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Thermal resistance of fifth-instar *Cydia pomonella* (L.) (Lepidoptera: Tortricidae) as affected by pretreatment conditioning

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Abstract

Codling moth (*Cydia pomonella* (L.)) is targeted for postharvest control by quarantine regulations in Japan and South Korea and by phytosanitation concerns in Europe. Heat treatments may be used to control *C. pomonella*. But possible increase of heat resistance in insect pests, caused by pretreatment thermal conditions during harvest and storage periods, may compromise the efficacy of subsequent thermal treatments. A heating block system was used to determine the effect of pretreatment conditioning on the thermal resistance of the fifth-instar *C. pomonella*. Results showed that pretreatment conditioning at 35 °C for 40, 120, 360 or 1080 min significantly increased the thermal resistance of *C. pomonella*. Among the above conditions 35 °C for 360 min resulted in the highest heat resistance for fifth-instars. The minimum treatment times required to reach 100% mortality for 300 larvae that went through thermal conditioning at 35 °C for 360 min 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 pretreatment conditioning. After a pretreatment at 35 °C for 360 min followed by a period of at least 120 min at 22 °C, fifth-instar thermal resistance returned to the level that had existed before pretreatment conditioning.

Keywords: Codling moth; Heating block; Pretreatment conditioning; Quarantine; Thermal death resistance; Recovery

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1. Introduction

Heat treatments are increasingly used or are being considered as an alternative control measure to provide quarantine security against insect pests in fresh and stored agricultural commodities in the wake of consumer concerns over the use of chemical treatments. The heating methods include forced hot air (Armstrong et al., 1989; Sharp, 1992), hot water (Yokoyama et al., 1991; Jones and Waddell, 1997), vapour heat (Hansen et al., 1992; Shellie and Mangan, 1994), microwaves (Ikediala et al., 1999), and radio frequency (RF) energy (Tang et al., 2000; Wang et al., 2001, 2002c). The most important variables for heating methods are target temperature and exposure time, which are predetermined in efficacy tests defining insect thermal resistance and thermal death kinetics under laboratory conditions. However, test insects are usually maintained at room temperature (20–25 °C) for at least 12 h before mortality studies. This general base-line information on insect mortality is widely used in developing thermal treatment protocols. Deviation of harvest and storage conditions from 20 to 25 °C prior to treatments may alter the efficacy of the treatments because the minimum time-temperature combinations required to kill certain insect populations may change with pretreatment conditions at sub-lethal warm temperatures (Jang, 1992; Waddell et al., 2000).

Codling moth larvae, *Cydia pomonella* (L.) (Lepidoptera: Tortricidae), are important cosmopolitan insect pests, and feed on many nuts and fruits (Barnes, 1991; Wearing et al., 2001; Hansen et al., 2002). *Cydia pomonella* is also an important target of quarantine regulations in Japan and South Korea and of phytosanitation concerns in Europe. To meet the quarantine requirements, all development stages of *C. pomonella* need to be killed by the thermal treatments. Based on previous studies (Wang et al., 2002a, 2004) on thermal lethality of *C. pomonella* using the heating block system, fifth-instars are the most heat tolerant stage, as also found by Yokoyama et al. (1991).

Increased heat resistance following exposure to sub-lethal temperatures between 32 and 42 °C, has been observed in *Drosophila* sp. (Feder et al., 1996), tephretid fruit flies (Jang, 1992; Beckett and Evans, 1997; Waddell et al., 2000), flesh flies (Yocum and Denlinger, 1992), and lightbrown apple moths, *Epiphyas postvittana* (Walker) (Lepidoptera: Tortricidae), (Lester and Greenwood, 1997). Hallman (1994) observed that third instars of the Caribbean fruit fly, *Anastrepha suspensa* (Loew) (Diptera: Tephritidae), reared at 30 °C, were significantly more heat tolerant than those reared at 20 °C. Lester and Greenwood (1997) reported that the lethal time for 99% mortality for fifth-instar lightbrown apple moths increased from 23 min without pretreatment thermal conditioning to 37 min after 8 h at 35 °C. Hallman and Mangan (1997) cautioned that field temperatures of infested fruits might alter the efficacy of quarantine heat treatments, which were developed using laboratory insects reared at constant temperatures. As a result, thermal conditioning of insects caused by warm weather may compromise the effectiveness of the treatment because of the increased insect heat resistance. It is important to determine the enhanced heat resistance of targeted insects as affected by various pretreatment conditions. Currently, there are no reported data about the effect of pretreatment conditions on heat resistance of *C. pomonella*.

