

## HIGH TEMPERATURE DISINFESTATION OF NECTARINES: LIGHTBROWN APPLE MOTH LARVAE

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### SUMMARY

A heat treatment, similar to that used to disinfest Hawaiian papaya of fruit flies, has potential for improving summerfruit exports by controlling New Zealand insect pests of quarantine importance. The mortality responses of lightbrown apple moth (*Epiphyas postvittana* (Walker)) larvae on 'Redgold' nectarines to a heat treatment were determined. Complementary log-log analyses of time-mortality data showed there were no differences in susceptibility between third and fifth instar larvae. The estimated time for 99% mortality of third and fifth instar larvae of 19.9 and 20.7 h at 40°C respectively was significantly reduced to predicted times of 12.7 and 13.3 h respectively at 42°C.

**Keywords:** quarantine, leafrollers, disinfestation, nectarines, high temperature

### INTRODUCTION

Nectarines, peaches and apricots are becoming increasingly important export crops for New Zealand, with export receipts of almost \$10M (Halsted 1990). More than 80% of these fruits are exported to Australia (Kasanji 1991) during the summer months where, if live pests are intercepted on arrival, the consignments are fumigated with methyl bromide. Fruit quality is reduced mainly by fumigation at higher temperatures than those recommended (G.F. McLaren, DSIR Plant Protection, pers. comm. 1989), and by the resultant delays of up to several weeks (K. Murray, AQIS<sup>1</sup>, pers. comm. 1989). As future exports depend on high fruit quality, increased volumes of New Zealand summerfruit to Australia are unlikely unless the high-temperature fumigation treatment and resultant delays can be avoided.

The main pests intercepted in Australia on summerfruit are New Zealand flower thrips, *Thrips obscuratus* (Crawford), mites and leafrollers. Although the pests intercepted are usually not identified to the species level by the AQIS, the mites are probably the two-spotted mite, and the leafrollers are probably the Australian lightbrown apple moth (LBAM) (*Epiphyas postvittana* (Walker)) and two other species endemic to New Zealand, *Planotortrix octo* (Dugdale) and *Ctenopseustis obliquana* (Walker).

A high temperature treatment is approved for disinfestation of Hawaiian papaya exports (potentially infested with fruit flies) to the mainland United States (Armstrong *et al* 1989; Anon. 1989). While heat treatments are known to carry some risk of damage to temperate fruit (Couey 1989), preliminary research in Central Otago in 1989 showed peach and nectarine quality was not adversely affected by 17 hours exposure at 38-40°C (McLaren 1989).

Previous research on 'Fantasia' nectarines predicted that all stages of *T. obscuratus* would be killed by exposure to a 12 h heat treatment. During treatment the infested fruit was warmed from ambient to 37.8-40.3°C over 2.5 to 3.5 hours at 95% RH, and kept at the final temperature for the remainder of the treatment (Waddell *et al* 1990).

LBAM was selected for this research as it is more heat resistant than the other leafroller species. The upper threshold for larval and pupal development of LBAM is

<sup>1</sup>Australian Quarantine and Inspection Service

31-32 °C and no eggs hatch above 31.3 °C (Danthanarayana 1975) whereas *C. obliquana* do not lay eggs at temperatures above 20.0 °C (Clare and Singh 1990).

The aim of this study was to compare the time-mortality responses of LBAM third and fifth instar larvae to heated air.

#### METHODS

'Redgold' nectarines were obtained from the DSIR Research Orchard, Clyde. The fruit received a full fungicide programme, but were kept free of insecticide sprays for at least 8 weeks prior to harvest to ensure no pesticide toxicity to the experimental insects.

The high-temperature, forced-air facility (external dimensions of 2.4 m x 1.2 m x 1.2 m) is constructed of 22-gauge galvanised iron insulated externally with Armaflex™ cladding. Fruit were contained in 20 plastic-coated, wire baskets (465 mm x 430 mm x 85 mm), 10 each side of the fan. Air, heated by four 2 kW heater elements and evenly dispersed by a plenum, was forced through each basket at approximately 0.7 m/s. The air was humidified using Autofil™ humidifiers, one located adjacent to each stack of 10 baskets, and the humidity regulated by a Hygrostat® humidity controller. The facility, loaded to capacity for each experiment, contained on average 110 kg of nectarines, or a mixture of nectarines and peaches when nectarine supplies were insufficient.

Third and fifth instar LBAM larvae were mass-reared on artificial diet (Singh 1983) in grid boxes (Clare *et al* 1987). Larvae were transferred to nectarines contained inside a fine nylon mesh bag, and placed on a basket in a single layer. Each basket contained approximately 36 nectarines, and at least 150 insects. The net bag was securely tied and randomly allocated to a treatment, including the untreated control. The netting was impervious to larvae and aided later recovery of the insects.

