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A cumulated lethal time model to evaluate efficacy of heat treatments for codling moth *Cydia pomonella* (L.) (Lepidoptera: Tortricidae) in cherries

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Abstract

Developing heat treatment methods to control insect pests in harvested commodities has traditionally relied on empirical trial-and-error approaches. There is a need for an effective means to systematically develop and assess heat treatments to save time and expense. In this study, we developed a cumulated lethal time model based on the efficacy of different hot water treatments for killing codling moth in cherries. Minimum temperature–time combinations to achieve complete kill of codling moth larvae in cherries were determined and compared with the prediction of the cumulated lethal time model. In experiments to validate the model, larval mortality of the codling moth, *Cydia pomonella* (L.) (Lepidoptera: Tortricidae), was evaluated in infested cherries subjected to various periods of temperatures in warm water baths. This model calculates the accumulative lethal effect of the complete process by using measured temperature–time data in the core of cherries along with established intrinsic thermal death kinetics information on the target insects. This model predicted minimum treatment times to achieve a total mortality of the pest population in cherries for different treatment temperatures. The results show that this model can be used to predict the thermal mortality of the insects in fruit for any pattern of heat treatment, provided that the temperature–time profile in the infested fruits is measured. This procedure should allow for rapid efficacy comparisons in a range of thermal treatments against codling moth larvae in different commodities.
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Keywords: Cumulated lethal time model; Thermal death kinetics; Codling moth; Cherries; Quarantine treatments; Methyl bromide

1. Introduction

The use of heat treatments for postharvest phytosanitation has become prominent against pests in fresh and stored agricultural commodities in replacing chemicals that will be discontinued due to regulatory actions. The basic variables for thermal treatments are time and temperature (Hallman and Armstrong, 1994; Sharp, 1994), and these are adjusted to minimize phytotoxicity. The sources of heat can be water (Follett and Sanxter, 2001; Shellie and Mangan, 2002), forced hot air (Armstrong et al., 1989; Sharp

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et al., 1991; Neven et al., 1996), or electromagnetic energy (Ikediala et al., 1999; Wang et al., 2001a, 2002b). Heat treatments can be single (Armstrong, 1982) or multiple exposures (Couey and Hayes, 1986). Treatment times to achieve effective control of insect pests in fruit depend on temperature, fruit size, heating medium, and intrinsic heat tolerance of insect pests (Hallman and Armstrong, 1994; Sharp, 1994). Knowledge of the minimally required thermal treatment to control infesting insects over a relatively large range of temperatures would provide flexibility for selecting suitable and effective thermal quarantine processes. However, efficacy tests have relied on a trial-and-error approach with infested fruit, which is expensive, labor-intense, and time consuming.

A systematic methodology is desirable to reduce development time and expense. An essential element in this approach is information on the intrinsic thermal death kinetics of targeted insect pests. Several different experimental and heat application methods have been used to gain such information (Jang, 1986, 1991; Wang et al., 2002a,b). Ikediala et al. (2000) and Wang et al. (2002a) developed a heating block system to study the thermal death kinetics of codling moth, *Cydia pomonella* (L.) (Lepidoptera: Tortricidae). The heating block system uniformly heats insects through direct contact between insects and relatively uniform isothermal metal surfaces. The heating rates are controlled between 0.1 and 20 °C/min by regulating the flow of electricity in heating pads that are attached to the outer sides of the two metal blocks with a cavity to form an insect chamber (Ikediala et al., 2000; Wang et al., 2002a). The heating block system has also been used to study the thermal death kinetics of navel orangeworm, *Amyelois transitella* (Walker) (Lepidoptera: Pyralidae), and Indian meal moth, *Plodia interpunctella* (Hübner) (Lepidoptera: Pyralidae) (Wang et al., 2002a,b; Johnson et al., 2003).

In heat treatments, the rise in fruit temperature depends upon fruit size and heating methods (Wang et al., 2001b). With intrinsic thermal death kinetic information for targeted insects, the cumulative lethal effect of a heating process can be evaluated from the measured temperatures during the treatment process, or the temperature–time history (Tang et al., 2000). A cumulative lethal effect model would be advantageous for predicting the efficacy of a proposed treatment to guide the development of a phytotoxic safe procedure and consequent mortality studies. The objectives of our study were to determine the minimum process time to control codling moth in cherries over a range of treatment temperature and time variations, including multiple exposures and different sources of heat, and to develop a cumulated lethal time model to evaluate insect control effectiveness.

