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Heating condition effects on thermal resistance of fifth-instar *Amyelois transitella* (Walker) (Lepidoptera: Pyralidae)

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Abstract

Successful development of a thermal treatment protocol depends on reliable information on fundamental thermal death kinetics of targeted insects under different heating conditions. The effects of heating rates (1, 10, and 15 °C min^{-1}), pre-treatment conditioning (30 °C + 6 h), and the difference between long-term laboratory cultures and recently isolated cultures on thermal mortality of fifth-instar navel orangeworm, *Amyelois transitella* (Walker), were studied using a heating block system. There was no significant difference in insect mortality resulting from heating rates of 10 and 15 °C min^{-1}. Temperature control at 1 °C min^{-1} was more uniform than for the other heating rates, resulting in reduced variability for insect mortality. The mean mortality at the heating rate of 1 °C min^{-1} was significantly lower than for the two faster heating rates only at 48 °C + 30 min. The pre-treatment conditioning of fifth-instar *Amyelois transitella* enhanced their thermotolerance only at certain temperature–time combinations. Fifth-instars from long-term laboratory and recently isolated cultures were equally susceptible to elevated temperatures. Therefore, thermal death kinetic information obtained from the long-term laboratory cultures can be used to develop thermal protocols against field pests.

Keywords: Heating block; Heating rates; Navel orangeworm; Pre-treatment; Thermal mortality

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1. Introduction

Navel orangeworm, *Amyelois transitella* (Walker) (Lepidoptera: Pyralidae), is a major insect pest infesting California tree nuts (walnuts, almonds, and pistachios) in the field. It causes post-harvest loss by directly feeding and by contaminating the product with insects, frass, and webbing. Methyl bromide (MeBr) fumigation of tree nuts is widely used to meet commercial phytosanitary requirements to control insect pests (Carpenter et al., 2000). MeBr is, however, listed as one of the chemical compounds that damages the atmospheric ozone layer (Anon., 1995). International agreements call for halting the manufacture and use of MeBr by the year 2005, although recently granted critical use exemptions may make the fumigant available to US nut processors until 2007. However, the future use of MeBr in the tree nut industry is at great risk due to reduced production, unavailability, increased price, or future restrictions under international agreements (United States Environmental Protection Agency, 1998).

Thermal treatments using hot air, hot water, and radio frequency (RF) energy have been studied to replace MeBr fumigation to control post-harvest pests in different commodities (Sharp et al., 1991; Yokoyama et al., 1991; Nelson, 1996; Jones and Waddell, 1997; Mangan et al., 1998; Tang et al., 2000). Wang et al. (2001a, 2002c, 2003) evaluated the possibility of using RF treatments to control insect pests in in-shell walnuts. A thermal disinfestation protocol based on RF energy is proposed in Wang et al. (2002c) using thermal death kinetic information of *Amyelois transitella* reared under laboratory conditions. Questions arise about whether this protocol can be effectively implemented in the tree nut industry to control wild insects that have experienced warm pre-treatment conditions around 30 °C during the nut harvest season. It is desirable to understand the effect on the thermal resistance of selected pests at slow heating rates (about 1 °C min^{-1}) using heated air (Wang et al., 2001b), as compared to the fast heating rates (about 10–15 °C min^{-1}) in RF systems (Wang et al., 2002c).

Enhanced thermotolerance following exposure to non-lethal high temperatures before reaching targeted temperatures has been reported in fruit flies (Beckett and Evans, 1997; Waddell et al., 2000), in flesh flies (Yocum and Denlinger, 1992), and in apple moths (Lester and Greenwood, 1997). Third-instar larvae of the Caribbean fruit fly, *Anastrepha suspensa* (Loew) (Diptera: Tephritidae), reared at 30 °C, were significantly more heat resistant than those reared at 20 °C (Hallman, 1994). Recently, Yin personal communication found that the minimum holding times required to completely kill 300 fifth-instar codling moths, *Cydia pomonella*, that went through pre-conditioning at 35 °C for 6 h were 30, 7, and 3 min at 48, 50, and 52 °C, respectively, as compared with 15, 5, and 2 min at those temperatures without pre-conditioning. Thermal conditioning of insects caused by the elevated pre-treatment temperatures during harvest, processing, or storage may influence treatment efficacy. However, the thermal resistance of insect pests might return to the level which existed before pre-conditioning if held at room temperature for a period of time (Jang, 1992).

