MORTALITY RESPONSE OF LIGHTBROWN APPLE MOTH TO A CONTROLLED ATMOSPHERE COLD STORAGE TREATMENT FOR APRICOTS

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ABSTRACT

The mortality response of lightbrown apple moth, *Epiphyas postvittana* (Walker), to a controlled atmosphere (CA) cold storage treatment for apricots was investigated. The mean estimated times for 99% mortality (LT₉₉) of 1st and 5th instar larvae inoculated onto apricots and exposed to 1.5% O₂, 3% CO₂ (CA) at 0°C, were 19.9 ± 0.13 and 22.4 ± 0.24 d, respectively. The treatment time for 50% mortality (LT₅₀) was similar for the two life stages. However, 5th instar larvae required longer treatment times than 1st instar larvae to achieve 99 and 99.9968% mortality (LT_{99.9968}). A 28 d treatment resulted in complete mortality of both life stages tested.

Keywords: lightbrown apple moth, controlled atmosphere, cold storage, apricot, disinfestation

INTRODUCTION

Apricot (*Prunus armeniaca* L.) is New Zealand's most valuable summerfruit export crop, traded mainly to the United States and Australia (Taylor 1996). Lightbrown apple moth, *Epiphyas postvittana* (Walker), is considered a quarantine pest on New Zealand apricots exported to the United States. Currently, consignments on which this pest is detected are denied entry to this market. Methyl bromide fumigation treatments against some insect pests infesting apricots have been identified overseas (Armstrong and Couey 1984; Johnson *et al.* 1947). However, with the worldwide move away from chemical usage for horticultural pest management, recent studies have focused on the potential of heat-based treatments to disinfest apricots after harvest. Unfortunately, neither hot air nor hot water treatments can control lightbrown apple moth (Birtles *et al.* 1991; Jones and Waddell 1994) without damaging New Zealand apricots (McLaren *et al.* 1992; 1995).

Controlled atmosphere (CA) cold storage (land-based and in-transit) is being considered by the New Zealand summerfruit industry to extend apricot storage life after harvest. Such treatments successfully prolong the storage life of New Zealand nectarines (Burmeister and Harman 1997), and overseas research suggests application of the same technology could be beneficial to apricots (Claypool and Pangborn 1972). These treatments can also be lethal to insects (Hallman 1994).

We present investigations on the time-mortality responses of 1st and 5th instar lightbrown apple moth on apricots, to a novel apricot storage atmosphere (1.5% O₂, 3% CO₂) at 0°C.

METHODS

Lightbrown apple moths were obtained from a laboratory culture maintained at HortResearch Auckland at 20 ± 1°C, 60% RH, 16:8 (L:D) h. Egg batches on wax paper and strips (30 x 100 x 2 mm, w x l x h) of artificial diet were placed in sealed plastic containers (volume 2 L) and kept at 20 ± 1°C, 60% RH, 16:8 (L:D) h. Emerging 1st instars were supplied 2-3 d after egg hatch. Fifth instars were mass-reared under the same conditions for 18 days in 'grid boxes' containing artificial diet (Clare *et al.* 1987).

Insects were inoculated onto insecticide-free count 40 'Sundrop' apricots. A preliminary trial highlighted that 1st instars need to burrow into the fruit to feed and survive. For this reason, establishment of this life stage was encouraged by pricking each apricot 11 times with a pin to provide entry holes. Fifteen fruit (one sample time of one replicate for one life stage) were placed on a plex tray inside a fine fabric mesh bag. Insects of one larval stage (160 1st or 100 5th instars) were inoculated directly onto the apricots using a fine, camel hair brush. Each bag was sealed, and placed inside a 20 litre plastic treatment bucket. One bag of each life stage was exposed to treatment in the same bucket with the bags separated by a plastic netting divider which prevented insect and fruit compression. Inoculated apricots were kept at $20 \pm 1^\circ\text{C}$, 70% RH, 16:8 (L:D) h for 3 d to facilitate insect establishment in preferred locations before treatment.

Experiments were carried out in 21 identical treatment buckets (with lids modified with a gas inlet and outlet and a rubber septum to enable gas sampling) located inside a cold store with an internal volume of 28.8m^3 ($4.8 \times 2.5 \times 2.4\text{m}$, L x W x H) and refrigeration unit rated at 2.4 W. The atmosphere in the buckets, generated from bottles of oxygen-free nitrogen, food grade CO_2 and a low pressure compressed air supply, was mixed in a manifold then split to all treatment buckets. The flow rate through each bucket (100 ml/min) ensured one complete atmosphere change every 3.3 hours. Bucket outlets were manifolded together into a single outlet to ensure even back pressure.

All CA treatment buckets were attached simultaneously to gas inlet lines inside the cold store (exposure time = 0 h), and atmosphere establishment and fruit cooling commenced immediately. Cold store air temperature, measured every hour with a thermistor probe located outside the treatment buckets at fruit level and connected to a Squirrel data logger (Grant 1206 model), was -0.05 ± 0.02 during the trial. Fruit surface and stone temperature were measured in the three buckets allotted to the longest treatment duration (32 d). From the fruit starting temperature of $20 \pm 1^\circ\text{C}$, apricot surface temperature (inside fabric bags inside buckets), measured with a thermistor probe held onto the surface of each of two fruit in each bucket (one positioned in the bottom and the other in the top of the bucket and attached with teflon tape (Ceelon)), reached target temperature after 43.0 ± 3.9 h. After equilibrium, mean fruit surface temperature within the buckets was $0.33 \pm 0.002^\circ\text{C}$. The fruit stone, measured with a thermistor probe inserted into the apricot centre of each of two fruits similarly in the 3 buckets, took on average 44.2 ± 3.1 h to attain target temperature and after this period averaged $0.29 \pm 0.001^\circ\text{C}$. The atmosphere composition in all buckets was sampled and analyzed throughout each experiment using a paramagnetic cell (Servomex 1111D transducer) and an infrared gas analyser (Servomex 1505 transducer). Concentration of O_2 and CO_2 was calculated by comparison with a standard reference gas containing 1.67% O_2 and 5% CO_2 (vol:vol). Target O_2 and CO_2 concentrations for CA treatment (actual mean composition $1.65 \pm 0.3\%$ O_2 , $3.25 \pm 0.4\%$ CO_2) were established approximately 1.1 and 0.4 d into the treatment, respectively. All experiments were performed in darkness.

