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Thermal resistance of different life stages of codling moth (Lepidoptera: Tortricidae)

S. Wang[a], X. Yin[a,b], J. Tang[a,*], J.D. Hansen[c]

[a] Department of Biological Systems Engineering, Washington State University, 213 L.J. Smith Hall, Pullman, WA 99164-6120, USA

[b] College of Plant Protection, Henan Agricultural University, Wen Hua Road No. 95, Zhengzhou, Henan 450002, People's Republic of China

[c] USDA-ARS Yakima Agricultural Research Laboratory, 5230 Konnowac Pass Road, Wapato, WA 98951, USA

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Abstract

Phytosanitation regulations in several international markets require postharvest treatments to control codling moth, *Cydia pomonella* (L.) (Lepidoptera: Tortricidae), in various commodities. Thermal treatments are gaining acceptance to replace chemical fumigation. Determining the most thermal-tolerant life stage is essential in the development of effective postharvest insect control protocols based on thermal energy. A heating block system was used to evaluate relative heat resistance of five different life stages of codling moth: white-ring eggs, black-head eggs, third-instar, fifth-instar, and diapausing larvae, at a heating rate of 15°C/min. The fifth-instar was the most heat-resistant life stage in the tested temperature range of 50–52°C except for diapausing larvae. Thermal death kinetic data of diapausing fifth-instar larvae were determined and also compared with the published TDT curve of non-diapausing larvae using the same heating block system. Both diapausing and non-diapausing larvae were dead after treatments at 50°C for 5 min and 52°C for 2 min. However, at the lower temperatures or shorter times, diapausing larvae had lower mortality than non-diapausing larvae.

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Keywords: Codling moth; Heating block; Heat treatment; Quarantine; Thermal death resistance

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*Corresponding author. Tel.: +1-509-335-2140; fax: +1-509-335-2722.
E-mail address: jtang@mail.wsu.edu (J. Tang).

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1. Introduction

Codling moth, *Cydia pomonella* (L.), is an important phytosanitary and quarantine pest of fruits and nuts destined for certain countries including Japan, South Korea, and Europe. Methyl bromide is currently used in commercial treatments against codling moth in cherries and nectarines (Guance et al., 1981; Yokoyama et al., 1990; Moffitt et al., 1992). However, methyl bromide is a toxic material that affects both target pests and non-target organisms. Methyl bromide has been identified by the US Environmental Protection Agency (EPA) under the Federal Clean Air Act (Anonymous, 1990) and by the Montreal Protocol (Anonymous, 1995) as a chemical that causes atmospheric ozone depletion. The EPA has mandated the removal of methyl bromide from the chemical register and the phase out of its production and importation into the United States by December 31, 2005. Although methyl bromide fumigation for postharvest quarantine treatments is exempted from legal restrictions, the future of methyl bromide use is very vulnerable because of possible sharp price increases due to reduced production, unreliable sources, or future restrictions under international agreements (USEPA, 1998). Over the past 5 years, the price of methyl bromide has increased by about four-fold (USEPA, 2001). Different thermal treatments have been investigated as alternatives, including forced hot-air and vapor heat to control fifth-instar codling moth larvae in apples and pears (Neven et al., 1996), heated controlled atmospheres for fifth-instar in cherries (Neven and Mitcham, 1996), hot water baths for codling moth eggs and larvae (Yokoyama et al., 1991; Jones and Waddell, 1997; Hansen et al., unpublished data), microwaves to control third-instar codling moths in cherries (Ikediala et al., 1999), and radio frequency (RF) energy to control third- or fourth-instar codling moths (Wang et al., 2001a) and fifth-instar navel orangeworms, *Amyelois transitella* (Walker) (Lepidoptera: Pyralidae) (Wang et al., 2002c) in in-shell walnuts. For quarantine treatments, Japan-MAFF requires treatment efficacy to be verified on the most resistant codling moth life stage (Waddell et al., 1990) that may be present in the treated commodity.

