Disinfestation of sweet corn for export using phosphine and controlled atmospheres

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PURPOSE OF THIS REPORT:

This is the final report for the project "Disinfestation of sweet corn for export using phosphine and controlled atmospheres"

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1. MEDIA SUMMARY

Sweet corn growers in Australia (annual production 62 million tonnes, ABS 2006) have the potential to become a strong export oriented industry. Sweet corn is exported to Japan because of good prices obtained there, but there are problems when live insects are found in shipments. The pests intercepted include corn earworm, native budworms, aphids, thrips, and mites. Effective postharvest disinfestation of produce is essential to satisfy the zero tolerance of live insects required by overseas quarantine authorities. Fumigation with methyl bromide is the current quarantine treatment, but has the disadvantage of causing browning of the green sheath covers of the corn cob. Methyl bromide is also known to induce "off flavours" in stored corn. To ensure good quality exports, an alternative is required. Methyl bromide depletes the ozone layer and because it is subject to phase out by 2015 in Australia under the Montreal Protocol, its cost rises each year as production and imports are curtailed.

The research on alternatives to methyl bromide was carried out at the Department of Agriculture and Food, Western Australia, South Perth, using international trial protocols. Several hundred experiments were conducted on all life stages of the prohibited pests. The key outcomes are:

- A new fumigant (combining ethyl formate with carbon dioxide) was tested in the laboratory and in commercial conditions and found to be very effective in destroying pests and preserving product quality.
- New and effective fumigation quarantine treatments have been developed against the corn earworm, *Helicoverpa armigera*, native budworm, *H. punctigera*, two spotted spider mite *Tetranychus urticae*, plague thrips *Thrips imaginis*, western flower thrips *Frankliniella occidentalis*, green peach aphid *Myzus persicae* and corn aphid *Rhopalosiphum maidis* at temperatures ranging from 10 to 20°C and exposure periods from 2-4 hours.
- The research data satisfies international standards of quarantine treatment required for market access of sweet corn from Australia to all countries imposing restrictions against specified pests.
- Fumigation combining ethyl formate with carbon dioxide at temperatures and exposure periods that fit within the cool chain process preserves the quality of fresh harvested sweet corn.
- Using alternative treatments to methyl bromide is a sustainable way of managing the sweet corn industry into the future.
- These new fumigation methods have high applicability for many pests found in other exported vegetable crops.

2. TECHNICAL SUMMARY

Nature of the problem: Australian sweet corn is often fumigated using methyl bromide when exported. The pests requiring fumigation are: corn earworm, *Helicoverpa armigera*, native budworm, *H. punctigera*, two spotted spider mite *Tetranychus urticae*, plague thrips *Thrips imaginis*, western flower thrips *Frankliniella occidentalis*, green peach aphid *Myzus persicae* and corn aphid *Rhopalosiphum maidis*. Methyl bromide is phytotoxic to sweet corn and shortens shelf life. Alternative fumigation treatments that are commercially viable and which preserve good quality are needed. To achieve market access, data proving effectiveness is required for bi-lateral negotiations.

Brief description of the science undertaken: Experiments to test the effects of fumigant gases on test insects were conducted in the laboratory. Tests included phosphine, propylene oxide, and ethyl formate as pure gases and in combination with carbon dioxide. Treatments at temperatures ranging from 10 to 20°C were carried out at the Department of Agriculture and Food, Western Australia, South Perth. Experiments were conducted on Australian sweet corn using international trial protocols. These protocols require data on (1) the pest life stage development; (2) the stage of each species that is most tolerant to the treatment; and (3) large scale trials proving that the treatment is effective under simulated export conditions where >10,000 insects must be exposed to the treatment in separate replicate trials with none surviving. The trial design was developed using the following procedures:

- Life history studies were conducted to determine the course of development of the immature stages and the dates when each stage (eggs, larval instars, pupae, adults) should be tested.
- The most tolerant stage fumigation trials were conducted by treating each stage using a series of doses and time periods at specified temperatures. The most tolerant stage was determined by comparing estimates of the LD_{50} and LD_{99} of each stage at each time period and specified temperature. Thereafter probit 9 level of treatment was determined for large scale trials.
- Large-scale semi-commercial fumigation trials were conducted in bulk bins using sweet corn in a series of replicated trials exposing >10,000 individuals of the most tolerant stage/s to selected treatment x time x temperature combinations.
- Of the fumigants tested, the only commercial product available was Vapormate® (a mixture of 16.7% ethyl formate and 83.3% CO_2) and this was used in all trials. Throughout each trial continuous measurements were made of fumigant concentrations and temperature of fruit and air.

