EFFECTS OF HEATING RATE ON THE MORTALITY OF LIGHTBROWN APPLE MOTH

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ABSTRACT

The effect of heating rate on mortality of fifth instar *Epiphyas postvittana* (Walker) was investigated. Insects were treated in air or water ramped to target temperatures over a range of times. Hot air treatments where the temperature of the air was increased from 20 to 43°C in 1, 2 and 4 h were associated with lethal times for 99% mortality (LT₉₉) that were 29.8, 45.8 and 44.6 minutes longer than the LT₉₉ for LBAM exposed to air at 43°C throughout the treatment. In contrast, no increase in tolerance was observed if the insects were immersed in water which was heated to a target of 43°C over similar ramp durations.

Keywords: lightbrown apple moth, *Epiphyas postvittana*, thermotolerance, heating rate.

INTRODUCTION

Heating as a quarantine treatment is a proven technology for a range of crops (Armstrong 1994; Waddell *et al.* 1997). Most heat treatments have been developed for tropical produce but research has also been carried out for temperate crops (Dentener *et al.* 1996; Neven *et al.* 1996; Yokoyama and Miller 1987). The recent thermal history of the insect has a significant effect on the efficacy of heat treatment protocols. For example, long exposure to elevated non-lethal temperatures may condition the insect to be more tolerant to subsequent treatment at lethal temperatures (Laidlaw *et al.* 1996). Both static (Beckett and Evans 1997; Lester and Greenwood 1997; Waddell *et al.* 1997) and ramped (Waddell *et al.* 1997) pretreatments can result in such conditioning.

When insects are heated inside fruit, the temperature experienced is effectively that of ramped heating. The details depend on the medium (air or water) temperature, the heat-transfer coefficient at the fruit-medium interface, thermal conductivity of the fruit (Armstrong 1994) and the location of the pest in the fruit. It is therefore important to determine how thermotolerance is altered when insects experience different thermal profiles.

The aim of this study was to assess possible effects of exposure of fifth instar LBAM to temperatures in the non-lethal range (20–43°C), over various ramp durations, on expression of LBAM mortality after subsequent treatment at a static 43°C in both hot air and hot water.

MATERIALS AND METHODS

Insects

Fifth instar LBAM were obtained from a laboratory colony reared on an artificial diet (Singh 1983; Clare *et al.* 1987) at 20°C, a photoperiod of 16:8 (L:D) h, and 60% RH. Approximately 30 larvae were placed in each plastic container with a stainless steel gauze lid and base as described by Jones *et al.* (1995).

Hot water treatments

Treatments were conducted in a waterbath system (Purbeck Limited, Auckland, N.Z.) consisting of four 25 litre water baths. Grant (Cambridge, U.K.) heater units (temperature accuracy $\pm 0.01^\circ\text{C}$) on each bath were independently controlled by a laptop computer running Workbench PC for Windows data acquisition and control software (Strawberry Tree, Sunnyvale, California, U.S.A.). Temperatures were verified for each trial using an electronic thermometer (Model RT200, Measurements Standards Laboratory of New Zealand, Industrial Research Limited, Wellington, N.Z.).

At the beginning of a ramped trial, six or seven containers of insects were simultaneously immersed in a water bath at 20°C, which was then heated to 43°C in 30, 35, 45, 60, 90, 120 or 240 minutes, and maintained at 43°C for the remainder of the trial. For static temperature trials six or seven containers were simultaneously immersed in a water bath at 43°C which was held at this temperature for the duration of the treatment. One or two containers were immersed in 20°C water in a second waterbath at the beginning of the treatment as a non-heated control. Individual containers were removed over a range of time intervals to allow determination of the actual time required to achieve 99 % mortality (LT₉₉). These containers were hydrocooled in the 20°C waterbath for 2 minutes and allowed to drain. Non-heated controls were removed after a period equivalent to the longest hot water immersion in each ramp trial including two minutes hydrocooling. All containers were stored in air at 20°C overnight before assessment. Each ramp trial was replicated at least four times.

Hot air treatments

Air treatments were carried out in the High Air Flow Controlled Atmosphere and Temperature (HAFCAT) fruit treatment facility (Dentener *et al.* 1996). Air velocity was set at 9 m/s, initial air temperature to 20°C and relative humidity to 85%. Between eight and ten containers of insects were placed in a single drawer for each treatment. One or two containers were kept at ambient temperature to act as non-heated controls. Ramping trials, where temperature was increased from 20 to 43°C over 60, 120 or 240 minutes and subsequently maintained at 43°C, and static trials, where the temperature was maintained at 43°C throughout the treatment, were carried out. Individual containers were removed from the HAFCAT unit at intervals to allow determination of LT_{99s} for each treatment. All containers were held overnight at 20°C prior to mortality assessment. Six replicates of the static trial and four or five replicates of each ramped trial were carried out.