Heat resistance of thermally conditioned fruit flies reverted to the level that existed before pretreatment conditioning within 2–3 h after return to 26 °C (Jang, 1992). If applicable to *C.*

pomonella, a holding period of 2–3 h at 20–26 °C could be added before a heat treatment to reduce the chance of increased heat resistance and to ensure treatment success.

The purposes of this study were: (1) to determine the effect of pretreatment conditioning on the thermal resistance of fifth-instar *C. pomonella*; (2) to compare the thermal mortality of fifth-instars under non-pretreatment conditions; and (3) to study possible recovery from thermal resistance caused by pretreatment conditioning.

2. Materials and methods

2.1. Test insects

Cydia pomonella larvae were reared at 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). Third-instars, in diet, were shipped in a large cooler chest (100 × 50 × 50 cm) from YARL to Washington State University (WSU) by overnight delivery. At WSU, larvae were reared in the conditions mentioned above until they developed to fifth-instars, which have distinguishing head capsule coloration.

2.2. Heating block system

Insect mortality tests were conducted using the heating block system developed at WSU (Wang et al., 2002b). The system consisted of top and bottom aluminium blocks, heating pads, an insect test chamber, and a data acquisition/control unit (Fig. 1). A PID control unit (CN616TCO, Omega Engineering Inc., Stamford, CT, USA) regulated a heating block temperatures via the solid-state relay, with a mean error of less than 0.3 °C from the set-point temperature. Detailed information on this heating block system can be found in Wang et al. (2002b). The heating block system has been used to determine the thermal death kinetics of *C. pomonella* (Wang et al., 2002a); Indian meal moth, *Plodia interpunctella* (Hübner) (Lepidoptera: Pyralidae) (Johnson et al., 2003); and navel orangeworm, *Amyelois transitella* (Walker) (Lepidoptera: Pyralidae) (Wang et al., 2002b). In this study, a heating rate of 15 °C/min was chosen for all the treatments to simulate the fast heating of commodities when subjected to electromagnetic energy (Tang et al., 2000).

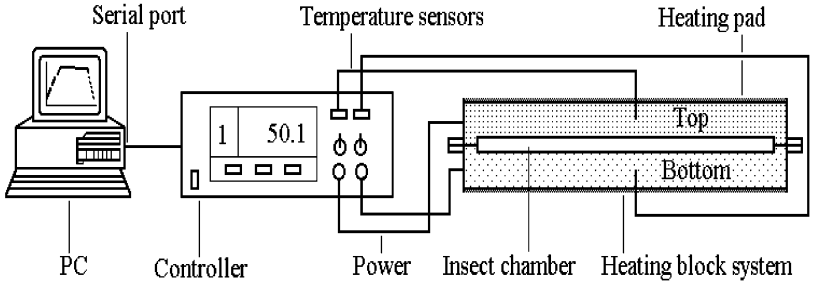

Fig. 1. Diagram of the heating block system (not to scale).

2.3. Pretreatment conditioning and heat treatments

We selected 35 °C for the pretreatment temperature to simulate the daytime air temperature during the nut harvest period in California. Fifth-instars were exposed to the pretreatment conditions for 40, 120, 360 or 1080 min at 35 °C before the thermal treatments to determine pretreatment resistance induction using the WSU heating block system. For the 40 and 120 min preconditioning, insect larvae were directly conditioned in the heating block system at 35 °C. On completion of pretreatment conditioning, the blocks were heated at 15 °C/min to the preset postconditioning treatment temperatures.

