suggested a better model prediction for third instars than for eggs.

Table 3 lists the lethal times and 95% CIs (from LT_{95} to $LT_{99.9968}$) predicted by the 0.5 order kinetic models for third instars and eggs. In this study, the observed exposure time for 100% mortality of all 300 test insects corresponded to the estimated lethal times to produce >99.67% mortality (to kill >299 insects out of 300). The results showed that the predicted $LT_{99.67}$ was slightly smaller than the observed time to achieve a complete kill of 300 insects. The 0.5 kinetic model seemed to have underestimated lethal times for eggs and third instars at each temperature compared with the experimental values. At the temperatures of 50 or 52°C, the lethal times of experimental data and predicted values were not much different from practical point of view. Lethal times increased with increasing insect sample sizes (e.g., from 50 to 31,250).

Table 2. Thermal death kinetic parameters for the 0.5 order kinetic model $(N/N_0)^{0.5} = -kt + c$, standard errors (SE) of the means and determination coefficients (r^2) for Mediterranean fruit fly eggs and third instars at four temperatures

Temp. (°C)	Eggs			Third instars		
	$k \pm$ SE	$c \pm$ SE	$r2$	$k \pm$ SE	$c \pm$ SE	$r2$
46	0.0422 ± 0.0040	1.0173 ± 0.0824	0.974	0.0165 ± 0.0007	1.0327 ± 0.0383	0.992
48	0.2139 ± 0.0175	1.0179 ± 0.0731	0.974	0.0674 ± 0.0035	1.0361 ± 0.0427	0.990
50	0.3681 ± 0.0497	1.0036 ± 0.1288	0.932	0.2545 ± 0.0177	0.9755 ± 0.0609	0.981
52	1.0471 ± 0.2280	0.6832 ± 0.1173	0.913	1.0417 ± 0.2082	0.9370 ± 0.1698	0.893

But the difference between $LT_{99.9968}$ and LT_{95} became small at higher temperatures. Therefore, adding a short holding time at high temperatures dramatically increased the thermal mortality of heat treatment.

TDT Curves and Activation Energy. TDT curves for eggs and third instars of Mediterranean fruit fly are presented in Fig. 3. The curve defines a boundary for minimum time–temperature combinations to achieve 100% mortality in a sample of 300 insects. The two curves were described by the linear regression equations $\log t = 12.697–0.247\ T$ ($r^2 = 0.967$) for eggs and $\log t = 15.365–0.295\ T$ ($r^2 = 0.999$) for third instars, where t is the time (minutes) and T is the treatment temperature (Celsius). The z values derived from the TDT curves were 4.1 and 3.6°C for eggs and third instars, respectively. The TDT curves showed that the third instars were more heat resistant than eggs, because longer time was needed to achieve a complete kill of third instars at each temperature, especially for lower temperatures (i.e., 46 and 48°C). With the z values determined from TDT curves, equation 3 yielded an activation energy for thermal death of eggs and third instars of 490.6 and 551.9 kJ/mol, respectively. An alternative method (equation 4) to calculate activation energy from the slope of an Arrhenius plot (Fig. 4) for rate constant, k, resulted in an estimation of 471.0 and 593.9 kJ/mol, respectively. The two methods resulted in very similar activation energy values with <10% difference.

The TDT curves (Fig. 3) show that the minimum exposure time required to kill all third instars decreased at a faster rate with increasing treatment temperature than that for eggs. Both the higher activation energy and steeper slope of the TDT curve suggested that the heat resistance of third instars was more sensitive to increase in treatment temperatures than that of eggs. The activation energy is useful in determining the sensitivity of insects to changes in temperature. The activation energy for thermal death of third instars of Mediterranean fruit fly calculated from the TDT curve was higher than that found for codling moth (473 kJ/mol) (Wang et al. 2002a), Indianmeal moth (506 kJ/mol) (Johnson et al. 2003), and navel orangeworm (519 kJ/mol) (Wang et al. 2002b). Jang (1986) determined the activation energy for thermal kill of Mediterranean fruit fly eggs and first instars to be 784 and 656 kJ/mol, respectively, which were slightly higher than our observations. The difference might be caused by different heating methods. Jang (1986) used a hot water immersion technique at temperatures of 45–48°C and also obtained an activation energy of 517 and 649 kJ/mol with 957 and 400 kJ/mol for eggs and first instars of melon fly, *Bactrocera cucurbitae* Coquillett and oriental fruit fly, *Dacus dorsalis* Hendel, respectively.