Major research findings and industry outcomes:

- Effective quarantine fumigation treatments against several sweet corn pests have been determined and supplied to the Australian Quarantine & Inspection Service, Biosecurity Australia and APVMA.
- Commercial scale application techniques have been developed for the sweet corn industry that are suitable for use by exporters as well as growers.
- The new fumigant does not cause injury to sweet corn and does not leave any residue.
- This work is expected to satisfy Quarantine requirements of overseas countries permitting the export of Australian sweet corn under a treatment schedule recommended as an outcome of this project.

Recommendations to industry: Industry should fund new R&D including use of partial pressure fumigation that will result in potentially effective control of fruit flies in vegetables to satisfy quarantine authorities.

Contribution to new technology: The work described in this report is an important contribution to new development of safe fumigants in horticulture and new application of fumigation techniques for sweet corn. Other fruit and vegetable industries should consider these findings and develop disinfestation protocols to their advantage for market access.

3. INTRODUCTION

There is considerable interest from overseas importers and from Australian exporters to develop markets for sweet corn during periods of seasonal scarcity in the Northern Hemisphere in Japan and other countries when exchange rates are favourable.

However, sweet corn is often fumigated using methyl bromide which is phytotoxic and shortens shelf life. The pests in Australian produce that results in prohibition of imports are: the cotton bollworm or corn earworm, *Helicoverpa armigera*, the native budworm or Australian bollworm, *H. punctigera*, two spotted spider mite *Tetranychus urticae*, plague thrips *Thrips imaginis*, western flower thrips *Frankliniella occidentalis*, green peach aphid *Myzus persicae* and corn aphid *Rhopalosiphum maidis.*

Rapid removal of field heat is important for preserving the quality of sweet corn while maintaining relative humidity at > 95% to reduce moisture loss. The challenge has been to develop a fumigation treatment that does not damage sweet corn while permitting treatments in the shortest possible period over a range of temperatures that fits within the cool chain process.

Quarantine trial protocols require extremely high levels of disinfestation to be demonstrated through a carefully conducted series of experiments. The first stage is to develop a standard infestation procedure that gives a reproducible method of obtaining sufficient numbers of the required life stages of the target pest. This is done through developing appropriate rearing methods to determine the life history of each pest. Thereafter, trials requiring a series of dose–response tests (replicated 3 times) to determine the LD_{50} and LD_{99} mortality values for each life-stage of each species, at each temperature and exposure period, are conducted. The results of these tests are used to select the life-stage most tolerant to each treatment and to plan and conduct a series of large-scale trials under simulated export conditions at a dose x time and temperature estimated to give Probit 9 [$LD_{99.99683}$] level of control.

Controlled Atmospheres

A controlled atmosphere (CA) trial shipment was prepared by another project wherein a 20ft CA container loaded with sweet corn in cartons was shipped from Manjimup arriving after 20 days in Nagoya, Japan on 27 April 2001. Oxygen levels were measured at an average of 5% while CO2 levels averaged 6%. The temperature was maintained between 1-3°C throughout the shipment except for a short period of temperature spikes coinciding with transhipment in Singapore. However, live *Helicoverpa* spp. and other insects were found on arrival in Japan and after the consignment was fumigated with methyl bromide it was in poor condition and unsaleable. The inclusion of CA as a treatment was made at the request of industry, however in small scale (CA) trials conducted in South Perth after the above shipment also proved that the treatment was ineffective.

Phosphine

Phosphine: Phosphine of approximately 85% purity was generated from a fresh supply of 1g pellets of aluminum phosphide (Detia, Germany) by immersing a pellet wrapped in filter paper under an inverted funnel in 5% sulphuric acid and allowing the gas to displace water in a gas-tight burette held over the funnel and fitted with a septum for drawing samples. The purity of the gas was measured against a certified standard supplied by BOC Special Gases Ltd (Sydney) using the thermionic specific detector (TSD) on a Varian 3400 Gas Chromatograph.

Tests were conducted in several replicates using phosphine alone, in combination with 25% carbon dioxide, and in carbon dioxide alone for 6, 16 and 20 hour exposures at 20°C. All insects were controlled at 1.1 ml/L phosphine in 20-hour exposures but required only 0.55 ml/L in elevated (25%) CO_2. The treated produce did not show any phytotoxic symptoms even after 8 days storage at 5°C. However, the long exposure period required for phosphine did not make it attractive for exporters. In addition phosphine is a

far more toxic product to handle and for occupational health and safety reasons the treatments were not taken to commercial scale.