For the 360 and 1080 min preconditioning, fifth-instars, held in plastic containers ($15 \times 10 \times 5$ cm), were placed in an incubator at 35 °C. Strips of cotton saturated with distilled water were placed in the containers to maintain a suitable humid environment. The containers were covered with fine plastic mesh screens to allow passage of air. Immediately after the preset preconditioning periods, insects were gently transferred into the heating block system and the blocks were then heated to the preset temperatures at a 15 °C/min heating rate (Fig. 2).

To determine the thermal resistance of insects after preconditioning, we selected minimum holding times of 10, 3 and 1 min for 48, 50, and 52 °C respectively, because these were just below the thermal-death-time (TDT) curve that was obtained in a previous study (Wang et al., 2002a) for codling moth without preconditioning. Additional experiments were conducted with holding times increased by 5 min increments for 48 °C, 2 min increments for 50 °C and 1 min increments for 52 °C until 100% mortality was achieved in all the tested replicates. For example, holding

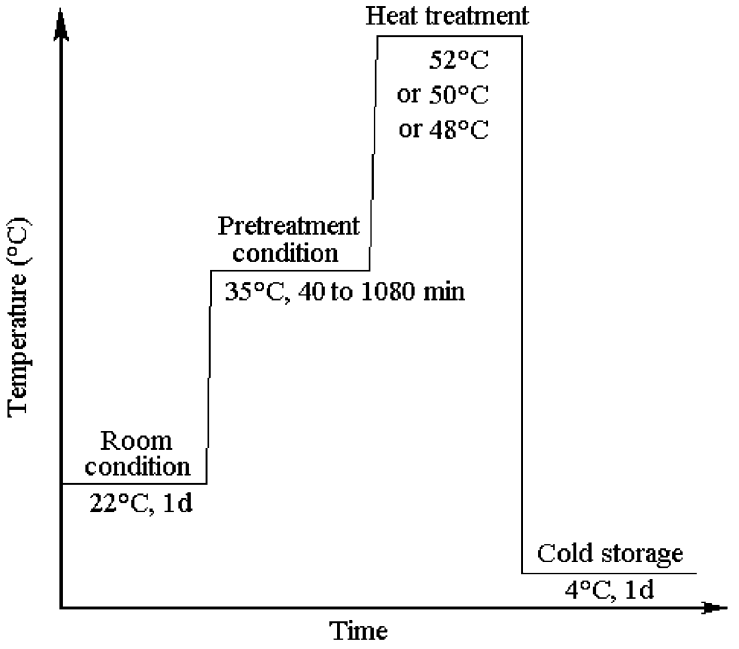

Fig. 2. Experimental conditions to determine the heat resistance of fifth-instar *C. pomonella* preconditioned at 35 °C using heat treatments at 48, 50 and 52 °C.

times of 10, 15, 20, 25 and 30 min were used at 48 °C. One hundred larvae were used for each run. Each treatment was repeated three times for a total of 300 larvae. After each treatment, insects were gently transferred to plastic containers covered with lids with a fine mesh screen. Because commercial thermal treatments for fresh fruits often include rapid posttreatment cooling to minimize the effect on product quality, the treated larvae in plastic containers were immediately placed in 4 °C cold storage for 1 day. After the cold storage, all plastic containers with larvae were placed at room temperature (22 °C), 60% r.h. and a 16:8 h (L: D) for 1 day to eliminate cold stupor before examination. The live and dead larvae were checked and counted once a day, over 6 days. Larvae that did not move after prodding were considered dead.

The general schemes for the above tests are illustrated in Fig. 2. Preconditioned controls for 0, 40, 120, 360 and 1080 min were not subjected to heat treatments and were held in plastic containers, with wet strips of cotton, at room temperature (22 °C) for 1 day before mortality evaluations.