The insect-infested fruits were kept at either 0.5 °C or 20 °C overnight prior to treatment. All the infested fruit were loaded into the facility, and a proportion removed after different periods of exposure to achieve a range of insect mortalities. Removed fruits were replaced in the facility with fruit previously stored in an incubator at approximately 40 °C to minimise temperature fluctuations. Four doors allowed individual baskets of fruit to be removed with minimal disruption to the airflow or temperature.

Three experiments were conducted to obtain 11 time-mortality responses for the third and fifth instar larvae recorded at intervals between 14 to 26 h, inclusive of a warm-up period for the fruit to 40.0 ± °C of approximately 2 h from 20.0 °C, or 4 h from 0.5 °C.

Temperature and humidity were recorded at 5 minute intervals using two data loggers, and the exact temperature determined using calibration lines. Five temperature probes were inserted into the fruit at different locations within the facility, and further temperature and humidity probes were located in the air stream. Fruit temperatures for each time increment were determined (mean ± SEM), and the corresponding coefficients of variation were calculated.

The fruit were weighed before and after exposure to heat to determine water loss from the fruit.

After exposure, the insect-infested fruit were held at 20.0 °C until assessment. The mortality of half the larvae at each exposure period was assessed 4 days after treatment, and the remainder 7 days after treatment. Each nectarine was examined externally, and then opened to retrieve any larvae that entered the open calyx of the fruit. The control insects were not exposed to the heated air, but were otherwise stored and assessed under the same conditions as the treated insects. Larvae were recorded as live or dead by the presence or absence of movement.

The computer software package 'S' (Becker *et al* 1988) was used to generate mortality-response curves based on the number of heat units received by each infested-fruit sample, and to estimate the time for 50% (LT<sub>50</sub>) and 99% (LT<sub>99</sub>) mortalities. Fruit-pulp temperatures were recorded for some baskets containing infested fruit, and the temperatures in the remaining baskets were estimated using the measured temperatures, and the relative positions of the baskets in the facility.

The contribution of any particular temperature 'T' to the heat units was assumed proportional to  $e^{hT}$ , and therefore the heat units received by each insect sample was:

$$H = \sum e^{hT_i} \Delta t_i$$

where the ' $\Delta t_i$ ' are time intervals over which the temperature is approximately constant (see Jang 1986). For simplicity, we used an approximation for  $1/(273 + T)$  in terms of 'T' which is valid over the small range of temperatures used. The parameter 'h' was estimated by a grid search. A confidence interval on the likelihood was used to infer a confidence interval for 'h'. The optimum value of 'h' was assumed to be the same for both instars and was estimated to be 0.22 with a 95% confidence interval for 'h':  $0.02 < h < 0.04$ . If  $h = 0.02$  the line produced is almost flat, and if  $h = 0.4$  a much steeper gradient is produced. A complementary log-log model (Maindonald 1988; Preisler and Robertson 1989) was used with 'log (H)' as the explanatory variable. If the temperature was fixed, then:

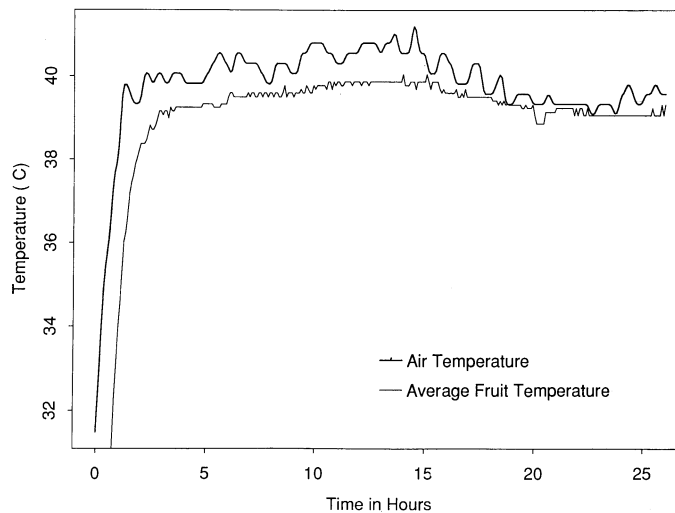
$$\text{Log}(e^{hT} t_{99}) = H_{99}$$

Alternatively,  $t_{99} = e^{H_{99} - hT}$  gives an estimate of the time required to give 99% mortality at a set temperature, 'T'. Confidence limits were calculated at the  $LT_{50}$  and  $LT_{99}$  levels using MLP (Ross 1980).

