TIME-MORTALITY RESPONSE OF LEAFROLLERS EXPOSED TO COMMERCIAL CONTROLLED ATMOSPHERE COOLSTORAGE

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SUMMARY

Commercial controlled atmosphere (CA) coolstorage was completely effective in disinfesting apples of lightbrown apple moth (*Epiphyas postvittana* Walker), brownheaded leafroller (*Ctenopseustis obliquana* Walker), and greenheaded leafroller (*Planotortrix excessana* Type A¹), pests which are subject to quarantine restrictions by the United States Department of Agriculture. CA coolstored apples have the potential to attract premium prices as a result of the better storage conditions compared with standard coolstorage. Sixty days continuous exposure to an atmosphere of 3% O₂ and 3% CO₂ at 0.5 °C resulted in 100% mortality of the mid-instars of all three species. Commercial CA coolstorage could substitute for inspection by USDA personnel in New Zealand on the final apple exports to the United States.

INTRODUCTION

Controlled atmosphere (CA) treatments have been reported to disinfest stored products (Bailey and Banks 1980), and also some fresh fruits (Klag 1985) of insect pests. Since complete kill under CA conditions at low temperatures requires longer treatment times than at higher temperatures, only produce that tolerate a long coolstorage period is suitable for CA disinfestation by this means. Even with a commodity that stores well, the periods required to kill insects can be too long to be commercially practical (Angerilli *et al* 1986).

More recently, the insecticidal effects of CA have been enhanced with more extreme environments thereby reducing the treatment period. Very low O₂, high CO₂ and high temperatures, either individually or in combination, have been investigated as ways of shortening the treatment duration with a range of successes for commodities that cannot be stored for extended periods (Nicolas and Sillans 1989; Soderstrom and Brandl 1985).

The use of CA for insect disinfestation of food is an environmentally acceptable quarantine treatment. Because of the time period required to kill insects under CA storage, the treatment could substitute for USDA inspection services only for the final shipments of New Zealand apples exported to the United States. Granny Smith apples are an economically important export crop that undergoes a preharvest pesticide programme to control leafrollers. However, when leafrollers are detected on harvested fruit by USDA inspectors in New Zealand, the consignment is rejected from the United States market. Although conventional CA coolstorage has been applied routinely to Granny Smith apples in New Zealand to extend their storage life, little information is available on the effect of these conditions (2-3% O₂, 2-3% CO₂, 0.5 °C) on insect pests of quarantine importance. Preliminary experiments have shown 90 days exposure to these conditions killed mid-instar larvae of lightbrown apple moth (LBAM), brownheaded leafroller (BLR) and greenheaded leafroller (GLR) on Red Delicious apples (Batchelor *et al* 1985). The eggs of leafrollers are not a quarantine issue as they are not found on the fruit at harvest (Thomas, pers. comm.). Tests were therefore confined to the larval life stages.

¹Foster and Dugdale 1988

Proc. 43rd N.Z. Weed and Pest Control Conf. 1990: 328-333

The aim of this study was to determine the most resistant species and life stage of these three economically important leafroller species stored under commercial CA coolstorage.

METHODS

Leafrollers were laboratory-reared on artificial diet (Singh 1983). Neonate first instar larvae were transferred directly onto the fruit from eggs laid on plastic sheets. Third and fifth instar larvae were mass-reared on artificial diet at $20.0 \pm 1.0^\circ\text{C}$ (Clare *et al* 1987) and released onto the fruit.

Granny Smith trees were kept free of insecticidal sprays for a period of 12 months prior to harvest. The fruits were placed in fine net bags inside cardboard boxes. Each box contained about 30 apples and 150 insects. The first instar larvae were confined to the calyx end of the fruit by sealing a layer of Parafilm® onto the fruit surface with Bluetack®. This aided insect establishment and improved recovery at the time of assessment. Following inoculation onto the fruit the leafrollers were left to establish for 72 h at $20.0 \pm 1.0^\circ\text{C}$ and then pre-conditioned for a further 16 h overnight at $12.0 \pm 0.5^\circ\text{C}$ before commencing CA coolstorage. Natural mortality of the insects plus mortality due to handling was determined by assessing a sample of infested apples on the day the CA treatment conditions were initiated (Day 0).