Propylene oxide + CO2

Propylene oxide: Analytical grade propylene oxide (>99% 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. Gas concentrations were analysed throughout every trial as described below for ethyl formate and carbon dioxide.

Propylene oxide + CO2 was tested extensively in a graded series of dosages ranging from 8 to 40 g/m^3 and >10% CO2. Complete control was obtained in laboratory tests at 20 and 15°C, in 3 and 4-hour exposures at 28 and 36 g/m^3 respectively. Small scale tests showed that the treated produce did not exhibit any phytotoxic symptoms even after 8 days storage at 5°C. However, since no commercial product had been licensed and approved in Australia (there is a product registered in the USA by Aberco) no further work could be done on a commercial scale for sweet corn exports.

Ethyl formate + CO2

As a result of extensive and intensive laboratory and field work with ethyl formate + CO2 and its availability in a formulation as a registered product efforts were focused on specifying the dosages of the active ingredients required for various insects for which registration was not available. The experiments for disinfesting sweet corn at several temperatures were conducted over a period of 6 years from 1999 to 2006 and are reported in detail below.

4. METHODS & MATERIALS

4.1. INSECTS TESTED

4.1.1 Corn earworm *Helicoverpa armigera* (Hübner) Lepidoptera: Noctuidae

The cotton bollworm or corn earworm, *H. armigera* is found in sweet corn after harvest. Test insects obtained from laboratory cultures were reared at 24 ± 1°C, 60% RH, 16:8 (L:D) cycle. Eggs were laid in batches on toweling paper placed inside 20 litre containers with adults. The paper was cut into suitable sizes before being placed with artificial cowpea media in plastic containers to develop. All required stages were obtained by continuous rearing.

4.1.2 Native budworm *Helicoverpa punctigera* (Wallengren) Lepidoptera: Noctuidae

The native budworm or Australian bollworm**,** *H. punctigera* is found in sweet corn after harvest. Rearing methods were similar to that described for *H. armigera.*

4.1.3 Two spotted spider mites *Tetranychus urticae* (Koch) Acari: Tetranychidae

TSM were reared in the laboratory on potted bean plants at 24±1°C, 65% RH and 16:8 (L:D) cycle. Life stages required for testing were collected with a moist soft hair brush by removing them from leaves using a desk top magnifying lens where required.

4.1.4 Western flower thrips *Frankliniella occidentalis* (Pergande) Thysanoptera: Thripidae

WFT were reared in the laboratory on marigold or chrysanthemum flowers obtained from small nursery stocks which had not been sprayed with insecticide. Rearing conditions were 24±1°C, 65% RH and 16:8 (L:D) cycle and dilute honey was provided in cages. Flowers were changed weekly and the life stages for treatment and control were obtained by shaking flowers over deep white tubs and collecting required stages with an aspirator.

4.1.5 Plague thrips *Thrips imaginis* (Bagnall) Thysanoptera: Thripidae

Rearing methods were the same as WFT. Flowers initially obtained from plant nurseries had predominant numbers of plague thrips and these were utilized for trials.

4.1.6 Green peach aphid *Myzus persicae* (Sulzer) Hemiptera: Aphididae

Aphids were reared in the laboratory on potted bean plants at 24±1°C, 65% RH and 16:8 (L:D) cycle. Life stages required for testing were collected with a moist soft hair brush by removing them from leaves using a desk top magnifying lens where required.

4.1.7 Corn aphid *Rhopalosiphum maidis* (Fitch) Homoptera: Aphididae

Field collected aphids were reared on potted bean plants as for green peach aphid.

4.2 LABORATORY FUMIGATION

Laboratory tests were done (1999 – 2006) in individually calibrated glass desiccators (by measuring the volume of water held) of 6.6 – 7.1 L each containing a magnetic stirrer rod in the base and the lid fitted with a self sealing septum. Preliminary fumigations were done at 10, 15, and 20°C to estimate the best dose response range. Subsequently, a range of concentrations usually 9 doses were used for a range of exposure periods. A minimum of 3 replicate trials to obtain a concentration x time product spanning a dose 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 doses at 100% mortality. Vials with test insects in medium were placed in the desiccators in advance of fumigation. Each vial contained a plastic cap cover into which a 5mm hole was punched and sealed with a strip of filter paper to allow entry of the fumigant while preventing escape of the insects. Each desiccator was placed on a magnetic stirrer which was run continuously for the duration of each trial.