Development of thermal phytosanitary treatments against targeted insects requires the use of a large number of insects ($>10{,}000$) for tests to obtain data on the thermal tolerance of the insects and for treatment on validation tests. It is impractical to collect wild *Amyelois transitella* larvae in orchards for these efforts. As a result, researchers rely on insects reared under optimal growth conditions in laboratories for mass production. Some laboratory colonies have been maintained for decades. A major concern in using lab-reared insects is that they may not have the same

thermal tolerance as wild insects, and the treatments developed using lab-reared insects may not be suitable to control field-infested commodities.

The thermal mortality results show that the fifth-instar larva of *Amyelois transitella* is the most heat-resistant stage (Wang et al., 2002c). The objectives of this study were: (1) to determine the effect of three heating rates (1, 10, and 15 °C min^{-1}) on thermal resistance of fifth-instar *Amyelois transitella* larvae using a heating block system; (2) to study the pre-treatment conditioning effect on the thermal resistance of fifth-instar *Amyelois transitella* larvae; and (3) to compare the thermal resistance of fifth-instar *Amyelois transitella* from long-established laboratory cultures with others from recently isolated cultures.

2. Materials and methods

2.1. Heating block system

An experimental thermal apparatus for insect mortality tests was developed at the Washington State University (WSU) in Pullman, WA and this heating block system consisted of top and bottom blocks, heating pads, an insect test chamber, and a data acquisition/control unit (Wang et al., 2002b). Two calibrated type-T thermocouples, inserted through sensor holes near the center of each block, monitored temperatures of the top and bottom blocks (Fig. 1). Heating rates and set-point temperatures were controlled by a Visual Basic software developed at WSU and PID controllers (i/32 temperature and process controller, Omega Engineering Inc., Stamford, CT) via two solid state relays. The relative humidity (r.h.) was around 50% inside the heating block. During the holding period at heating rates of 1, 10, and 15 °C min^{-1}, the block temperatures obtained from the temperature sensors for controllers deviated from the set point by less than 0.3 °C (Johnson et al., 2003).

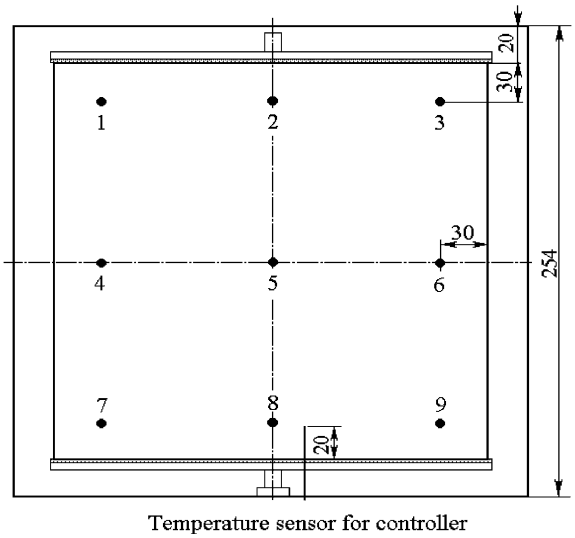

Fig. 1. Temperature sensor distributions (1–9) on the bottom block together with the sensor from the controller (all dimensions are in mm).

2.2. Insects

Fifth-instar larvae of *Amyelois transitella* were reared at the USDA San Joaquin Valley Agricultural Sciences Center, Parlier, CA, on a wheat bran diet (Tebbets et al., 1978) at 27 °C, 60% r.h. and a photoperiod of 14:10 h (L:D). Before treatments with the heating block system, larvae were placed in plastic cups (475 ml) with diet, packed in an insulated shipping carton, and shipped via overnight delivery to WSU, Pullman, WA. The insects were left at room temperature for several hours before testing. We used only actively moving fifth-instar larvae in the tests.