2. Materials and methods

2.1. Fruit infestation

Codling moth larvae were obtained from the colony reared at the USDA-ARS Yakima Agricultural Research Laboratory, in Wapato, Washington, where they were maintained on a soy–wheat germ-starch artificial diet at ≃27 °C, 40–58% RH, with a 16:8 h light:dark photoperiod (Toba and Howell, 1991). Third instars were used to infest fruit because this was the required stage in previous quarantine tests of codling moth on cherries exported to Japan (Moffitt et al., 1992; Hansen et al., 2000). In 2001, immature 'Bing' cherries were obtained from commercial sources in California for early season fruit (average [±S.D.] size: 12.3 [±0.7] Row, 5.8 [±0.8] g) and from an organic orchard at the USDA-ARS Moxee Farm in Washington state for late season fruit (average [±S.D.] size: 12.0 [±0.7] Row, 6.3 [±0.8] g). In 2002, immature 'Bing' cherries were obtained from California for early season fruit (average [±S.D.] size: 11.9 [±0.7] Row, 6.8 [±1.0] g) and from the Moxee Farm for late season fruit (average [±S.D.] size: 11.7 [±0.7] Row, 6.9 [±1.0] g). The preharvest history of the California cherries is unknown, but no insecticides were applied to the Washington cherries. Each cherry was infested by placing a larva on the stem end so that 50 infested fruit made a treatment replicate. The infested cherries were then held overnight at room temperature (25 °C) in a rearing room to allow the larvae to penetrate into the fruit. In preparation for treatment, the cherries were placed in a 18-mesh fiberglass bag (with 1 mm openings) sealed with medium-size paper clips. To simulate local commercial operations, the California fruit were cooled to 4 °C before treatment, whereas the Washington cherries were treated directly after removal from the rearing room. Treatment evaluations were conducted the day after treatment.

Table 1
Observed survival of codling moth larvae in cherries treated for different durations in water baths in 316 separate treatment runs

Bath temperature (°C)	Duration in baths with survivors (min)	Duration in baths with no survivors (min)
48	5–14	15
49	9	10–11
50	5–8	9
51	6–8	8–9
52	4–6	7
53	2–6	6–7
54	2–4	4–6

2.2. Water bath treatments

The infested Washington and California cherries were submerged for different times in 316 water bath treatments ranging in temperature from 48 to 55 °C, including a double bath intended to raise the fruit core temperatures in a series of treatment plateaus in order to reduce possible phytotoxic effects from the heat (Table 1; Hansen et al., 2001; Feng et al., 2004). The water was heated by a 150 l Vangard Model #6E727 (Rheem Mfg. Co., Montgomery, Alabama) water heater with one-phase electrical connection at 240 V, a maximum of 4500 W, and the water temperature controlled by a microprocessor. A Bell & Gossett (Morton Grove, Illinois) Model NRF-22 circulator (115 V, 60 Hz) moved the water at 83 l/min with 10.5 kg/cm^2 pressure to the holding tank from the water heater and back through 25.5 mm diameter black vinyl tubing. The oblong (94 cm long × 74 cm wide × 58 cm high) holding tank was composed of pre-formed fiberglass, wrapped with aluminum coated fiberglass sheet that provided additional insulation. The prebath and hydrocooling tanks were low density polyethylene 38 l Rubbermaid (Rubbermaid Home Prod., Wooster, Ohio) bins (61 cm long × 41 cm wide × 23 cm high). Temperatures were measured using Omega (Stamford, Connecticut) nine-count, three-wire 0.00385 platinum 100 RTD probes; Model HYP4-16-1.5-100-EU-48-RP was used to measure internal fruit temperatures and Model RTD-810 was used to measure bath temperature with an accuracy of 0.2 °C. Temperature data were collected using a data acquisition board (Measurement Computing, Middlebora, Massachusetts) composed of a CIO-EXP-RTD expansion board and a CIO-DA5802/16 ISA board with an Instacal (Measurement Computing, Middlebora, Massachusetts), version 5, 12 A board driver. A customer developed Visual Basic 6 (Microsoft Corporation, Redmond, Washington) application program directed the data to specific text files, which were later exported to Quattro Pro (Corel Corp., Ottawa, Ontario), version 7, spreadsheets for storage, graphics, and analysis.