### RESULTS AND DISCUSSION

The air temperature stabilised rapidly at approximately 40.0 °C in each experiment, and the rate of fruit heating was slower than that for air (Figure 1). The mean relative humidity in all experiments was approximately 93%. The mean fruit weight loss for the three trials was less than 2.5%. Previous research showed 'Redgold' nectarines could tolerate up to 5% weight loss without showing signs of shrivelling (McLaren *et al* 1990).

**Fig. 1:** Air and mean fruit-pulp temperatures (initial fruit pulp temperature of 20.0 °C).



As there were no statistical differences in the larval mortalities between pre-treatment storage at 0.5 or 20 °C, or between insect mortalities assessed 4 and 7 days after the treatments, the results were pooled.

The extent of the overlap of confidence limits indicates there were no significant differences at 40 °C between the  $LT_{50}$ 's for LBAM third and fifth instar larvae, and between the  $LT_{99}$ 's for the same two larval stages (Table 1).

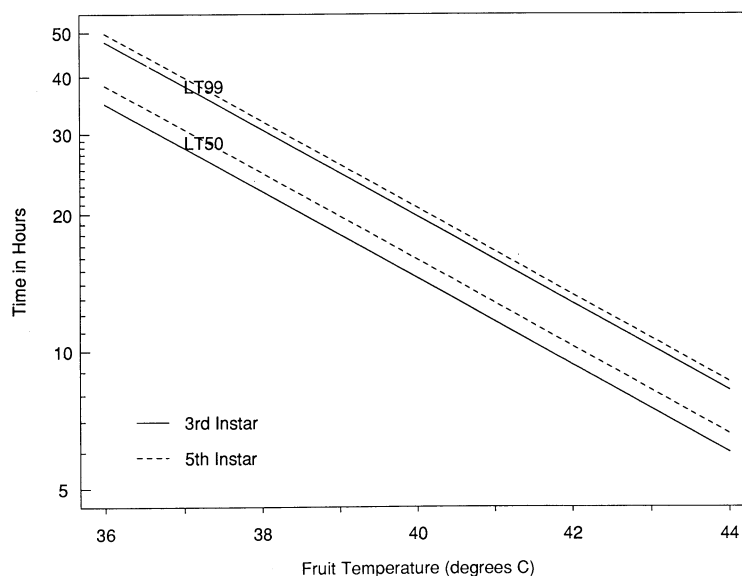
**TABLE 1: The estimated time (95% fiducial limits) in hours for 50% ( $LT_{50}$ ) and 99% ( $LT_{99}$ ) lightbrown apple moth larval mortality after exposure to heated air at 40.0°C, and predicted  $LT_{50}$  and  $LT_{99}$ , at 42.0°C.**

Temperature	Instar	$LT_{50}$	$LT_{99}$
40	Third	14.47 (13.63-21.80)	19.89 (18.54-21.76)
	Fifth	15.90 (15.44-16.33)	20.70 (19.69-22.20)
42	Third	9.32 ( 8.78-14.04)	12.74 (11.94-14.00)
	Fifth	10.24 ( 9.94-10.52)	13.33 (12.68-14.30)

Confidence limits generally underestimate overall statistical variability and are too narrow. They may be used for comparing the time-mortality responses between two instars at a given temperature (Table 1), but not for comparing the effects of different temperatures on each of the instars.

Comparisons between third and fifth instar responses to temperature are illustrated by plotting the time-mortality responses for 50% and 99% mortality at different temperatures (Figure 2). The figure can be used to estimate the time for mortality at temperatures between 38-42°C. The estimates are most reliable at around 40.0°C, the temperature at which the experiments were conducted.

**Fig. 2: The estimated time-mortality responses of third and fifth instar lightbrown apple moth larvae to heated air, based on their  $LT_{50}$  and  $LT_{99}$  responses at 40.0°C.**



The estimated time for 99% mortality of third and fifth instar LBAM larvae was 19.9 and 20.7 h at 40°C. These results are not significantly different. The  $LT_{99}$  for all stages of thrips was between 3.70 and 6.08 h at this temperature (Waddell *et al* 1990). The estimated  $LT_{99}$ 's for third and fifth instar larvae were significantly reduced to predicted times of 12.7 and 13.3 h at 42°C which should also kill the more heat-sensitive thrips. Preliminary experiments on 'Fantasia' nectarines showed they were not damaged by 24 h exposure to 42°C (pers. comm. M. Lay-Yee, DSIR Fruit and Trees).

Heated air shows promise as a disinfestation treatment for controlling pest species of quarantine importance on summerfruit, providing the treatment is not detrimental to fruit quality. Further experiments on the quality of summerfruit after the heat treatment, and the time-mortality responses of mites, are in progress.

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