Thermal Resistance Comparison between Eggs and Third Instars. At temperature–time combinations that did not result in complete kill (Table 4), significant differences between the third instars and eggs of Mediterranean fruit fly were observed for all four treatments ($P < 0.05$). Specifically, the observed thermal mortality for eggs was higher than that for third instars. That is, third instars were more heat resistant than eggs. The same conclusion was reached based on the TDT curves shown in Fig. 3. Therefore, based on the

Table 3. Comparison of lethal times (minutes) obtained by the 0.5 order kinetic model for Mediterranean fruit fly eggs and third instars at four temperatures

Insects	Temp. (°C)	Observed min. time for 100% mortality of 300 insects (min) ($\approx LT_{99.67}$)	Lethal time (min)							
			LT_{95}	95% CI	LT_{99}	95% CI	$LT_{99.67}$	95% CI	$LT_{99.9968}$ (probit 9)	95% CI
Eggs	46	25	18.8	15.4–22.2	21.7	17.8–25.6	22.7	18.5–26.9	24.0	19.6–28.4
	48	5	3.7	3.2–4.2	4.3	3.8–4.9	4.5	3.9–5.1	4.7	4.1–5.3
	50	3	2.1	1.6–2.6	2.5	1.9–3.1	2.6	2.0–3.2	2.7	2.0–3.4
	52	0.67	0.44	0.19–0.69	0.56	0.26–0.86	0.60	0.28–0.92	0.65	0.3–1.0
Third instars	46	60	49.0	45.6–52.4	56.5	52.5–60.5	59.1	54.9–63.3	62.2	57.7–66.7
	48	15	12.1	11.1–13.1	13.9	12.7–15.1	14.5	13.2–15.8	15.3	13.9–16.7
	50	4	3.0	2.7–3.4	3.4	3.0–3.8	3.6	3.2–4.0	3.8	3.3–4.3
	52	1	0.7	0.4–1.0	0.8	0.5–1.1	0.8	0.5–1.1	0.9	0.6–1.3

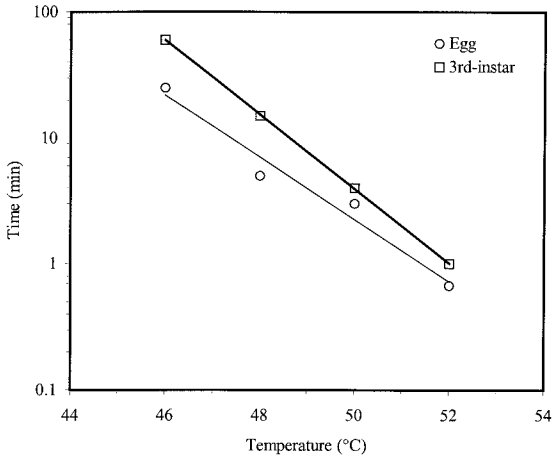

Fig. 3. TDT curves for eggs and third instars of Mediterranean fruit fly (n = 300) at a heating rate of 15°C/min. Lines represent linear regression equations: $\log t = 12.697 - 0.247\,T$ ($r^2 = 0.967$) for eggs and $\log t = 15.365 - 0.295\,T$ ($r^2 = 0.999$) for third instars, where t is time (minutes) and T is temperature (Celsius).

HBS studies, thermal treatments with a relative high heating rate (e.g., up to 15°C/min) designed to control third instars of Mediterranean fruit fly in citrus also should be effective against eggs. Our results suggest that in developing treatment protocols, third instars can be used in the efficacy studies. One caveat to this method of testing thermal death kinetics is that the role of humidity in enhancing mortality was not controlled. Both larvae and eggs are sensitive to desiccation, but the third instars are more tolerant than less developed larval stages. Eggs, which are normally laid on or near the fruit surface, are also resistant to desiccation. Therefore, an ideal testing system would allow the control of heating rate, final holding temperature, and relative humidity around the insects.

Table 4. Comparative heat resistance of eggs and third instars of Mediterranean fruit fly (n = 300 for three replicates)

Temp (°C)	Exposure (min)	% Mortality ± SE	
		Eggs	Third instars
46	20	99.2 ± 1.4**	41.5 ± 3.9**
48	2	63.4 ± 1.6**	16.5 ± 13.6**
50	2	99.6 ± 0.7*	84.0 ± 16.5*
52	0.17	85.0 ± 10.6**	23.5 ± 14.2**

t Test performed on arcsine-transformed data, * $P \leq 0.05$; ** $P \leq 0.01$ (SAS Institute 1989).

Thermal Resistance Comparison with Other Insects. The Mediterranean fruit fly eggs had comparable heat resistance to fifth instars of Indianmeal moth larvae at 50 and 52°C (Johnson et al. 2003). Third instars of Mediterranean fruit fly was more heat resistant than Indianmeal moth larvae at the two low temperatures (i.e., 46 and 48°C) but had comparable heat resistance at the two high temperatures (Fig. 5). However, it had similar heat resistance at low temperatures but lower heat resistance at high temperatures compared with fifth instars of codling moth (Wang et al. 2002a). Third instars of Mediterranean fruit fly were less heat resistant than fifth-instar navel orangeworm (Wang et al. 2002b). In general, the heat resistance of third instars of Mediterranean fruit fly was more sensitive to temperature changes than the other three larvae as reflected by the slope of the TDT curves in Fig. 5.