All heated water contained 50 mg/l sodium hypochlorite to reduce disease on fruit. Washington cherries were subjected to a single bath in the holding tank at specified temperatures and durations followed by hydrocooling in a 4 °C water bath. California cherries were first submerged in a 40 °C bath for 3–6 min (a prebath treatment), then subjected to a warm water bath in the holding tank, as was done with the Washington cherries. After hydrocooling in a 4 °C water bath, the treated fruit were returned to a 25 °C holding room, held overnight, then dissected the following day to determine larval survival. Moribund larvae were placed on immature organic apples and inspected periodically until they died or pupated, as described in detail in Hansen et al. (2000). During evaluation, missing larvae were considered dead. Temperature and survival data were entered into Quattro Pro spreadsheets. Internal temperatures were illustrated by using the graphics program in Quattro Pro and univariate statistics were calculated by using the appropriate Quattro Pro function statements.

2.3. Cumulative lethal effect of heat treatments

Typical fruit core temperature–time profiles for single and double exposure treatments are shown in Fig. 1. Total insect mortality was dependent on the cumulative thermal exposure during the course of the treatment. The cumulative lethal effect of the treatment at a specific location can be estimated if the actual temperature–time history of that location was recorded in the treatment. In evaluating the efficacy of

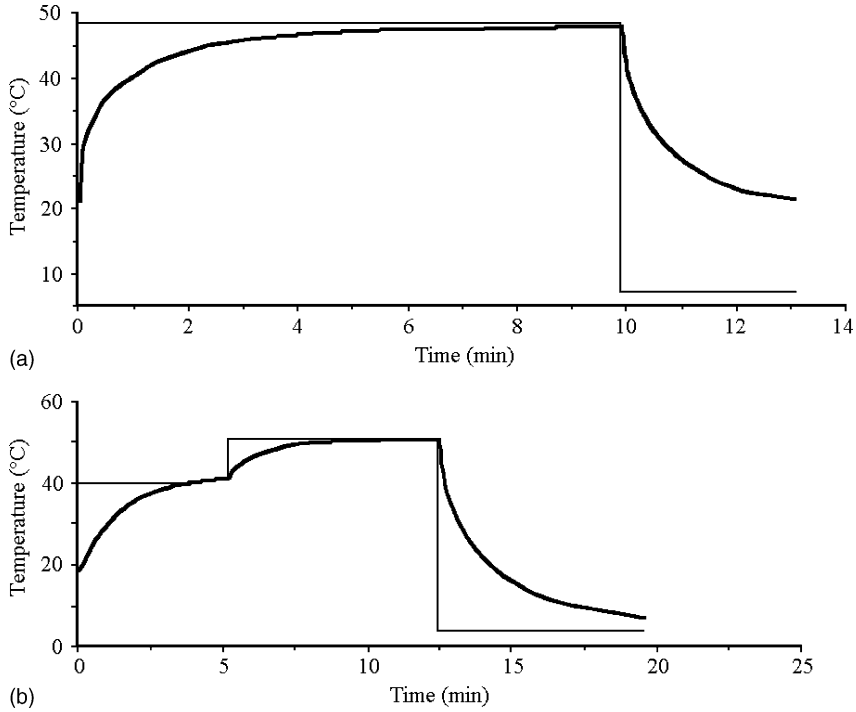

Fig. 1. Typical temperature–time history of the fruit core of a cherry in warmed water: (a) in a single bath; (b) in a double bath.

a treatment, we generally considered the least heated part of the fruit to be near the seed (the fruit core). For 1st order kinetics, we used the relationship, called the *cumulated lethal time model*, to determine the accumulative lethal effect for any given combination of temperature–time (Tang et al., 2000), in terms of equivalent total lethal time M_{ref} (min) at a reference temperature, T_{ref} (°C):

$$M_{\text{ref}} = \int_0^t 10^{(T(t)-T_{\text{ref}})/z}\,\mathrm{d}t \tag{1}$$

where $T(t)$ is the recorded core temperature as a function of time t (in min), and z the temperature difference required for a 10-fold change in the thermal death time curve (°C). An average z value of 4 °C was used in this study based on the results of Ikediala et al. (2000) and Wang et al. (2002a) for codling moth larvae. According to Wang et al. (2002a), this value is not influenced by the heating rate between 1 and 18 °C/min.