The thermal mortality of various life stages of the codling moth has been determined by heating infested commodities (Neven et al., 1996), heating insects in glass vials or metal tubes in a water bath (Yokoyama et al., 1991), or by directly immersing insects in heated water (Jones and Waddell, 1997). Jones et al. (1995) reported that the first-instar was more tolerant than other life stages of codling moth at 43–49°C while Yokoyama et al. (1991) observed that the fifth-instar was the most heat-resistant life stage at 49–51°C. These discrepancies might have been caused by the test methods. The temperatures where insects were located in the commodity or tubes influenced the insect thermal mortality. In addition, temperatures of the insects varied with the commodity or tube size, the heating medium temperature and heating methods (Hansen, 1992; Wang et al., 2001b). With the direct water immersion methods, insect mortality may be affected by the actual heating rate in the sample and possible insect suffocation during long exposures.

A heating block system was developed at Washington State University (WSU), Pullman, WA that provides accurate heating rates and temperature controls in treating different insects. This system was used to study the thermal death kinetics of fifth-instar codling moth (Ikediala et al., 2000; Wang et al., 2002a), fifth-instar Indian meal moth (Johnson et al., 2003), and fifth-instar navel orangeworm (Wang et al., 2002b) at heating rates between 1 and 18°C/min. The same system was also used to determine the most heat-resistant life stage of navel orangeworms for developing thermal treatment protocols against field pests in in-shell walnuts (Wang et al., 2002c).

Until now, no data have been reported on thermal mortality of diapausing codling moth and on thermal resistance of different codling moth life stages at high heating rates (e.g. $>10°C/min$), which is commonly experienced in small fruits when heated in water (Wang et al., 2001b) and in commodities when heated in RF systems (Tang et al., 2000; Ikediala et al., 2002).

The objectives of this study were: (1) to determine the most thermal-resistant life stage of codling moth at a heating rate of 15°C/min using the WSU heating block system; and (2) to determine the thermal death kinetics of diapausing larvae, and compare them with those of the non-diapausing fifth-instars.

2. Materials and methods

2.1. Test insects

Adult female codling moths lay eggs on the surface of fruit. After egg hatching, larvae eat into the core of the fruit where they may be transported into storage and shipped to market after harvest. In walnuts, codling moths may over-winter in waterproof silken cocoons as diapausing larvae. Codling moth eggs are not found on walnuts during the time of treatment but may be found on apples. In thermal treatments, all egg and larval stages of codling moth must be killed. In this study, we selected white-ring eggs, black-head eggs, third-instars, fifth-instars, and diapausing larvae in our comparative tests for heat resistance.

Codling moth larvae were reared by the USDA-ARS Yakima Agricultural Research Laboratory (YARL) in Wapato, Washington, USA, on a soya-wheat germ starch artificial diet (Toba and Howell, 1991) at about 27°C, 40–50% r.h., with a photoperiod of 16:8 h (L:D). Diapause was induced by rearing larvae at 24°C, 80–85% r.h., with a photoperiod of 8:16 h (L:D). After 2 weeks, corrugated cardboard strips were placed in the diet to serve as sites for cocoon formation. Cocoons were transferred to 2°C, 40% r.h., at 0:24 h (L:D) for an additional 6 weeks to induce diapause. Insect mortality tests were conducted using the heating block system at WSU. Eggs on wax paper sheets, larvae in diet, and diapausing larvae in cardboard strips were shipped from YARL to WSU by overnight delivery.

2.2. Treatment procedure

The heating block system developed at WSU heated the insects at 15°C/min. The height (3 mm) of the insect chamber in the system ($254 \times 254 \times 18$ mm) was used in this study based on the size of fifth-instar codling moth. A PID control unit (CN616TCO, Omega, CT) regulated the heating block temperatures via a solid-state relay with a mean error of less than 0.3°C from the set temperature. Detailed information on this heating block system can be found in Wang et al. (2002b).

