ETHYL FORMATE + CO_2 FUMIGATION OF TABLE GRAPES FOR LIGHT BROWN APPLE MOTH

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Light Brown Apple Moth (LBAM) *Epiphyas postvittana* (Walker) (Lepidoptera: Tortricidae) were obtained from laboratory cultures reared at 22 ± 1°C; 60% RH; 16:8 (L:D) cycle. Eggs were laid in batches on the inner surface of white ridged opaque plastic cups. The cups were cut into strips which were placed with artificial media in sealed plastic containers (lunch boxes). All stages were reared in this way. Rearing and handling methods were developed to ensure good survival (near 100%) of treated and control insects.

Laboratory fumigation: Tests were done in individually calibrated glass desiccators (6.6 – 7.1 L) each containing a magnetic stirrer rod in the base; the lid fitted with a self sealing septum. A range of 9 doses were used for exposure periods ranging from 1 to 6 hours. A minimum of 3 replicate trials to obtain a concentration x time product spanning a mortality range between 15 and >95% was done to obtain a good estimate of LD_{50} and LD_{99} by probit regression methods (Finney 1971) with at least two further doses to confirm 100% mortality. Each desiccator was placed on a magnetic stirrer which was run continuously for the duration of the trial. Vials with test insects were placed in the desiccators in advance of fumigation. CO_2 approximately 15% was introduced into each desiccator using a weighed amount of laboratory produced dry ice, before the desiccator was sealed. Analytical grade ethyl formate (>99.7% purity) was injected as liquid through the septum of the desiccator using a gas tight syringe into a petri dish placed above test insects and allowed to volatilize. Ethyl formate and CO_2 samples were analysed on a Varian 3400 Gas Chromatograph (GC) using Flame Ionisation (FID) and Thermal Conductivity (TCD) detectors respectively with a Poropak Q packed column (operating temperatures: oven 70°C; injector 140°C; detector 250°C). The first sample was taken 10 minutes after application to verify the applied dose and thereafter at 30 minute intervals throughout each trial. Fumigated insects were reared for the required period according to species and stage to assess mortality and corrected (Abbott 1925) based on untreated controls in every test. Trials of insect species and life stages were conducted over a number of years from 2001 to 2009.

Fumigation in refrigerated shipping containers: Trials were done in 40 ft / 12 m (68m^3) ISO refrigerated shipping containers. The test fumigant Vapormate® was supplied by BOC Gases Ltd. in 'G' size cylinders (Gas Code 279): a mixture consisting of 16.7% ethyl formate and 83.3% CO_2. The gas was passed through a heat-exchange unit consisting of a coil

immersed in boiling water. The fans at the rear of the container were switched on for the entire fumigation period, including the 30 minute degassing period at the end of each trial. The container was fitted with 3 nylon gas sampling lines along the front, middle and rear to ensure coverage of all areas within the container. The required treatments were applied, and ethyl formate was measured (by gas chromatography) every 30 minutes for the whole period of each trial. CO_2 was measured at 10 minute intervals (Vaisala CARBOCAP®) to maintain concentrations >10% for successful ethyl formate fumigation. Thermistor probes at 3 positions in air and 3 in fruit logged temperature °C (Grant Squirrel ^{TM}) at 10 minute intervals. Vials with test insects were placed in fruit before fumigation started and retrieved after de-gassing. Mortality was assessed and corrected as in laboratory trials. Moulded polystyrene packaging adsorbed approximately 4-5% of applied ethyl formate dose in Vapormate® fumigation. About 25 – 30% sorption loss in free ethyl formate gas occurred. Fumigations were successful where final ethyl formate concentration was 50% of applied dose. There was no significant sorption of CO_2. Refrigerated containers were excellent for fumigation provided drainage holes and vents were sealed to satisfy a pressure decay test (200 to 100 Pascals >15 seconds, De Lima 1994). Shipping containers were vented for ca. 30 minutes after fumigation for safe removal of fruit to cold rooms for storage at 1°C.

Laboratory test data were analysed using GenStat (2008) and container trial data were assessed using the equations of Couey and Chew (1986).

Results and Discussion: The estimates at LD_{50} and LD_{99} and 95% fiducial limits in laboratory tests (Table 1) show that late eggs are the most tolerant stage at 10, 15 and 20°C. An increase of ca. 10g/m^3 is required for each 5°C decrease in temperature. Trials in refrigerated containers (Table 2) showed that >99.95% control (95% CL) was achieved in all stages of LBAM.

The results reported herein are relevant to the practical application of these fumigants (ethyl formate + carbon dioxide) for disinfestation of light brown apple moth in table grapes. The produce was treated during the cool down process directly after harvest as well as after pre-chilling in the cold room to cover the major events in the table grape cool chain process.

References:

Abbott, W.S. (1925) J. Econ. Entomol. 18: 265-267.
Couey, H. M. and Chew, V. (1986) J. Econ. Entomol. 79:887-890.
De Lima, C.P.F. (1994) In Proc. 6th Int. Wkg .Conf. Stored Product Protection, Canberra, Australia, vol. 1, 71 - 77.
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GenStat (2008) Release 11.1 VSN International Ltd. Rothamsted, UK.

Table 1: Summary of Light Brown Apple Moth trials (2000 – 2009). Estimated response (LD_{50} & LD_{99} ±95% Fiducial Limits) of eggs, larvae and pupae exposed for 3 & 4 hours at 10, 15 and 20°C to ethyl formate + >10% CO_2.