2.4. Holding tests under ambient conditions

Mortality results of all preconditioned experiments showed that pretreatment at 35 °C for 6 h induced the highest level of resistance and these conditions were chosen for further tests to identify possible recovery. After preconditioning, fifth-instars were placed at 22 °C for 0.5, 1 or 2 h based on previous studies with fruit flies (Jang, 1992; Waddell et al., 2000) (Fig. 3). After placement for up to 2 h at 22 °C, larvae were treated in the heating block system by using three temperature–time combinations (48 °C for 15 min, 50 °C for 5 min or 52 °C for 2 min), which resulted in 100% mortality (Wang et al., 2002a). Larvae conditioned at 35 °C for 6 h were directly treated at 48 °C for 15 min, 50 °C for 5 min, or 52 °C for 2 min to serve as a control for the recovery tests. The same

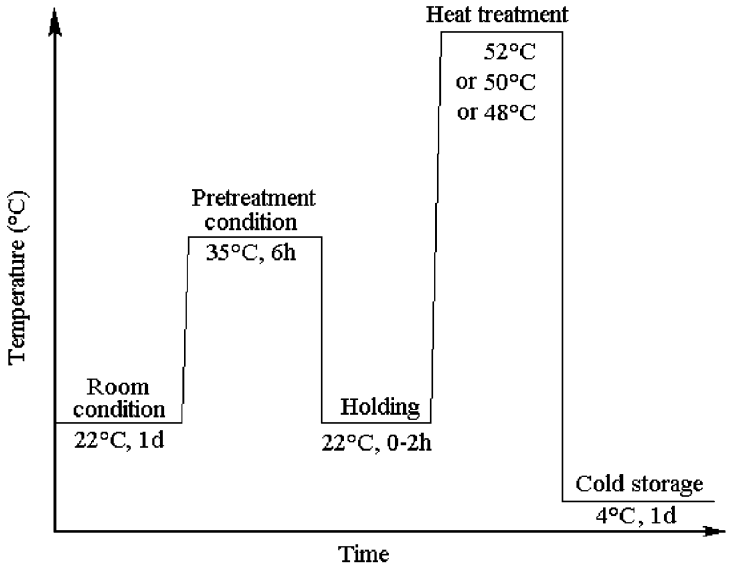

Fig. 3. Experimental conditions to treat recovered fifth-instar *C. pomonella* after pretreatment conditioning at 35 °C for 6 h.

procedures as those of the preconditioning tests were followed for posttreatment handling and mortality evaluations.

2.5. Statistical analyses

Larval mortality was calculated as the percentage of dead larvae relative to total treated larvae (N_0) for each treatment ($((N_0 - N) \times 100/N_0)$, where N is the number of surviving larvae. Treatment mortality was corrected based on the control mortality using Abbott's (1925) formula. Significant differences ($P \leqslant 0.05$) among mean values were identified using least significant difference (LSD) t-test (SAS Institute, 1999). Mean values and standard deviations for each temperature–time combination were obtained from three replicates.

3. Results and discussion

3.1. Thermal mortality as affected by pretreatment conditioning

Control mortalities (mean ± SD) were 7.6 ± 3.4% (non-preconditioned), 10.0 ± 3.6% (preconditioned at 35 °C for 40 min), 5.3 ± 1.5% (120 min), 8.3 ± 3.2% (360 min), and 9.7 ± 4.0% (1080 min). There was no significant mortality difference between preconditioned controls and non-preconditioned control samples, suggesting handling and long preconditioning had little effect on tested insects. To take into account the effect of control mortality differences, all mortality data were corrected before further statistical analysis, and means and standard deviations of fifth-instar mortality were corrected for each preconditioned test after the heat treatments. Table 1 lists

Table 1
Mortality (mean ± SD, %) of fifth-instar *C. pomonella* treated at different holding times at three temperatures as affected by different pretreatment times at 35 °C (3 replicates each with 100 larvae)