The New Zealand Apple and Pear Marketing Board commercial CA coolstorage facilities located at Henderson, Auckland were used for the duration of the trial. The temperatures and atmospheres of the stores were recorded daily. The target CA conditions for Granny Smith apples were 3% O₂ and 3% CO₂ (with the remainder N₂), and the target temperature was 0.5°C . The atmospheric gases in each CA coolstore were sampled from one location within the store and the concentration determined using O₂ (Sybron-Taylor Servomex, Crawley, Sussex) and CO₂ (Analytical Development Corporation, Hertfordshire) analysers. The air temperature of the CA stores was monitored continuously. Insect samples were removed from the CA coolstores after 7, 32, 60, and 88-90 days CA storage and the infested fruit kept at $20.0 \pm 1.0^\circ\text{C}$ for 1 day. Each apple was examined externally and internally for the presence of larvae which were categorised as live, dead or moribund. Moribund insects were retained on artificial diet for a further week and then categorised as live or dead.

Four CA coolstores were used to replicate the experiment for the third and fifth instars of LBAM and GLR. However, only three CA coolstores were available at the time of the experiment for the third and fifth instar BLR. The first instars for all species involved one replicate, and, where insect numbers were sufficient for a partial second replicate, they were allotted to the shorter treatment periods in a second CA coolstore.

Mortality-response curves were fitted using GENSTAT (GENSTAT 5 1987). A complementary log-log model was fitted to the data,

$$\log(-\log(1-p)) = a + b.t$$

where 'p' is the predicted mortality, 't' is the storage time (in days), 'a' is a measure of the Day 0 mortality and 'b' is the slope of the line. The constants 'a' and 'b' were estimated for each set of data. A line was produced when y was plotted against 't' and $y = (\log(-\log(1-p)))$. The complementary log-log model accurately described the data and allowed a simpler or more coherent analysis than was possible with logits and probits (Maindonald 1988).

RESULTS AND DISCUSSION

The average weekly O₂ and CO₂ levels were both very close to the target atmospheres of 3%. The mean O₂ level for five CA coolstores used was 3.2% (± 0.42 s.d.) and the CO₂ level was 2.9% (± 0.45 s.d.). The temperatures were also close to the target of 0.5°C with a mean of 0.6°C (± 0.15 s.d.).

Life stage comparison

The lines fitted for each mortality response were determined from only 2 or 3 points of less than 100% kill. The model fitted to the data allowed a reasonably consistent interpretation of the results between species and instars. However, this limitation necessitates a degree of caution when interpreting the results.

The slope 'b' as a measure of the rapidity of the approach to high mortality indicated that the lines for the different instars of the different species were close to parallel (0.141 for LBAM, 0.152 for BLR, 0.190 for GLR) except for fifth instar LBAM (0.319) and the first instar GLR (0.333). These exceptions may be due to variation in the Day 0 mortality. The order of the plotted lines showed the relative susceptibility of the different instars (Figures 1 to 3). When the estimated times for 50% mortality (ET_{50}) were compared fifth instar leafrollers were more resistant to CA coolstorage than third instars, which were in turn more resistant than first instars, irrespective of species ($P < 0.05$) (Tables 1 and 2).

Species comparison

GLR was more resistant than BLR which in turn was more resistant than LBAM to the CA coolstorage treatment ($P < 0.05$) when the ET_{50} 's of the fifth instars were compared. At the ET_{99} level LBAM was again the least resistant. For first and third instars, BLR was consistently the most resistant to the treatment, followed by LBAM which was in most cases significantly more resistant than GLR ($P < 0.05$).

TABLE 1: The percentage mortality (based on total larvae recovered) of lightbrown apple moth (LBAM), brownheaded leafroller (BLR) and greenheaded leafroller (GLR) exposed to commercial CA coolstorage when artificially placed on Granny Smith apples. Replicates combined.