Due to difficulties of collecting insects from the field in suitable numbers for testing, recently isolated *Amyelois transitella* cultures obtained from nut orchards in the fall of 2001 were compared to long-established *Amyelois transitella* cultures. The recently isolated cultures were reared on a mix of wheat bran diet and walnuts, and held at 27 °C, 60% r.h., and a photoperiod of 14:10 h (L:D). At the time of testing, recently isolated cultures had been reared for about five generations in the laboratory whereas the long-term laboratory cultures had been reared for more than 100 generations. Fifth-instar larvae of all cultures were shipped to WSU by overnight delivery for comparison tests.

2.3. Heat treatments

Three heating rates of 1, 10, and 15 °C min^{-1} were used to raise insect temperatures to a selected level between 46 and 52 °C. Complete kill of 600 fifth-instar *A. transitella* larvae required a minimum exposure time of 140, 50, 15, 6, and 1 min at 46, 48, 50, 52, and 54 °C based on the thermal-death-time (TDT) curve at a heating rate of 18 °C min^{-1} (Wang et al., 2002b). To compare the effect of the three heating rates (ramp periods) on insect mortality, four temperature-holding time combinations, 46 °C + 100 min, 48 °C + 30 min, 50 °C + 10 min, and 52 °C + 1 min, were deliberately selected to obtain a thermal mortality of less than 100%. Control larvae were placed in the unheated block chamber at 22 °C for 100 min. The 200 insect larvae were distributed uniformly on the block surface before each run and each treatment was repeated three times using three different batches of test insects. The treatments were conducted in random order for all the tests.

In order to evaluate the effect of temperature distributions over the heating block surface on the insect mortality at the three heating rates, nine fast response thermocouple temperature sensors with self-adhesive backing (SA1-T, Omega Engineering Inc., Stamford, CT) were used (Fig. 1). All the sensors were calibrated against a pre-calibrated mercury-in glass thermometer in an oil bath set at 22 and 50 °C to ensure measurement accuracy. Temperatures at nine different locations were monitored while the heating block temperature was raised from 22 to 50 °C at a heating rate of 1, 10, or 15 °C min^{-1}. The measured data from these nine sensors were recorded every 5 s by a data logger (DL2e, Delta-T Devices Ltd., Cambridge, UK).

Tree nut harvest season in California lasts from early September to mid-November when the daytime air temperature is around 30 °C for up to 6 h. It is desirable to evaluate the influence of these warm conditions on thermal resistance of insect pests. For this purpose, the insect chamber containing live larvae was pre-heated from 22 to 30 °C and held at 30 °C for 6 h, and then heated at 15 °C min^{-1} to a final temperature for a given time period. The effects of the pre-treatment conditioning on the insect mortality were evaluated at the same four temperature–time

combinations as used for the heating rate tests. Pre-conditioned control larvae were placed in the heated block chamber at 30 °C for 6 h without further heat treatments.

To compare the heat resistance between the recently isolated (five generations) and the long-term laboratory (>100 generations) cultures, three temperature–time combinations, 48 °C + 25 min, 50 °C + 10 min, and 52 °C + 4 min, were selected for the heating rate of 15 °C min^{-1}. For all the insect mortality tests, 200 larvae were treated at a time and each treatment was repeated three times for a total of 600 larvae. Post-treatments followed the same procedures for all tests.

2.4. Insect mortality evaluation and statistical analysis

At the end of each treatment, the insects were transferred to a plastic container in less than 10 s. Cardboard strips were provided for pupation sites. Because we anticipated that commercial treatments would include rapid post-treatment cooling of product 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 one day. After the cold storage, the larvae were held at 23 °C, 60% r.h. and a 14:10 h (L:D) photoperiod for one day to minimize the effect of cold stupor before examination. Insects were considered dead if the body color became black. Moribund and surviving larvae were observed for an additional five days. Pupation or adult emergence was not used in the evaluation because the time to pupation and adult emergence took several weeks.