Temp. (°C)	Treatment time (min)	Mortality (%) for pretreatment conditioning time at 35 °C (min)				
		0	40	120	360	1080
48	10	97.4 ± 0.5a*	89.6 ± 9.6a	—	—	—
	15	100a	98.2 ± 0.6b	79.2 ± 1.9c	91.7 ± 3.9ab	82.1 ± 7.5abc
	20	100a	100a	95.8 ± 1.0b	96.0 ± 1.7b	97.0 ± 0.7b
	25	100a	100a	100a	98.5 ± 1.3a	100a
	30	100a	100a	100a	100a	100a
50	3	96.9 ± 1.1a	92.5 ± 4.5ab	82.7 ± 1.7b	78.7 ± 9.5ab	80.2 ± 5.3ab
	5	100a	100a	96.1 ± 23.a	90.9 ± 4.2a	95.1 ± 3.0a
	7	100a	100a	100a	100a	100a
52	1	90.1 ± 8.2a	95.1 ± 3.5a	85.9 ± 1.4a	85.3 ± 10.8a	90.7 ± 2.1a
	2	100a	100a	96.4 ± 1.3b	99.3 ± 1.2a	100a
	3	100a	100a	100a	100a	100a

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

thermal mortality results of preconditioned larvae at 35 °C subsequently treated at 48, 50 and 52 °C. Without preconditioning, all fifth-instars were killed after being treated for 15, 5 and 2 min at 48, 50 and 52 °C, respectively, which confirms results from a previous study (Wang et al., 2002a). There was no significant difference ($P > 0.05$) in mortality between non-preconditioned and preconditioned insects at 35 °C for 40 min for all treatments except for treatments of 48 °C + 15 min. Preconditioned fifth-instars at 35 °C for 120, 360 or 1080 min exhibit significantly less mortality than non-preconditioned larvae at the 48 °C + 15 min, 48 °C + 20 min, 50 °C + 3 min, and 52 °C + 2 min treatments ($P < 0.05$). The average mortality of insects conditioned at 35 °C for 360 min was the lowest among the preconditioned periods for all treatments at 50 °C. The time required for achieving 100% mortality for larvae preconditioned at 35 °C for 360 min at 48, 50, and 52 °C increased by 15, 2, and 1 min, respectively, over those not conditioned. These observations are similar to those found on other insects. Lester and Greenwood (1997) reported that lightbrown apple moth larvae were the most tolerant after preconditioning at 43 °C for 8 h using hot-water immersion. Waddell et al. (2000) also observed that eggs of the Queensland fruit fly, *Bactrocera tryoni* (Froggart) (Diptera: Tephritidae), were the most heat resistant after preheating at 38 °C for 7 h.

Fig. 4 summarizes relationships between preheating times at 35 °C and minimum times to achieve 100% mortality of fifth-instars held at 48, 50 and 52 °C. Sub-lethal thermal conditioning at 35 °C for 120, 360 and 1080 min significantly increased the thermal resistance of *C. pomonella* for all three-treatment temperatures. Preconditioning at 35 °C for 40 min increased heat resistance of insect pests only for the treatment at 48 °C. At the treatment temperature of 48 °C, the minimum times required to reach 100% mortality increased more than those at 50 and 52 °C. By increasing the exposure for 1 min at 52 °C, all larvae preconditioned at 35 °C from 40 to 1080 min died in each of the treatments. When developing thermal protocols for insect pests in nuts and fruits, a treatment protocol at high temperatures may avoid problems associated with sub-lethal thermal conditioning.