To determine the most heat-resistant life stage of codling moth, nine temperature-holding time combinations were selected: 48°C for 2 min, 48°C for 5 min, 48°C for 10 min, 50°C for 2 min, 50°C for 3 min, 50°C for 5 min, 52°C for 0.5 min, 52°C for 1 min, and 52°C for 2 min (Fig. 1). These treatments were equal to or slightly less than the conditions needed to kill all insects in a given sample as defined by a thermal death-time (TDT) curve developed at the heating rate of

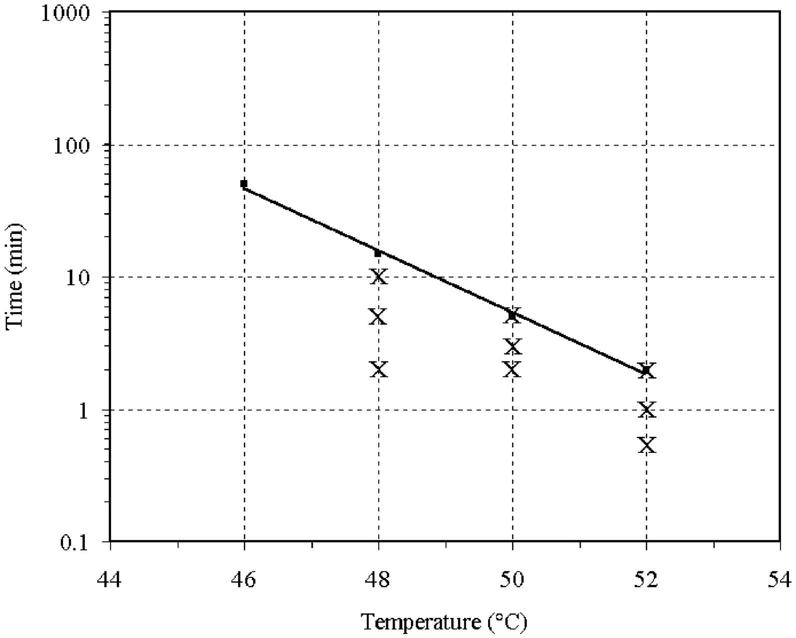

Fig. 1. Treatment conditions (X) used for heat-resistance evaluations of different life stages of the codling moth, compared with the TDT curve (—) for fifth-instars (Wang et al., 2002a).

18°C/min in a previous study (Wang et al., 2002a). Control insects were placed in the unheated block chamber at room temperature (22°C) for the longest exposure time used in the heat treatments. Including the control, 200 eggs or larvae were treated for each temperature and holding time combination, and all treatments were replicated three times.

Because commercial treatments would include rapid post-treatment cooling of fruits to minimize the effect on product quality, the treated larvae were immediately moved to cold storage at 4°C and stored at this temperature for 1 day. After the cold storage, the larvae were held at 22°C, 60% r.h., and a 16:8 h (L:D) for 1 day to minimize the effect of cold torpor before examination. Procedures for observing treated insects were similar to those described in Wang et al. (2002a). Larval mortality was calculated as the percentage of dead larvae relative to total treated larvae for each treatment. Mean values and standard deviations were obtained from three replications for each temperature–time combination.

The same experimental design for treating larvae was used for thermal mortality tests with eggs. After adult codling moths laid eggs on wax paper sheets, the sheets were cut into strips, each containing approximately 200 eggs. Each strip was then uniformly spread with tap water and placed in the heating block to ensure a good contact between the strip and the block surface before heat treatments. The wax paper with eggs faced down to the bottom block surface. The untreated eggs for controls were also spread with tap water. Three replicates and one control were used for both white-ring and black-head eggs. After treatments, the eggs were held at 26°C, 40% r.h., and a 16:8 h (L:D) for hatching. Egg mortality was calculated as the percentage of unhatched eggs relative to total treated eggs.

Three treatment times were selected for each temperature in thermal death kinetic studies on diapausing larvae. They included 40, 45, and 50 min at 46°C; 5, 10, and 15 min at 48°C; 2, 3, and 5 min at 50°C; and 0.5, 1, and 2 min at 52°C. These treatment times represented the longest holding periods used at each treated temperature for non-diapausing fifth-instars in a previous study (Wang et al., 2002a). The insect mortality data for diapausing larvae were compared to those obtained for non-diapausing fifth-instars from that study.