Mortality was calculated as the percentage of dead larvae relative to total treated larvae for each treatment. Mean values and standard deviations were calculated from three replications for each temperature–time combination. Treatment mortality was corrected based on the control mortality using the Abbott's (1925) formula. An arcsine transformation was used to normalize the data before analysis. Effects of heating rate on insect mortality were compared using the SAS analysis of variance test (ANOVA) procedure (SAS Institute, 1999). For each temperature–time combination where ANOVA showed significant differences ($P \leqslant 0.05$), means for heating rate were separated using least significant difference (LSD). Mean mortality for pre-conditioned and unconditioned insects was compared using *t*-tests (SAS Institute, 1999).

3. Results and discussion

3.1. Heating rate effect on thermal mortality

Control mortality (mean ± SD) for fifth-instar larvae was 7.8 ± 2.5% and 2.3 ± 3.6% after 100 min of exposure at 22 °C and after 6 h of exposure at 30 °C, respectively. This indicated that there were no adverse effects from handling or the elevated pre-treatment temperature. Table 1 shows the corrected mortality (mean ± SD) of fifth-instar larvae at different temperature–time combinations at the heating rates of 1, 10, and 15 °C min^{-1}. There were no significant differences in thermal mortalities among heating rates for any of the temperature and time combinations, except for the 48 °C + 30 min treatment, where mortality for the 1 °C min^{-1} heating rate was significantly lower ($F = 8.2$, $df = 2,6$, $P < 0.05$) than the faster heating rates. Evans (1987) and Neven (1998) found that both the lesser grain borer, *Rhyzopertha dominica* (L.) (Coleoptera:

Table 1
Corrected mortality (mean ± SD, %) of fifth-instar *Amyelois transitella* held under constant conditions before treatment at three different heating rates (200 insects per each of three replicates)

Temperature + holding time	1 °C min^{-1}	10 °C min^{-1}	15 °C min^{-1}
46°C + 100 min	71.4 ± 6.4a	76.1 ± 11.4a	79.9 ± 14.1a
48°C + 30 min	45.4 ± 2.4b	76.7 ± 12.2a	73.8 ± 9.9a
50°C + 10 min	77.5 ± 3.2a	89.6 ± 8.1a	88.9 ± 5.1a
52°C + 1 min	63.0 ± 6.3a	40.3 ± 16.5a	41.1 ± 19.3a

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

Bostrichidae), and *C. pomonella* larvae had to be exposed to 80 and 46 °C for longer times at slower heating rates (0.13–0.2 °C min^{-1}) to achieve 99.9% and 95% mortality, respectively. Thomas and Shellie (2000) also observed that the exposure times to achieve 99% Mexican fruit fly (Diptera: Tephritidae) mortality at 44 °C were 62 and 42 min at heating rates of 0.175 and 1.4 °C min^{-1}, respectively. It was clear from Table 1, however, that the effect of ramp time at 1 °C min^{-1} on developing insect thermotolerance became insignificant at higher temperatures, especially at 52 °C. A similar finding was observed for the fifth-instar larvae *C. pomonella* (Wang et al., 2002a).

A careful examination of the mortality data reveals that variability for each treatment is much smaller at 1 °C min^{-1} than at 10 and 15 °C min^{-1}. The large variations in mortality data at 10 and 15 °C min^{-1} can be partially explained by temperature overshoots and slightly non-uniform heating in the heating block (Fig. 2). For example, the hottest spot (51.2 °C) was located in the center and the coldest spots (48.8 °C) were located in the four corners at the heating rate of 10 °C min^{-1} while reaching the set point of 50 °C. The overall maximum standard deviations for temperatures over the nine positions were 0.8 and 1.2 °C for the heating rate at 10 and 15 °C min^{-1}, respectively, when the mean block temperature reached the set point of 50 °C. After 30 s of reaching 50 °C, however, the temperature difference between the hottest location among the nine measured positions and the set point of 50 °C was reduced to less than 0.6 °C both for heating rates of 10 and 15 °C min^{-1} (Fig. 2). At that moment, the mean temperatures (±SD) over the nine positions were 49.4 ± 0.5 and 49.3 ± 0.6 °C for 10 and 15 °C min^{-1}, respectively. No temperature overshoots were observed for any position at the heating rate of 1 °C min^{-1} (Fig. 3). The temperatures at those positions were well controlled both for the ramping and holding periods. For example, the average temperature and standard deviation over the nine positions were 49.4 and 0.2 °C, respectively, when reaching the set point of 50 °C (Fig. 3).