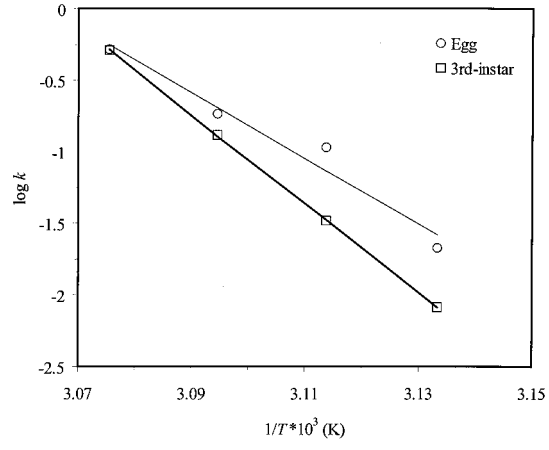

Fig. 4. Arrhenius plot for temperature effects on thermal death rate constant for eggs and third instars of Mediterranean fruit fly. The straight lines ($\log k = 70.322 - 22.948*1000/T$ and $\log k = 95.098 - 31.016*1000/T$) were obtained by linear regression for eggs ($r^2 = 0.963$) and third instars ($r^2 = 0.999$).

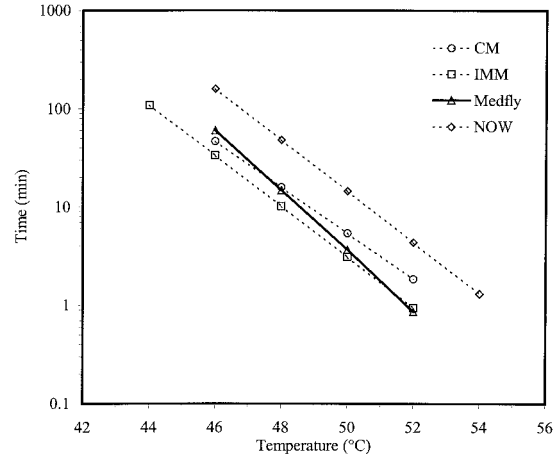

Fig. 5. Minimum time-temperature combinations for complete kill of 600 third instars of Mediterranean fruit fly (MFF) compared with those for 600 fifth instars of codling moth (CM), Indianmeal moth (IMM), and navel orangeworm (NOW).

Acknowledgments

We thank Ruti Akiva for technical assistance. This research was supported by grants from The United States-Israel Binational Agricultural Research Development Fund (U.S.-3276-01), USDA Initiative for Future Agriculture and Food Systems (00-52103–9656), and Washington State University IMPACT and Agricultural Research Centers.

References Cited

Abbott, W. S. 1925. A method of computing the effectiveness of an insecticide. J. Econ. Entomol. 18: 265–267.

Andreuccetti, D., M. Bini, A. Ignesti, A. Gambetta, and R. Olmi. 1994. Microwave destruction of woodworms. J. Microwave Power Electromagnetic Energy 29: 153–160.

Hallman, G. J., and J. L. Sharp. 1994. Radio frequency heat treatments, pp. 165–170. *In* J. L. Sharp and G. J. Hallman [eds.], Quarantine treatments for pests of food plants. Westview Press, San Francisco, CA.

Hansen, J. D., and J. L. Sharp. 1998. Thermal death studies of third-instar Caribbean fruit fly (Diptera: Tephritidae). J. Econ. Entomol. 91: 968–973.

Heard, T. A., N. W. Heather, and R. J. Corcoran. 1991. Dose mortality relationships for eggs and larvae of *Bactrocera tryoni* (Diptera: Tephritidae) immersed in hot water. J. Econ. Entomol. 84: 1768–1770.

Heather, N. W., R. J. Corcoran, and R. A. Kopittke. 1997. Hot air disinfestation of Australian 'Kensington' mangoes against two fruit flies (Diptera: Tephritidae). Postharvest Biol. Technol. 10: 99–105.

Ikediala, J. N., J. Tang, and T. Wig. 2000. A heating block system for studying thermal death kinetics of insect pests. Trans. ASAE 43: 351–358.

Jang, E. B. 1986. Kinetics of thermal death in eggs and first instars of three species of fruit flies (Diptera: Tephritidae). J. Econ. Entomol. 79: 700–705.