Ethyl formate: 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.

Carbon dioxide: was initially trialed at several concentrations before adopting 10 - 12% CO2 for mixture with ethyl formate for these trials. CO_2 was introduced into each desiccator using a weighed amount of laboratory produced dry ice (Frigimat® Cub Dry Ice Maker, ScienceLab.com, Inc. Houston, Texas).

Gas concentrations were verified using electronic sensors (CO2) and by Gas Chromatography using a Thermal Conductivity Detector (TCD). Before fumigation, the calculated volume of air displaced by each gas (ethyl formate and CO_2) was removed from the desiccator using a gas tight syringe. Ethyl formate and propylene oxide were analysed on a Varian 3400 Gas Chromatograph (GC) using a Flame Ionisation Detector (FID) with a 'Poropak Q" packed column (operating temperatures: oven 70°C; injector 140°C; detector 250°C). The first sample was taken at 10 minutes after application to verify the applied dose and thereafter at 30 minute intervals throughout each trial. Trials of insect species and life stages were conducted over a number of years from 1999 to 2006.

4.3 FUMIGATION IN SHIPPING CONTAINERS

For practical purposes only commercially available ethyl formate + CO2 was tested. The test fumigant Vapormate® was supplied by BOC gases in 'G' size cylinders (Gas Code 279): a mixture consisting of 16.7% Ethyl Formate and 83.3% CO_2. Trials were done in 20ft / 6m (28 m^3) ISO refrigerated shipping containers loaded with test produce. The gas was passed through a heat-exchange unit consisting of a coil immersed in boiling water. The unit was purpose built from stainless steel (diameter 20 cm and height of 80 cm) containing 25 litres of water. This unit raised the temperature of the input mixture to >90°C, which

was measured using an in-line thermometer. This process ensured that the gas was thoroughly volatilised before it entered the fumigation space. Vapormate® was introduced into the container through a 25mm (od) PVC tube running along the top for the entire length of the container or chamber. Holes (0.6mm) were drilled every 50 cm along the length of the tube and the end was capped. This setup ensured excellent distribution of the gas in the fumigation process and equilibration was achieved throughout within 30 minutes from the start. The fans at the rear of the container or fumigation room were switched on for the entire fumigation period, including the 30 minute degassing period at the end of each trial. The container or fumigation chamber was fitted with 3 nylon gas sampling lines along the front, middle and rear end to ensure coverage of all areas within the container: position 1 bottom/rear of the container; position 2 centre/centre of the container; position 3 front/top of the container (near the door).

The required treatments were applied, and gas concentration was measured (by taking samples for gas chromatography) every 30 minutes for the whole trial. Two samples were extracted from each location using a SGE Australia Gas sampler GAV-200 with re-useable 200 ml Mylar sample bags. Before samples were taken, the sample lines were evacuated using 20 strokes of a vacuum pump (a bicycle style pump with the plunger reversed) to ensure that measurements reflected the levels of fumigant at time of sampling, rather than measuring the stale gas in the sample tube. Bags were tested for gas-tightness by immersing them in water containing detergent and squeezing them to force air out of any holes in the bag or through the septum.

Gas concentrations were also measured using ethyl acetate or ethyl formate Dräger Gas monitoring tubes as a check (and for use by quarantine officers in future auditing). These monitoring tubes were also used at the end of the fumigation, after venting to assess if any gas was present. Measurements of CO_2 were taken at the above 3 positions during the fumigation using an ANRI BM-2 meter (0 – 100% CO_2) which provides an alternate means of measuring the applied levels of ethyl formate in Vapormate®. Carbon Dioxide levels were also measured using a Vaisala Type GMT221 Carbon Dioxide Transmitter using probe type GMP221 (Vaisala, Helsinki Finland) to measure in the range of 0-20% CO_2. Subsequently, Vaisala CARBOCAP® Hand-Held Carbon Dioxide Meter GM70 with data logging capabilities was tested. Measuring CO_2 is a potentially cheaper alternative to the use of Dräger detector tubes and can be used to assess fumigation since the meter can be used for several years and will soon recover initial investment.

Temperatures were measured by placing thermistor probes at 3 positions in air and 3 points in fruit as the container was loaded. The probes were connected to a digital data logger (Grant Squirrel ^{TM}) programmed to record data at 10 minute intervals throughout each trial.