Samples were removed after 4, 7, 12, 17, 22, 28 and 32 d exposure (times designed to cover 20-100% mortality), then stored at $20 \pm 1^\circ\text{C}$, 70% RH, 16:8 (L:D) h until mortality assessment 7 d later. Control fruit were exposed to air at $20 \pm 1^\circ\text{C}$, 70% RH, 16:8 (L:D) h for 7 d. Apricots were dissected and individual larvae were examined under a binocular microscope (30x magnification). Insects were probed with a pin and recorded as live (movement) or dead (no movement). The location of each larva on the apricot (external: cheek, suture, stem; internal: tunnel, stone; mesh bag) was also noted. Three replicates of each larval stage were executed.

Time-mortality data for each replicate were fitted using the complementary log-log (clog-log) model (Preisler and Robertson 1989) with time as the explanatory variable to derive estimated days for 50, 99 and 99.9968 (probit 9) % mortality (LT_{50} , LT_{99} and $\text{LT}_{99.9968}$, respectively). These estimates were calculated as the time to achieve a mortality of $c + (1 - c) \times m$, where c was the control mortality and m the estimated proportion mortality. For 5th instars, it was necessary to use a log transformation on the time axis to achieve a linear response. For each life stage a geometric mean LT and its associated standard error (SEM) were estimated, from which a 95% confidence interval (CI) was calculated. Non-overlap of the 95% CIs is equivalent to a test for difference *ca.* $P = 0.01$.

RESULTS AND DISCUSSION

First and 5th instar mortality increased steadily with increasing in treatment duration (Fig. 1). A 28 d CA cold storage treatment resulted in complete mortality of both life stages tested. Mean LT₅₀s for 1st and 5th instars were similar after treatment (10.8±0.35 and 12.3 ± 0.02 d, respectively; Table 1). However, 5th instar larvae required longer treatment times than 1st instar larvae to achieve higher levels of mortality (LT₉₉ and LT_{99,9968}). To attain a probit 9 level of security, 1st and 5th instars required treatment exposures of 23.9 ± 0.40 and 29.0 ± 0.42 d, respectively.

TABLE 1: Mean LT₅₀, LT₉₉ and LT_{99,9968} (days) derived from time-mortality data of lightbrown apple moth exposed to 1.5% O₂, 3% CO₂ at 0°C on apricots.

Instar	n _{total}	Mean LT ₅₀ (95% CI)	Mean LT ₉₉ (95% CI)	Mean LT _{99,9968} (95% CI)
1 st	2,262	10.8 (9.4-12.4)	19.9 (19.4-20.5)	23.9 (22.5-25.4)
5 th	2,548	12.3 (12.2-12.4)	22.4 (21.4-23.5)	29.0 (27.2-30.9)

Previous work on apples (Waddellet al. 1990), determined the mortality responses of 1st and 5th instar lightbrown apple moth under commercial CA coolstorage with twice

FIGURE 1: Mean percentage mortality of lightbrown apple moth larvae exposed for various times to 1.5% O₂, 3% CO₂ at 0°C on apricots. Data are shown for 1st instars [equation for fitted response where *m* is the mortality at time *t*: $\log(-\log(1-m)) = -2.5370(\pm 0.1801) + 0.2045(\pm 0.0103)t$], and 5th instars [$\log(-\log(1-m)) = -8.2841(\pm 0.1150) + 3.1552(0.0480)\log(t)$].

the O₂ concentration (3.2% O₂, 2.9% CO₂, 0.6°C) tested here. Although based on only two relevant treatment durations, mean LT_{99s} for 1st and 5th instars calculated from this earlier work [17.8 (17.4-18.2) and 18.1 (16.0-20.5) d, respectively] were less than ours on apricots. However, both studies found no larvae of this species survived a 32 d treatment exposure.

Although the position of larvae on the fruit did not influence the mortality responses to the CA cold storage treatment in our study, different instars displayed different locational preferences. Most 1st instars were found inside tunnels (51.5%). The fruit cheek was the next most popular location (21.1%) followed by the mesh bag (11.4%), fruit stem (7.8%), suture (5.9%) and stone (2.3%). In contrast, almost all the 5th instars were either off the fruit in the bag (72.4%) or positioned externally on the apricot cheek (24.2%). The stem, suture and stone were preferred sites for only 2.2, 0.7 and 0.4% of 5th instars respectively, while none were found in tunnels. The readiness of 1st instars to tunnel into apricots was previously noted by Jones and Waddell (1994) in a study where 1st instar establishment was unaided. This finding contradicts distributional observations on apples (Whiting *et al.* 1997). The tendency of 1st instars to burrow into apricots not only negatively impacts the viability of short heat-based treatments to disinfest this fruit (Jones and Waddell 1994), but also complicates translation of lightbrown apple moth mortality response data derived on apricots to other host fruit types.

Further research should determine the mortality responses of other quarantine pests intercepted on export apricots to this CA cold storage treatment, and assess the tolerance of New Zealand varieties to treatment exposures shown to effectively control these pests.

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