We used the concept of equivalent thermal lethal time at a fixed reference temperature to compare the efficacy of different treatments at different temperatures. Wang et al. (2002a) reported an equation to calculate cumulative thermal lethal time M (min) at a treatment temperature T_h (°C) with a fixed ramping rate of α (°C/min) from an initial temperature T_o (°C):

$$M(T_h) = \frac{z}{2.303\alpha}\left(1 - 10^{(T_o - T_h)/z}\right) + t_1, \tag{1}$$

where t_1 is the holding time (min) at T_h (°C), and z is the temperature difference required for a 10-fold change in the TDT curve ($z = 4$°C from Wang et al., 2002a). Based on the observed semi-log time and temperature relationships for a given degree of mortality (i.e. TDT curve) for codling moth, navel orangeworm, and Indian meal moth (Wang et al., 2002a, b; Johnson et al., 2003), thermal lethal time at any given temperatures (T_h) can be converted by the following relationship to an equivalent thermal lethal time at a fixed reference temperature (T_{ref}) with the same lethal effect (Tang et al., 2000):

$$M(T_{ref}) = 10^{(T_h - T_{ref})/z} M(T_h). \tag{2}$$

In this study, we selected 48°C, 50°C, and 52°C as our treatment temperatures T_h, and 50°C as our reference temperature T_{ref}, though one can use other reference temperatures such as 48°C and 52°C. The calculated equivalent thermal lethal time based on Eqs. (1) and (2) at 50°C provided a common base to compare the lethal effect of all treatments, regardless of the treatment temperature.

Treatment mortality was corrected based on the control mortality using Abbott's (1925) formula. An arcsine transformation was used to normalize the data before analysis. Effects of time–temperature treatments on insect mortality were compared using the SAS analysis of variance test procedure (SAS Institute, 1989). Significant differences ($P \leqslant 0.05$) among the mean values were identified using least significant difference t-tests (SAS Institute, 1989). Thermal mortality curves, showing the relationship between the logarithmic value of the ratio of the initial number of insects (N_0) to the number of surviving insects (N) and the exposure time, were developed for each treatment temperature. Where mortality was 100%, a value of 0.1 was used for N. The standard deviation was used to plot the error bar at each treatment level.

3. Results and discussion

3.1. Thermal resistance of different life stages

The mortality (mean ± SD) in unheated controls was 20.7 ± 5.0%, 16.5 ± 4.1%, 24.0 ± 2.7%, 2.7 ± 2.0%, and 1.7 ± 0.3% for white-ring eggs, black-head eggs, third-instars, fifth-instars and diapausing larvae, respectively. The relatively high mortalities in the controls for eggs and third-instars were the result of the difficulty in handling the small and delicate insects. The control mortality of fifth-instars was small, and the effect of handling and shipment was, therefore, negligible. However, differences in control mortality for all five life stages were significant ($P<0.05$). All mortality data were, therefore, corrected to take into account the control mortality before further statistical analysis, and means and standard deviations of codling moth mortality were corrected for each life stage after the heat treatments (Table 1).

Complete control of codling moth was achieved at 50°C for 5 min and 52°C for 2 min for all life stages (Table 1). With sub-lethal treatments, fifth-instar diapausing larvae were the most heat-resistant life stages ($P<0.05$). There was no significant difference in mortality between white-ring and black-head eggs for all treatments except for 48°C for 5 min and 50°C for 2 min, in which white-ring eggs were more heat-resistant than black-head eggs. Third-instars were the least heat-resistant life stage for the treatments of 48°C for 5 min, 48°C for 10 min, and 50°C for 3 min ($P<0.05$). For the treatments at 52°C, third-instars were not significantly different from both eggs. In the sub-lethal treatments at temperatures of 50–52°C, fifth-instars were more heat-resistant than, or at least have a similar heat resistance as, eggs and third-instars, except for diapausing larvae. Although the mortality for non-diapausing fifth-instars was consistently less than that for diapausing larvae, the difference was significant only at sub-lethal temperature–time combinations. For 50°C for 5 min and 52°C for 2 min treatments, we observed no difference among the five tested life stages because of a complete kill. Therefore, when developing treatment protocols that need to kill all insect pests at 50°C and 52°C, it makes virtually no difference which life stages are chosen for the efficacy study.