Instar	Days in storage	Leafroller species					
		LBAM		BLR		GLR	
		Total larvae	% mortality	Total larvae	% mortality	Total larvae	% mortality
First	0	147	29.9	206	22.8	177	16.4
	7	85	70.6	170	48.8	76	84.2
	32	79	100	147	100	87	100
	60	91	100	76	100	91	100
	90	88	100	61	100	83	100
Third	0	327	25.7	386	9.8	434	9.2
	7	331	46.8	285	33.7	261	39.9
	32	232	100	274	100	269	100
	60	245	100	327	100	313	100
	88-90	381	100	308	100	671	100
Fifth	0	689	1.5	379	5.0	561	2.0
	7	518	12.7	364	12.9	393	7.4
	32	468	100	360	99.7	426	99.8
	60	536	100	309	100	428	100
	88-90	509	100	363	100	429	100

TABLE 2: The estimated time (ET) in days for larvae of the lightbrown apple moth (LBAM), brownheaded leafroller (BLR), and the greenheaded leafroller (GLR) to achieve 50% (ET_{50}) and 99% (ET_{99}) mortality when exposed to commercial CA coolstorage (95% confidence intervals).

Species	Instar	ET_{50}	ET_{99}
LBAM	First	6.7 (6.5 – 7.0)	17.8 (17.4 – 18.2)
	Third	9.6 (9.4 – 9.9)	21.0 (20.5 – 21.5)
	Fifth	12.3 (10.7 – 14.0)	18.1 (16.0 – 20.5)
BLR	First	9.0 (8.9 – 9.2)	19.8 (19.6 – 20.0)
	Third	12.0 (11.8 – 12.1)	23.7 (23.5 – 23.9)
	Fifth	18.0 (17.8 – 18.3)	30.1 (29.8 – 30.4)
GLR	First	4.8 (4.6 – 4.9)	9.9 (9.1 – 10.0)
	Third	9.9 (9.4 – 10.4)	19.3 (18.4 – 20.2)
	Fifth	19.6 (18.7 – 20.6)	29.5 (28.1 – 30.9)

Fig. 1: Time-mortality response of first, third and fifth instars of lightbrown apple moth in CA coolstorage on Granny Smith apples.

Fig. 2: Time-mortality response of first, third and fifth instars of brownheaded leafroller in CA coolstorage on Granny Smith apples.

Fig. 3: Time-mortality response of first, third and fifth instars of greenheaded leafroller in CA coolstorage on Granny Smith apples.

After 32 days in CA coolstorage, complete mortality was achieved of all three larval instars of LBAM, and of the first and third instars of BLR and GLR. A storage period of 60 days CA coolstorage gave complete kill of the fifth instar larvae of BLR and GLR (Table 1).

This research indicated that the fifth instar GLR was the most resistant life stage, and that 60 days commercial CA storage would be sufficient to kill this stage on Granny Smith apples. Further large-scale experiments, using more than 28,000 insects, confirmed LBAM, BLR and GLR mid-instar larvae were killed after exposure to 3% O₂ and 3% CO₂ at 0.5°C for 60 days (Dentener *et al* in press).

It is very likely that the kill of leafrollers is independent of apple cultivar as they are surface feeding insects, and therefore directly exposed to the CA conditions. Previous research has shown that the substrate type affects the time-mortality response of codling moth eggs in coolstorage (Yokoyama and Miller in press), but the influence of different fruit cultivars did not significantly affect egg mortality (Moffitt and Burditt 1990; Waddell *et al* unpublished). It is therefore likely that a period of 60 days exposure to CA coolstorage (of 3% O₂, 3% CO₂ at 0.5°C) will also be sufficient to disinfest Red Delicious apples of leafrollers rather than the period of 90 days reported by Batchelor *et al* (1985).

ACKNOWLEDGEMENTS

We wish to thank Dr P. Singh, Mr D.J. Rogers and the staff of the Rearing Group, DSIR Plant Protection, for providing the leafrollers; The New Zealand Apple and Pear Marketing Board for funding the research and providing access to the commercial CA coolstores and Mr J.H. Maindonald, DSIR Applied Maths, for statistical analyses.

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