Jang, E. B., J. T. Nagata, H. T. Chan, and W. G. Laidlaw. 1999. Thermal death kinetics in eggs and larvae of *Bactrocera latifrons* (Diptera: Tephritidae) and comparative thermotolerance to three other tephritid fruit fly species in Hawaii. J. Econ. Entomol. 92: 684–690.

Johnson, J. A., S. Wang, and J. Tang. 2003. Thermal death kinetics of fifth instar *Plodia interpunctella* (Lepidoptera: Pyralidae). J. Econ. Entomol. 96: 519–524.

Lurie, S. 1998. Postharvest heat treatments. Postharvest Biol. Technol. 14: 257–269.

Mangan, R. L., and S. J. Ingle. 1992. Forced hot air quarantine treatment for mangoes infested with West Indian fruit fly (Diptera: Tephritidae). J. Econ. Entomol. 85: 1859–1864.

Mangan, R. L., and S. J. Ingle. 1994. Forced hot air quarantine treatment for grapefruit infested with Mexican fruit fly (Diptera: Tephritidae). J. Econ. Entomol. 87: 1574–1579.

Mangan, R. L., K. C. Shellie, S. J. Ingle, and M. J. Firko. 1998. High temperature forced-air treatments with fixed time and temperature of 'Darcy' tangerines, 'Valencia' oranges, and 'Rio Star' grapefruit. J. Econ. Entomol. 91: 933–939.

Nascimento, A. S., A. Malavasi, J. S. Morgante, and A.L.A. Duarte. 1992. Hot water immersion treatment for mangoes infested with *Anastrpha fraterculus, A. oblique,* and *Ceratitis capitata* (Diptera: Tephritidae) in Brazil. J. Econ. Entomol. 85: 456–460.

Nelson, S. O. 1996. Review and assessment of radio-frequency and microwave energy for stored-grain insect control. Trans. ASAE 39: 1475–1484.

Rössler, Y. 1975. Reproductive differences between laboratory-reared and field-collected populations of the Mediterranean fruit fly, *Ceratitis capitata.* Ann. Entomol. Soc. Am. 68: 987–991.

SAS Institute. 1989. SAS/STAT user's guide, version 6, 4th ed., vol. 2. SAS Institute, Cary, NC.

Schiffman-Nadel, M., E. Chalutz, J. Wax, and M. Dagan. 1975. Reduction of chilling injury in grapefruit by thiabendazole and benomyl during long term cold storage. J. Am. Soc. Hort. Sci. 100: 276–280.

Sharp, J. L., and W. P. Gould. 1994. Control of Caribbean fruit fly (Diptera: Tephritidae) in grapefruit by forced hot air and hydrocooling. J. Econ. Entomol. 87: 131–133.

Sharp, J. L., and G. J. Hallman. 1992. Hot air quarantine treatment for carambola infested with Caribbean fruit fly (Diptera: Tephritidae). J. Econ. Entomol. 85: 168–171.

Tang, J., J. N. Ikediala, S. Wang, J. D. Hansen, and R. P. Cavalieri. 2000. High-temperature-short-time thermal quarantine methods. Postharvest Biol. Technol. 21: 129–145.

Waddell, B. C., G. K. Clare, and J. H. Maindonald. 1997. Comparative mortality responses of two Cook Island fruit fly (Diptera: Tephritidae) species to hot water immersion. J. Econ. Entomol. 90: 1351–1356.

Wang, S., J. N. Ikediala, J. Tang, J. D. Hansen, E. Mitcham, R. Mao, and B. Swanson. 2001a. Radio frequency treatments to control codling moth in in-shell walnuts. Postharvest Biol. Technol. 22: 29–38.

Wang, S., J. Tang, and R. Cavalieri. 2001b. Modeling fruit internal heating rates for hot air and hot water treatments. Postharvest Biol. Technol. 22: 257–270.

Wang, S., J. N. Ikediala, J. Tang, and J. D. Hansen. 2002a. Thermal death kinetics and heating rate effects for fifth-instar codling moths (*Cydia pomonella* (L.)). J. Stored Prod. Res. 38: 441–453.

Wang, S., J. Tang, J. A. Johnson, and J. D. Hansen. 2002b. Thermal-death kinetics of fifth-instar *Amyelois transitella* (Walker) (Lepidoptera: Pyralidae). J. Stored Prod. Res. 38: 427–440.

Wang, S., J. Tang, J. A. Johnson, E. Mitcham, J. D. Hansen, R. P. Cavalieri, J. Bower, and B. Biasi. 2002c. Process protocols based on radio frequency energy to control field and storage pests in in-shell walnuts. Postharvest Biol. Technol. 26: 265–273.

Wardowski, W. F., W. Grierson, and G. J. Edwards. 1973. Chilling injury of stored limes and grapefruit as affected by differentially permeable packing films. HortScience 8: 173–175.

Received 7 August 2003; accepted 30 April 2004.