The calculated equivalent lethal times at 50°C for all treatments are also presented in Table 1. The relationship between the percentage mortality and the equivalent lethal time for different life stages of codling moth is shown in Fig. 2. It was clear that as soon as the equivalent lethal time at 50°C was greater than 5 min, codling moths were completely killed, regardless of life stages. The treatment of 50°C for 3 min was equivalent to that of 48°C for 10 min in terms of similar M_{ref} values (3.12 min versus 3.20 min) and similar insect mortalities (Table 1). As shown in Fig. 2,

Table 1
Corrected thermal mortality (mean ± SD, %) of codling moths after heating at a rate of 15°C/min (three replicates) in a heating block system as a function of temperature–time combinations and the corresponding cumulated lethal time M_{ref} (min) at 50°C

Temperature(°C) holding time (min)	Equivalent M_{ref} (min) at 50°C	White-ring eggs	Black-head eggs	Third-instars	Fifth-instars	Diapausing fifth-instars
48, 2	0.67	60.2 ± 17.3a	72.9 ± 5.3a	17.8 ± 5.1b	21.8 ± 16.7b	0.9 ± 0.3c
48, 5	1.62	65.0 ± 4.1c	87.3 ± 3.4b	96.2 ± 2.4a	70.2 ± 11.8c	1.0 ± 0.9d
48, 10	3.20	91.9 ± 4.1c	91.4 ± 2.5c	100a	97.4 ± 0.5b	1.1 ± 0.0d
50, 2	2.12	90.1 ± 5.2c	95.6 ± 2.2ab	97.4 ± 1.1a	92.3 ± 0.1bc	2.7 ± 1.0d
50, 3	3.12	98.1 ± 0.6b	95.6 ± 1.4b	99.6 ± 0.8a	96.9 ± 1.1b	1.6 ± 1.4c
50, 5	5.12	100a	100a	100a	100a	100a
52, 0.5	1.95	92.2 ± 4.7a	92.6 ± 1.7a	93.1 ± 1.7a	70.6 ± 17.2b	0.7 ± 0.3c
52, 1	3.53	97.9 ± 0.4ab	98.2 ± 1.6a	98.5 ± 1.4a	90.1 ± 8.2b	1.9 ± 0.5c
52, 2	6.70	100a	100a	100a	100a	100a

Different letters within a row indicate that means are significantly different ($P<0.05$).

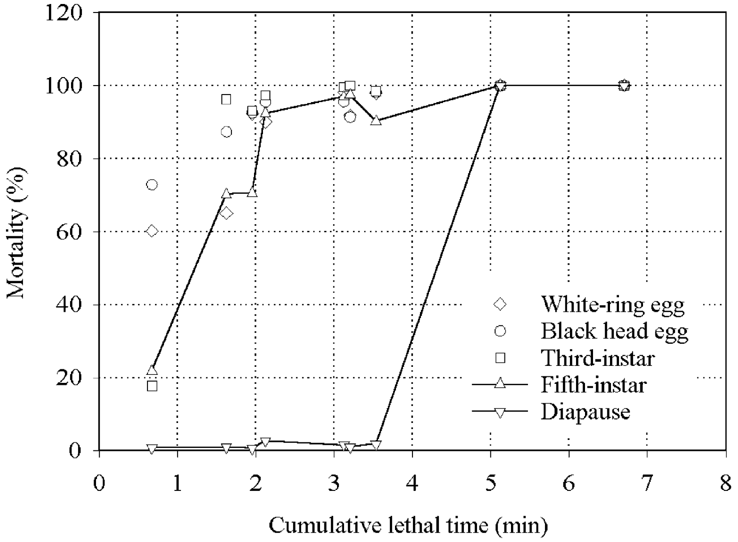

Fig. 2. Relationship between the equivalent cumulated lethal time (M_{ref}) at 50°C and the mean percentage mortality over three replicates of different life stages of codling moth.

except for the diapausing larvae, the fifth-instar was in general the most heat-resistant life stage. As demonstrated above, the equivalent lethal time M_{ref} concept could be used as an effective tool to compare relative heat resistance of different life stages.