In this study, we used 48 °C as our reference temperature T_{ref} for Eq. (1) based on our earlier heating block studies (Wang et al., 2002a), although a different reference temperature (e.g., 50 or 54 °C) can also be used. The equivalent total lethal time M_{ref} at one temperature T_1 can be translated to a lethal time of another temperature T_2 with the same mortality by the equation:

$$M_{\text{ref}}(T_1) = fM_{\text{ref}}(T_2) \tag{2}$$

where f is the conversion factor that can be calculated by the following equation for a given value of z:

$$f = 10^{-(T_1-T_2)/z} \tag{3}$$

Thus, the cumulated lethal time at temperatures of 50, 52, 54 and 55 °C can be translated to that at 48 °C by the following relationship with the same lethality effect:

$$M_{\text{ref}}(\text{Temp}) = fM_{\text{ref}}(48) \tag{4}$$

and

$$f = 10^{-(\text{Temp}-48)/z} \tag{5}$$

Based on the f values calculated from Eq. (5) for codling moths ($z = 4$ °C), 1 min full exposure at 48 °C has the same lethal effect as 0.316, 0.100, 0.0316 and

0.0178 min exposures at 50, 52, 54 and 55 °C, respectively.

The following relationship between the mortality of codling moth and the equivalent cumulated lethal time at 48 °C was derived from a 0.5th order thermal death kinetic model (Wang et al., 2002a):

$$\text{Mortality (\%)} = \{1 - [-0.0691 M_{\text{ref}}(48) + 0.9584]^2\} \times 100 \quad (6)$$

3. Results and discussion

3.1. Temperature–time history of fruit cores in water bath

Temperature profiles in cherries depended upon whether the treatment was a single or a double bath. In the single bath treatment, the temperature of the fruit core initially increased rapidly then flattened out as it approached the bath temperature (Fig. 1a). The typical profile in cherries treated in the double water bath was similar but with a stepwise increment for the warmer bath (Fig. 1b). Initially, the small size of the cherries allowed for the internal temperatures to change quickly because of the large temperature difference and relatively short distance between the core of the fruit and the surrounding water at the fruit surface. As the fruit core temperatures approached that of the water baths, the rate of temperature increase declined to produce a "plateauing" characteristic of conduction heating (Hansen, 1992; Wang et al., 2001b). Thus, the lethal effect of the treatment starts to accumulate before the fruit core reaches the bath temperature.

3.2. Minimum treatment time at different temperatures to control third instar codling moth

Mortality of third instar codling moth larvae in cherries varied among the 316 different water bath treatments ranging in temperature from 48 to 54 °C (Table 1). At each water bath temperature, insect mortality increased with treatment time to reach 100% mortality. The minimum time required to achieve total mortality of third instar codling moth larvae in cherries decreased sharply with increased water bath temperature (Fig. 2). Based on the predicted thermal death kinetic model for codling moth, the regression for cherries was

$$\log(t) = 4.037 - 0.061T \quad (7)$$

with $R^2 = 0.980$ for California cherry in water baths

$$\log(t) = 4.135 - 0.063T \quad (8)$$

with $R^2 = 0.927$ for Washington cherry in water baths

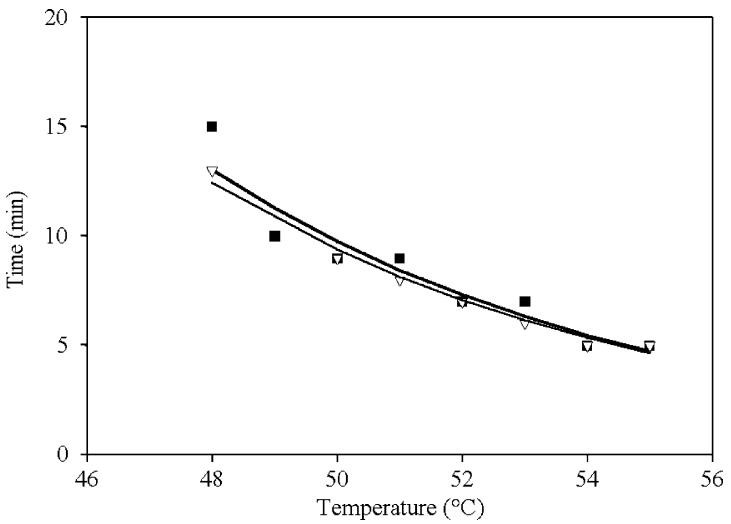

Fig. 2. Complete mortality of codling moth larvae with regression line ($\log(t) = a + bT$) in California (▽) and Washington cherries (■) from durations in hot water baths; $r^2 > 0.92$.