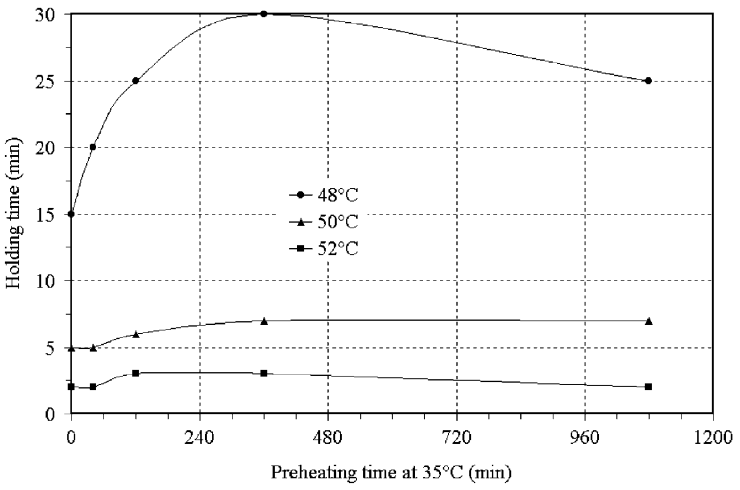

Fig. 4. Pretreatment effect on minimum treatment time required for 100% mortality of fifth-instar *C. pomonella* at temperatures of 48, 50 and 52 °C (3 replicates for 300 larvae).

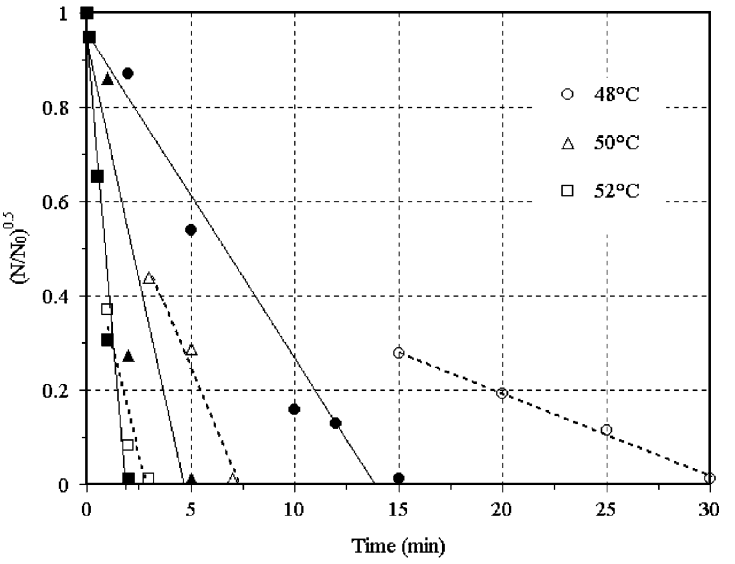

Fig. 5. Thermal mortality curves of fifth-instar *C. pomonella* without (solid) and with pretreatment at 35 °C for 6 h (dot and empty symbol) at three different temperatures. N_0 = number of treated insects, N = number of surviving insects.

Mortality data in this study were compared with thermal mortality curves obtained in Wang et al. (2002a), which examined *C. pomonella* larvae not preconditioned under sub-lethal thermal conditions. Thermal mortality curves, showing the relationships between the 0.5th reaction order value for the ratio of the surviving (N) to the initial number (N_0) of insects and the exposure time, were developed for both with and without preconditioning at 35 °C for 360 min at 48, 50, and 52 °C (Fig. 5). The conditioned fifth-instar *C. pomonella* generally had lower mortality (higher N/N_0 values) than non-conditioned larvae at each treatment temperature. The mortality lines with preconditioning shifted with decreased slopes to longer exposure times. The increased thermal resistance due to thermal conditioning was more dramatic for treatments at 48 °C than at 50 and 52 °C.

3.2. Effect of the holding time at room temperature on thermal mortality

The mortality (mean ± SD, %) of the fifth-instars is presented in Table 2 for different holding times at 22 °C after preconditioning at 35 °C for 360 min. Though there was no significant mortality difference statistically after holding at 22 °C, the average mortality value of conditioned insects gradually increased with increasing holding time. After holding for 2 h at room temperature, the insects were all killed by treatments with the treatment temperature of 50 °C for 5 min, with the treatment temperature of 52 °C for 2 min, and the treatment temperature of 48 °C for 15 min. The higher the treatment temperatures, the less holding time at 22 °C was needed to achieve 100% mortality.