Wang et al. (2002a, 2004) studied the accumulated effect of ramping time on the thermal mortality of fifth-instar larvae *C. pomonella*. For the 10 and 15 °C min^{-1} heating rates, the cumulative effect of the ramping period on the insect mortality was equivalent to 0.2 and 0.1 min exposure at the final temperature. As a result, one would not expect to see much difference in the observed thermal mortality between these two heating rates. Indeed, there was no significant difference of mortality between 10 and 15 °C min^{-1} ($P > 0.05$) as shown in Table 1. For 1 °C min^{-1}, however, the cumulative effect on the insect mortality for the ramping period was equivalent to 1.8 min exposure at the final temperature. This might have led to the higher

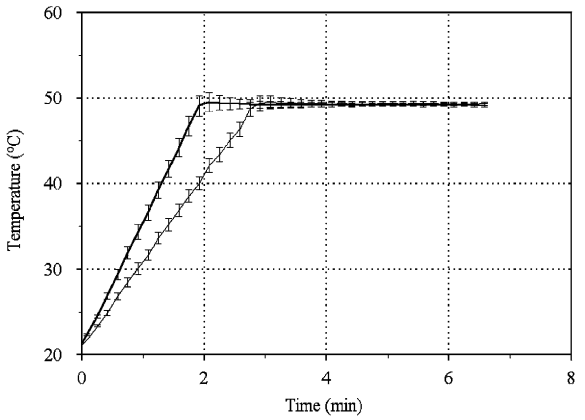

Fig. 2. Average and standard deviation values of temperature–time histories over nine positions on the bottom block surface from 22 to 50 °C at the heating rates of 10 (—) and 15 °C min^{-1} (—).

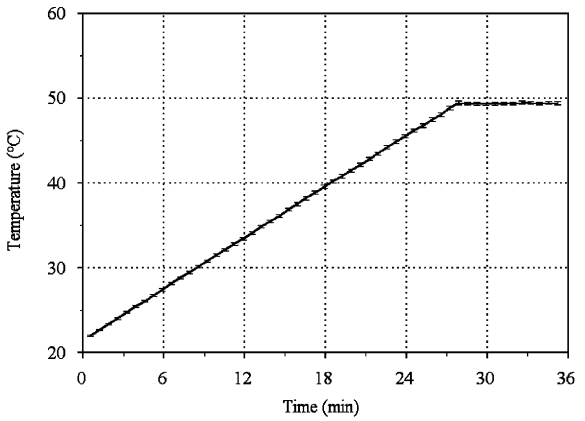

Fig. 3. Average and standard deviation values of temperature–time histories over nine positions on the bottom block surface from 22 to 50 °C at the heating rate of 1 °C min^{-1}.

mortality for treatments at set temperatures and short holding times (e.g., 52 °C + 1 min) for the heating rate of 1 °C min^{-1}, as compared with faster heating rates.