while the model obtained by the heating block study was

$$\log(t) = 12.41 - 0.234T \quad (9)$$

with $R^2 = 0.993$ from the heating block study

where t is the treatment time (min) and T the treatment temperature (°C). The first two models (Eqs. (7) and (8)) expressed the minimum time to reach the 100% kill of insects in cherries obtained from the efficacy studies and (Eq. (9)) for codling moths when the insects are fully exposed to heat in heating block system. The required exposure time at 48 °C for the efficacy study was shorter than that from the heating block study. This was probably due to insect suffocation during long heating times in water baths, which has low oxygen saturation capacity (Mortimer, 1981), and less heat tolerance for third instars than for fifth instars (Wang et al., 2004). At higher temperatures, the minimum required time for efficacy tests (with infested cherries) was longer than that for the heating block tests (insect fully exposed). This was reasonable because the slow heat transfer resulted in fruit core temperatures lower than the fruit surface and water bath temperatures.

3.3. Cumulative lethal effects on insect mortality of a thermal treatment

Although initial temperatures of treated fruit varied because of pretreatment handling, the procedure for measuring the lethal effect was the same. Lower initial fruit core temperatures and hydrocooled temperatures contributed little to the total insect lethality according to Eq. (1). Temperature–time history for each treatment was used to calculate its equivalent full larval exposure time to 48 °C using Eq. (1). The experimentally determined mortality and calculated equivalent exposure times (for 48 °C) for each treatment were then plotted in Fig. 3 together with the predicted insect mortality by the heating block study using Eq. (6).

Variations in treatment protocols did not allow for sufficient predictions in efficacy (the subefficacious treatments) for lethal times less than 20 min, but greater lethal times were consistent with effective treatments (Fig. 3). The 20 min accumulated time at 48 °C would be appropriate for phytosanitation procedures where complete efficacy is not required or where the pest occurs at low infestation levels. Quarantine treatments requiring probit-9 security, or 99.9968% morality (Baker, 1939), would need at least 35 min because no survivors were found beyond this lethal

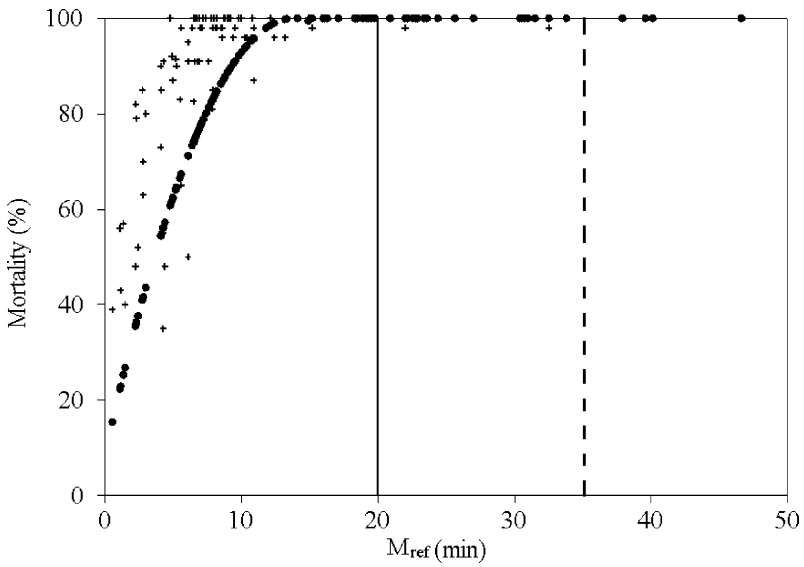

Fig. 3. Relationship between cumulated lethal times (M_{ref}) set at 48 °C and percent mortality of codling moth larvae: (+) experimentally determined insect mortality; (●) predicted mortality based on cherry core temperature from the heating block system; (—) sufficient for most phytosanitation treatments; and (- - -) minimum for quarantine treatments.

time. Therefore, it is possible to develop a treatment using cumulated lethal times calculated from measured temperature–time history of at the least heated portion of the fruit. This method is useful to predict mortality in new treatment conditions whereas probit analysis was used to estimate the treatment parameters; treated populations of over 30,000 are required and often the data used to determine these parameters were inadequate to accurately provide a treatment starting point (Robertson et al., 1994). Thus, a treatment goal based on cumulated lethal times can be specified before protocol development.

The equivalent total lethal times calculated by Eq. (1) take into account the effect of the entire temperature–time history for each treatment, including the effects of the ramp up time. Because each time segment was involved, it did not matter how the treatments were set up. Thus, even before efficacy of a treatment can be tested with codling moth larvae, the internal temperature history can be recorded, the cumulated lethal time for a reference temperature calculated to estimate possible efficacy of the treatment to the target insect pests. For example, if the cumulative lethal times at 48 °C are greater than 35 min, then this procedure can be evaluated as a potential quarantine treatment.