Mortality differences are shown in Fig. 6 between fifth-instar *C. pomonella* that were not conditioned and those conditioned at 35 °C for 360 min. The induced heat resistance from

Table 2
Mortality (mean ± SD, %) of fifth-instar *C. pomonella* at three temperature–time combinations as a function of holding time at 22 °C after pretreatment conditioning at 35 °C for 6 h (3 replicates for 300 larvae)

Temperature (°C)	Treatment time (min)	Mortality (%) for holding time at 22 °C (h)			
		0	0.5	1	2
48	15	92.7 ± 2.1*	97.0 ± 1.0	98.3 ± 1.5	100
50	5	93.0 ± 3.6	98.3 ± 1.5	100	100
52	2	99.0 ± 1.7	99.3 ± 1.2	100	100

*There was no statistical difference for all recovery tests ($P > 0.05$).

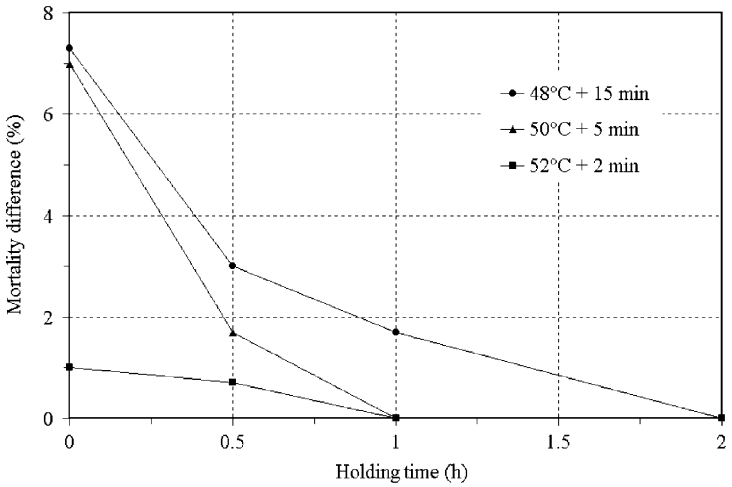

Fig. 6. Mortality differences of fifth-instar *C. pomonella* between non-conditioned samples and the samples conditioned at 35 °C for 6 h as a function of the holding times at 22 °C using three different temperature–time combinations.

pretreatment conditioning decreased with holding time at room temperature. The first 0.5 h holding at 22° C rapidly reduced the heat resistance of fifth-instars for treatments at 48 °C + 15 min and 50 °C + 5 min. The induced thermal resistance completely disappeared at 2 h holding at 22 °C for all samples.

The observed increase in thermal resistance of insects may be related to heat shock proteins induced by sub-lethal temperatures and other environmental stresses (Jang, 1992; Lester and Greenwood, 1997; Dahlgaard et al., 1998; Waddell et al., 2000). Jang (1992) reported that major heat shock proteins in fruit fly induced by conditioning at 33–41 °C disappeared after recovery for 2 to 3 h at 26 °C.

These results suggest that increased thermal resistance from warm weather (30–40 °C) can be reduced in insect pests. Extrapolating to commercial conditions, infested commodities can be stored at room temperature for a certain period before heat treatments for controlling pests in infested fruits and nuts. Future studies are needed to confirm this hypothesis.

4. Conclusions

The WSU heating block system was used to determine the effect of pretreatment conditioning on the thermal resistance of fifth-instar *C. pomonella*. Pretreatment conditioning for 40, 120, 360 or 1080 min at 35 °C increased the thermal resistance. The insect was most heat resistant after 360 min conditioning. Because of the influence of thermally induced heat resistance, *C. pomonella* that went through sub-lethal thermal conditioning required longer heat treatments than those that did not experience such conditioning. But the induced heat resistance due to pretreatment conditioning at 35 °C for 360 min could be reduced with holding times at room temperature and completely disappeared after a holding period of 2 h at 22 °C.

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