Plastic or glass vials (5cm long 2cm diameter fitted with plastic caps with 0.5 cm vent hole sealed with filter paper) containing live insects of all stages were placed throughout the cartons containing fruit produce before the fumigation started and were retrieved after de-gassing and reared for the required period according to species and stage to assess mortality. Mortality was corrected (Abbot 1925) based on untreated controls in every test.

Tests for sorption in plastic bins revealed that 3-4% loss of applied dose could be attributed to packaging and was not a significant issue for Vapormate® fumigation. Applied doses were raised by 5% in all trials to account for sorption. Sorption did not occur on stainless steel surfaces in refrigerated containers. Generally a 25 – 30% loss occurs in the large scale fumigation process and starting doses were raised to compensate for this. All fumigations were successful where final dose was at near 60% of initial applied dose. Refrigerated containers were found to be excellent for fumigation provided drainage holes and vents were taped. Cold rooms that were more modern and had a good seal were similarly found to be suitable for fumigation. In non-refrigerated shipping containers sealing that satisfied a pressure decay test - 200 to 100 Pascals in >10 seconds (De Lima 1994, 2001) were found suitable for fumigation with Vapormate®.

4.4 STATISTICAL ANALYSIS

The data were analysed using the GenStat package (GenStat 2008). The probit model uses a generalised linear procedure, assuming a binomial distribution for the number of responses and a probit link function between the number of responses and the logarithm (log_{10}) of the dose. Tests on data using the logit link function and the complementary log-log function did not significantly reduce the residual deviance and the probit link function was retained in analysis. A logistic curve was fitted using probit transformation to give the best fit to converge and obtain the variance-covariance matrix for the analysis of variance table. The Fieller procedure was used to calculate the fitting process for the estimates at LD_{50} and LD_{99} and of the lower and upper fiducial limits.

5. RESULTS

5.1 RESULTS OF LABORATORY FUMIGATION TRIALS

Seven insect species that are of concern to Australia's trading partners including Japan, Taiwan, USA, etc. were tested.

5.1.1 *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae)

The results (Tables 1-3) show that in general at all temperatures eggs are the most tolerant stage.

Table 1: Summary of *Helicoverpa armigera* trials (1999 – 2006). Estimated response (LD_{50} & LD_{99}) of eggs, young larvae and adults exposed for 2-4 hours at 10°C to ethyl formate + 10% CO_2.

Stage tested	Time treated	Number treated	Slope ±SE	Chi-sq (df= 6) [1]	LD_{50} (g/m^3) ±95% CL	LD_{99} (g/m^3) ±95% CL
Eggs	2h	12,792	5.89±0.58	4.45	24.47 (23.12 - 25.77)	56.21 (51.86 - 61.98)
	3h	15,440	6.13±0.65	3.51	21.98 (20.73 - 23.16)	50.48 (46.73 - 55.43)
	4h	12,794	6.23±0.63	2.24	17.87 16.78 - 18.89)	41.04 (38.01 - 44.99)
Early larval instars	2h	12,240	6.36±0.78	2.97	24.82 (23.18 - 26.35)	54.28 (49.79 - 60.54)
	3h	14,360	7.16±0.92	3.44	21.23 (19.76 - 22.58)	46.43 (42.73 - 51.52)
	4h	14,864	5.85±0.73	5.31	16.57 (15.33 - 17.71)	36.24 (33.41 - 40.10)
Late larval instars	2h	11,396	8.95±1.07	3.65	28.07 (26.69 - 29.38)	52.14 (48.53 - 57.05)
	3h	13,124	9.38±1.12	3.29	23.71 (22.49 - 24.85)	44.06 (41.17 - 47.94)
	4h	12,652	7.91±0.94	2.04	22.56 (21.38 - 23.65)	41.92 (39.2 - 45.56)

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

Table 2: Summary of *Helicoverpa armigera* trials (1999 – 2006). Estimated response (LD_{50} & LD_{99}) of eggs, young larvae and adults exposed for 2-4 hours at 15°C to ethyl formate + 10% CO_2.