3.2. Pre-treatment conditioning effect

Table 2 shows the effect of pre-treatment conditioning (30 °C + 6 h) with a heating rate of 15 °C min^{-1} on mortality of fifth-instar larvae. Pre-treatment conditioning at the elevated but non-lethal temperature of 30 °C appeared to reduce mean larval mortality when compared with those without conditioning, but the reduction was significant ($P < 0.05$) only for treatments at 46 °C + 100 min and 50 °C + 10 min. Yocum and Denlinger (1992), Beckett and Evans (1997), and Waddell et al. (2000) also observed reduced mortality in insects pre-treated under warm but

Table 2
Effect of a pre-treatment conditioning (30 °C + 6 h) on mortality (mean ± SD, %) of fifth-instar *Amyelois transitella* after heating at 15 °C min^{-1} (200 insects per each of 3 replicates)

Temperature + holding time	Without conditioning	With conditioning
46 °C + 100 min	79.9 ± 14.1*	45.4 ± 11.2*
48 °C + 30 min	73.8 ± 9.9NS	64.6 ± 13.0NS
50 °C + 10 min	88.9 ± 5.1*	57.6 ± 13.2*
52 °C + 1 min	41.1 ± 19.3NS	21.4 ± 3.6NS

t-test performed on arcsine-transformed data within rows, *, $P \leqslant 0.05$; NS, not significant.

non-lethal conditions prior to heat treatment. It is clear from our study that insects, after pre-treatment conditioning at sub-lethal warm temperatures, had to be exposed to a treatment temperature for a longer time to achieve the same mortality than those without conditioning. The enhanced thermotolerance was possibly caused by the expression of heat-shock proteins (Thomas and Shellie, 2000). Extending treatment time or slightly raising the treatment temperature may meet the needs in controlling insect pests with increased thermal resistance as a result of pre-treatment conditioning (Yin, personal communication). They also reported that exposure of insects to 22 °C for 2 h is adequate to completely eliminate the effect of warm weather conditioning on *C. pomonella*. Instead of extending treatment time or raising temperatures, conditioning of nuts at room conditions for a certain period before treatments may also help reduce the enhanced heat resistance of *Amyelois transitella* due to pre-treatment conditioning at sub-lethal warm temperatures. Further studies are needed, both to determine the effects that higher pre-treatment temperatures used in mechanical nut dehydration may have on treatment efficacy, and to determine the recovery times at room conditions to reduce the increased heat resistance of fifth-instar larvae of *A. transitella*.

3.3. Comparison between recently and long-term isolated cultures

Table 3 shows a comparison of the relative heat resistance between the recently isolated and long-term laboratory *Amyelois transitella* cultures. The mean mortality of fifth-instar larvae from the long-term laboratory cultures appeared to be lower than that of the recently isolated cultures, but the difference was not significant ($P > 0.05$). This suggests that the thermal treatments developed based on long-term laboratory-reared insects should be equally effective against wild insects. However, it is important to test additional field populations to confirm the findings based on a single recently isolated culture.

4. Conclusions

The thermal mortality of fifth-instar larvae of *Amyelois transitella* appears to be influenced by a variety of different treatment conditions, including heating rate, pre-treatment conditioning and the source of test insects. The temperature–time histories and temperature distributions over the heat block can partially explain the insect mortality differences observed at the three heating rates.

Table 3
Mortality (mean ± SD, %) of fifth-instar *Amyelois transitella* from the long-term laboratory (100 generations) and the recently isolated cultures (five generations) after heating at 15 °C min^{-1} (200 insects per each of three replicates)

Temperature + holding time	Long-term culture	Recent culture
48 °C + 25 min	72.4 ± 15.0	98.3 ± 1.7
50 °C + 10 min	82.7 ± 16.9	78.3 ± 11.3
52 °C + 4 min	95.0 ± 5.0	95.7 ± 2.6

t-tests performed on arcsine-transformed data within rows indicated no significant differences ($P > 0.05$).

Statistically, the effect of heating rate considered in this study on the thermal mortality of fifth-instar larvae was, however, negligible. The pre-treatment conditioning at 30 °C for 6 h increased the thermal resistance of *Amyelois transitella*. This study underlines the importance of keeping infested nuts in a cool condition for a period of time to avoid pre-treatment conditioning. There was no significant difference between the observed thermal mortalities of long-term laboratory and recently isolated cultures. Therefore, the thermal kinetic information obtained from long-term laboratory *Amyelois transitella* cultures may serve as an adequate base for developing the practical thermal treatment protocol against wild insects.

Acknowledgments

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