Mortality assessment

A binocular microscope (10 x magnification) was used while assessing mortality of insects. Each insect was prodded with a pin several times. Insects that moved were recorded as live whilst those which did not were recorded as dead.

Statistical analyses

Analyses of individual treatments used the model:

$$\log (-\log (1-p)) = a + bt,$$

where p = expected mortality and t = time in minutes (Preisler and Robertson 1989). This gave approximate linearity and determined the estimated time for 99% mortality (LT₉₉). The LT₉₉ was designed to give a mortality of:

$$c + (1-c) 0.99,$$

where c was the control mortality. LT_{99s} calculated are exclusive of the ramp period of the treatment.

The model was fitted using a robust version of the generalised linear model analysis available in S-PLUS (Chambers and Hastie 1991). This assumed that variance was proportional to that of a binomial distribution. The robust version reduced the weight given to points lying away from the main body of the data.

Error bars shown in Figures 1 and 2 represent 95% confidence limits. Estimates of LT₉₉ were compared using overlap of 95% confidence limits. Non overlap of 95% confidence limits is equivalent to a test for difference at approximately $P = 0.01$. Use of the 1% significance level in place of the 5% significance level provides a safeguard against finding too many significant differences. The safeguard is comparable to that provided by the formal use of a multiple comparison test.

RESULTS

Hot water treatments

No increase in tolerance with increasing ramp duration was observed in the hot water treatments (Figure 1). Static trials at 43°C resulted in an LT₉₉ of 21.3 (95% confidence limits; 16.5-27.5) minutes. Ramped trials did not produce significantly longer LT_{99s} than the static trials; 30.6, 30.8, 31.7, 29.3, 29.9 and 15 minutes for 30, 35, 45, 60, 90 and 120 minute ramps respectively. Considerable mortality occurred during

the 120 minute ramp (31.3 - 80%) before the target temperature was reached. This explains the lower LT_{99} for this ramp duration. LT_{99} s for 240 minute ramp trials could not be calculated as complete mortality was attained prior to completion of the ramp.

FIGURE 1: Mean LT_{99} of fifth instar light brown apple moth heated in water from 20-43°C over ramp durations of 0, 30, 35, 45, 60, 90 or 120 minutes. Vertical bars are 95% confidence limits on the mean of at least four replicates.

Hot air treatments

Static trials using the HAFCAT facility at 43°C produced an LT_{99} of 104 (95% confidence limits; 97.7 - 110.7) minutes (Figure 2). Ramping the temperature from 20 to 43°C conferred thermotolerance on the insects with ramped trials exhibiting longer LT_{99} s than the static 43°C trial. LT_{99} s for the 60, 120 and 240 minute ramps were not significantly different from each other suggesting that thermotolerance does not increase with ramp duration. On average the mean LT_{99} of ramped trials was 38.5% longer than the non-ramped LT_{99} .

FIGURE 2: Mean LT_{99} of fifth instar light brown apple moth heated in air from 20-43°C over ramp durations of 0, 60, 120, 180 or 240 minutes. Vertical bars are 95% confidence limits on the mean of at least four replicates.

DISCUSSION

Lester and Greenwood (1997) and Beckett and Evans (1997) reported thermotolerance of LBAM when static hot air pretreatments were followed by hot water immersion. Our research indicates that thermotolerance is also induced during ramped hot air treatments. Neven *et al.* (1996), using moist and vapour forced air, also observed thermotolerance with codling moth larvae which increased tolerance to temperature extremes if slower heating rates were used. Research in which insects were heated in air-filled vials in water (Neven 1998) also produced evidence of thermotolerance. These findings are in contrast to the results we observed when both the ramp and static components of the treatment occurred in hot water, in which case no evidence of thermotolerance was found. This suggests that the medium in which heating takes place may be important. Oxygen availability to insects may be restricted when heating takes place in water, perhaps explaining the lack of thermotolerance under these conditions. Yocum and Denlinger (1994) reported that thermotolerance induced by previous exposure to high temperatures can be reduced or eliminated by anoxia and suggested that physiological processes generating tolerance can only proceed under aerobic conditions. Insects heated to 40°C for varying ramp times under controlled atmosphere conditions (1% O₂, 1% CO₂) did not exhibit thermotolerance (Whiting and Hoy 1998), supporting this conjecture.

Klein and Lurie (1992) suggested that slower heating rates during hot air treatments maintain apple quality compared with fast rates. However, insect tolerance of hot air treatment appears to be enhanced by these slower rates. It is likely that the extent of thermotolerance will vary between insect species and that ramping will affect various commodities differently. Ramping rate and medium are important factors which should be considered, together with time and temperature, when defining disinfection treatments.

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