3.4. Comparison with degree-minute (DM) model

A commonly used method to account for the cumulated lethal effect is the so-called degree-minute model (Shellie and Mangan, 1994; Nyanjage et al., 1998). This model suggests that the cumulated temperature (in °) beyond a threshold value times the duration of exposure (in min) is a critical factor that yields a certain level of insect mortality, regardless of the treatment process. A general degree-minute model can be expressed with the following equation:

$$\mathrm{DM} = \int_0^t [T(t) - T_s]\,\mathrm{d}t \tag{10}$$

where DM is the degree-minute value, $T(t)$ the temperature in fruit (°C) as a function of time t, and T_s the threshold temperature (°C). For temperature treatments where insects are fully exposed to a constant temperature, Eq. (10) is reduced to

$$\mathrm{DM} = [T(t) - T_s]t \tag{11}$$

Threshold temperature varies with treatment methods, fruit and insects. Selection of this value is very arbitrary. For example, 30 °C was selected for "Valencia" orange fruit changes using moist forced air heating (Shellie and Mangan, 1994), 42 °C for lethal temperature of Queensland fruit fly eggs, *Bactrocera tryoni* (Froggatt) (Diptera: Tephritidae) (Waddell et al., 2000), and 48 °C for larvae of the warehouse beetle, *Trogoderma variabile* Ballion (Coleoptera: Dermestidae) (Wright et al., 2002). Table 2 shows the differences of calculated lethal effects for four treatment temperature–time combinations between degree-minute model, where we selected 42 °C as the threshold temperature for codling moth larvae, and the kinetic model. Clearly, the degree-minute model took the linear accumulation of the temperature–time history while assuming the temperature effect on insect mortality was constant. This model did not reflect the real effect of temperatures because lethality only increased 2.0 times at 54 °C from that at 48 °C. In fact, the lethal effect sharply increased with increasing temperature. As shown in Fig. 2, the treatment time to achieve the same lethal effect (complete kill of 600 insects) dramatically decreased with increased temperature. The cumulated lethal time calculated based on 0.5th order thermal death kinetic model for coding moth (Wang et al., 2002a) was 31.6 times larger at 54 °C than at 48 °C for the same 2 min holding (Table 2).

3.5. Advantages of the cumulated lethal time model

The use of the cumulated lethal time model facilitates the development of new thermal postharvest sanitation treatments. Because it involves the coolest part of the fruit, it is conservative in its approach and it can be assumed that efficacy applies to internal pests located closer to the fruit surface. Different types of treatments can be included for parallel comparison, including temperature rampings at different rates, different holding times, and consecutive or multiple thermal exposures, to yield the best possible treatment that has minimum impact on fruit quality while providing required lethality to targeted insect pests. Because internal temperatures are measured, the heat can be from different sources, including forced hot air, water baths, and electromagnetic energy. Also, the approach can be used for different types of commodities. In our

Table 2
Comparison of temperature effects on the cumulated lethal effect between the degree-minute model and the kinetic model

Hypothetical treatment conditions, cherry temperature (°C) + holding time (min)	Degree-minute model (°C min)[a]	Cumulated lethal time M_{ref} (48 °C), based on an 0.5th order kinetic model (min)
48 + 2	12	2.0
50 + 2	16	6.3
52 + 2	20	20.0
54 + 2	24	63.3

[a] Threshold temperature selected to be 42 °C.

example, the tests were conducted with codling moth in cherry, which is not a natural host, but the procedure can be applied to other commodities as well. Furthermore, because the cumulated lethal times (at a reference temperature) include the treatment temperature profile, the procedure compensates for slow heating due to fruit size. This would be particularly helpful in identifying treatments against internal pests in commodities that have a wide range of host sizes, such as codling moths in apples or fruit flies in mangoes.

Efficacy testing causes a major expense in the development of postharvest treatments. Because the cumulated lethal time approach involves the use of fruit only, the cost and time of using infested fruits are not expended in the exploratory phases of treatment development. Sequential and multiple exposures can be explored. Particular sites within the fruit, regardless of fruit shape, can be selected for temperature monitoring. Therefore, potential treatments can be identified, and the phytotoxic effects and quality factors can be identified before large-scale confirmatory tests using infested fruits. This should decrease developmental time and save on expensive resources and labor. Rapid development of thermal postharvest treatments would greatly benefit the fruit export industry.

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