Stage tested	Time	Number treated	Slope ±SE	Chi-sq (df= 6)*	LD50 (g/m^3) ±95% FL	LD99 (g/m^3) ±95% FL
10°C Trials						
Early Eggs	3h	16,840	9.11±1.56	2.04	21.92 (20.85 - 22.92)	36.60 (34.66 - 39.08)
	4h	16,448	8.85±1.52	2.51	18.70 (17.75 - 19.60)	31.23 (29.50 - 33.42)
Late Eggs	3h	15,360	6.75±0.93	0.94	21.49 (20.41 - 22.51)	42.21 (39.82 - 45.17)
	4h	14,400	7.42±1.03	3.12	18.76 (17.79 - 19.69)	36.86 (34.76 - 39.45)
Early instars (1-3)	3h	16,488	6.55±1.81	1.87	16.47 (15.53 - 17.33)	26.63 (25.15 - 28.54)
	4h	14,684	8.61±1.85	0.14	14.48 (13.63 - 15.26)	23.40 (22.07 - 25.12)
Late instars (4-6)	3h	5,650	10.13±1.79	1.81	20.30 (19.08 - 21.41)	36.04 (33.85 - 38.88)
	4h	6,240	6.37±1.44	2.77	17.87 (16.76 - 18.89)	31.74 (29.81 - 34.23)
Pupae	3h	1,680	10.57±1.63	2.34	20.90 (19.84 - 21.89)	35.51 (33.55 - 37.99)
	4h	1,644	7.47±1.46	2.16	18.38 (17.41 - 19.28)	31.22 (29.47 - 33.42)
15°C Trials						
Early Eggs	3h	21,480	11.49±1.72	3.18	19.79 (18.87 - 20.65)	31.41 (29.78 - 33.49)
	4h	24,448	8.68±1.40	3.14	18.54 (17.67 - 19.36)	29.44 (27.92 - 31.36)
Late Eggs	3h	19,460	10.29±1.59	2.67	21.03 (20.01 - 21.98)	35.46 (33.56 - 37.86)
	4h	20,282	8.18±1.43	1.78	19.19 (18.24 - 20.08)	32.36 (30.63 - 34.55)
Early instars (1-3)	3h	15,282	11.50±1.80	2.17	15.49 (14.33 - 16.51)	27.30 (25.55 - 29.60)
	4h	16,420	9.23±1.56	1.40	12.42 (11.46 - 13.29)	21.89 (20.36 - 23.92)
Late instars (4-6)	3h	5,296	11.36±2.01	1.56	19.99 (18.96 - 20.95)	32.49 (30.68 - 34.83)
	4h	4,628	6.07±1.52	2.75	16.74 (15.84 - 17.59)	27.21 (25.68 - 29.17)
Pupae	3h	1,464	11.37±1.91	1.17	20.00 (19.03 - 20.91)	32.66 (30.94 - 34.84)
	4h	2,008	7.00±1.43	2.77	15.09 (14.29 - 15.84)	24.64 (23.27 - 26.35)
20°C Trials						
Early Eggs	3h	23,284	7.89±1.47	1.39	15.48 (14.48 - 16.38)	27.24 (25.55 - 29.42)
	4h	21,880	5.62±1.48	1.05	12.55 (11.69 - 13.32)	22.09 (20.75 - 23.78)
Late Eggs	3h	13,418	8.14±1.33	1.87	16.67 (15.85 - 17.44)	26.63 (25.22 - 28.44)
	4h	14,432	7.04±1.35	2.14	14.00 (13.29 - 14.67)	22.36 (21.15 - 23.92)
Early instars (1-3)	3h	14,424	5.41±1.44	1.76	12.79 (11.66 - 13.70)	19.85 (18.42 - 22.05)
	4h	16,600	4.83±1.55	1.44	9.80 (8.67 - 10.73)	15.21 (13.99 - 16.90)
Late instars (4-6)	3h	6,846	6.04±1.42	2.36	13.34 (12.20 - 14.32)	21.94 (20.39 - 24.14)
	4h	5,480	4.48±1.64	0.76	10.93 (9.89 - 11.82)	17.97 (16.64 - 19.80)
Pupae	3h	1,192	7.08±1.48	1.63	13.64 (12.57 - 14.58)	23.27 (21.71 - 25.39)
	4h	1,582	5.61±1.59	1.30	12.32 (11.33 - 13.19)	21.03 (19.62 - 22.91)

*ANOVA – 3 degrees of freedom (df) are taken for fitting the logistic curve for 9 doses (non-linear probit regression)

Table 2: Summary of large scale Light Brown Apple Moth trials (2005 – 2009) in refrigerated containers using Vapormate® in 3 replicated tests for 4 h at 10°C, 3.5 h 15°C, and 3 h 20°C

	Fumigation 10°C			Fumigation 15°C			Fumigation 20°C		
Stage tested	Number of insects*	S	Estimated mortality 95% CL	Number of insects*	S	Estimated mortality 95% CL	Number of insects*	S	Estimated mortality 95% CL
Early eggs	25,320	0	99.88%	26,160	0	99.88%	27,000	0	99.89%
Late eggs	26,880	0	99.88%	24,840	0	99.88%	26,280	0	99.88%
Early instars	10,440	0	99.71%	10,920	0	99.72%	11,640	0	99.74%
Late instars	6,720	0	99.55%	9,960	0	99.70%	9,720	0	99.69%
Pupae	6,240	0	99.52%	5,400	0	99.44%	5,880	0	99.49%
Total	80,880	0	99.96%	80,040	0	99.96%	80,520	0	99.96%

*estimate based on total of 120 vials in 3 replicate tests; S = survivors