3.2. Thermal mortality of non-diapausing and diapausing fifth-instars

Survival (mean ± SD) of diapausing fifth-instar codling moths in unheated controls (98.3 ± 0.3%) was not different from that of non-diapausing larvae (97.3 ± 2.0%), suggesting no differential and adverse effects of shipping and handling. Consequently, mortality data for the thermal treatments were used directly for analyses without corrections. Thermal mortality values of diapausing larvae as a function of the last three exposure times at temperatures of 46°C, 48°C, 50°C, and 52°C were presented in Fig. 3, together with the completely determined thermal death kinetics of non-diapausing larvae (Wang et al., 2002a). At each temperature, thermal mortality of diapausing larvae was very low (less than 5%) before a critical treatment time for each temperature, after which the mortality increased sharply to 100%. The temperature–time combination for complete kill of diapausing codling moth was the same as that of the non-diapausing larvae. This observation suggests that the final TDT curve developed for fifth-instars (Wang et al., 2002a) was still applicable for diapausing larvae. It seems that diapausing larvae had higher heat resistance until reaching a critical break point. This phenomenon is very similar to the vitality of lentil seeds when treated with heat (Tang and Sokhansanj, 1993) in which lentil seeds lost all germination ability after only a few extra degrees of temperature, but before that critical point, there was hardly any loss of vitality.

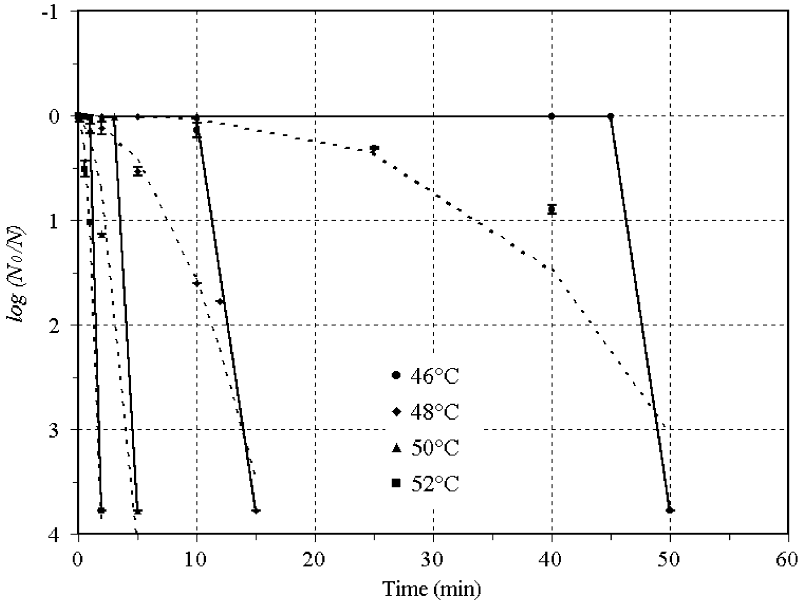

Fig. 3. Thermal mortality curve of fifth-instar diapausing (—) and non-diapausing ((- - -) from Wang et al., 2002a) codling moths at four temperatures. Each point represents three samples of 200 larvae. N_0 and N stand for initial and final numbers of live insects. $N = 0.1$ was used when 100% mortality was achieved.

4. Conclusions

Efficacy studies for thermal treatments require the most heat-resistant life stage of codling moth to be used for obtaining effective insect controls. A heating block system was used to determine the most heat-resistant life stage among white-ring eggs, black-head eggs, third-instar, fifth-instar, and diapausing larvae. In sub-lethal heat treatment conditions, the fifth-instar was the most applicable life stage for efficacy study in the tested temperature range of 50–52°C because of easy handling. From thermal death kinetic data for non-diapausing and diapausing fifth-instar larvae, the time required to achieve 100% mortality of 600 insects for both larvae were the same, although diapausing larvae seemed to be more heat-resistant than non-diapausing larvae under sub-lethal treatment conditions. An efficacy test with fifth-instar diapausing larvae will be needed to confirm the results obtained by the heating block system.

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