Stage tested	Time treated	Number treated	Slope ±SE	Chi-sq (df= 6) [1]	LD_{50} (g/m^3) ±95% CL	LD_{99} (g/m^3) ±95% CL
Eggs	2h	15,424	6.75±0.64	2.80	22.93 (21.72 - 24.08)	48.37 (45.10 - 52.60)
	3h	14,296	6.62±0.65	1.44	20.72 (19.58 - 21.78)	43.70 (40.85 - 47.37)
	4h	14,180	6.99±0.70	5.62	18.32 (17.28 - 19.30)	38.64 (36.08 - 41.93)
Early larval instars	2h	16,012	7.51±0.78	3.31	22.49 (21.29 - 23.61)	43.61 (40.78 - 47.33)
	3h	11,224	7.76±0.83	2.37	20.35 (19.23 - 21.39)	39.46 (36.94 - 42.74)
	4h	15,318	6.94±0.72	1.35	18.03 (17.01 - 18.99)	34.97 (32.70 - 37.91)
Late larval instars	2h	12,962	7.52±0.77	3.54	23.00 (21.80 - 24.13)	44.07 (41.22 - 47.81)
	3h	17,108	8.17±0.90	2.55	20.37 (19.26 - 21.40)	39.03 (36.55 - 42.27)
	4h	12,290	7.12±0.74	1.47	18.40 (17.37 - 19.37)	35.26 (32.99 - 38.22)

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

Table 3: Summary of *Helicoverpa armigera* trials (1999 – 2006). Estimated response (LD_{50} & LD_{99}) of eggs, young larvae and adults exposed for 2-4 hours at 20°C to ethyl formate + 10% CO_2.

Stage tested	Time treated	Number treated	Slope ±SE	Chi-sq (df= 6) [1]	LD_{50} (g/m^3) ±95% CL	LD_{99} (g/m^3) ±95% CL
Eggs	2h	15,316	6.23±0.69	5.74	22.84 (21.48 - 24.11)	47.38 (43.94 - 51.99)
	3h	13,224	7.73±0.89	3.55	19.59 (18.36 - 20.72)	40.64 (37.75 - 44.48)
	4h	12,470	6.25±0.70	1.03	17.39 (16.26 - 18.43)	36.07 (33.53 - 39.43)
Early larval instars	2h	12,406	8.82±1.13	3.93	24.73 (23.25 - 26.08)	45.53 (42.28 - 50.06)
	3h	15,336	10.76±1.59	3.44	21.08 (19.76 - 22.28)	38.82 (36.08 - 42.58)
	4h	14,640	6.27±0.79	5.42	18.52 (17.30 - 19.62)	34.10 (31.77 - 37.28)
Late larval instars	2h	14,226	6.75±0.73	2.29	21.87 (20.39 - 23.22)	44.23 (40.97 - 48.67)
	3h	15,092	7.64±1.01	1.83	18.55 (17.23 - 19.74)	37.51 (34.80 - 41.17)
	4h	16,280	6.17±0.79	5.74	15.80 (14.63 - 16.86)	31.95 (29.55 - 35.16)

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

5.1.2 *Helicoverpa punctigera* (Wallengren) (Lepidoptera: Noctuidae)

The results (Tables 4-6) show that in general at all temperatures eggs are the most tolerant stage.

Table 4: Summary of *Helicoverpa punctigera* trials (1999 – 2006). Estimated response (LD_{50} & LD_{99}) of eggs, young larvae and adults exposed for 2-4 hours at 10°C to ethyl formate + 10% CO_2.

Stage tested	Time treated	Number treated	Slope ±SE	Chi-sq (df= 6) [1]	LD_{50} (g/m^3) ±95% CL	LD_{99} (g/m^3) ±95% CL
Eggs	2h	12,220	5.52±0.56	2.75	26.66 (25.32 - 27.96)	58.83 (54.36 - 64.76)
	3h	18,300	8.13±0.85	5.58	22.89 (21.69 - 24.05)	50.51 (46.79 - 55.43)
	4h	16,240	6.47±0.67	2.39	20.57 (19.45 - 21.62)	45.38 (42.14 - 49.64)
Early larval instars	2h	14,825	7.76±0.89	2.69	25.82 (24.30 - 27.25)	53.41 (49.27 - 59.10)
	3h	15,200	8.94±1.14	3.77	22.68 (21.30 - 23.97)	46.93 (43.38 - 51.76)
	4h	14,936	6.05±0.76	1.90	19.41 (18.16 - 20.56)	40.16 (37.26 - 44.07)
Late larval instars	2h	14,460	7.22±0.81	4.04	27.32 (26.07 - 28.51)	49.69 (46.51 - 53.95)
	3h	14,280	9.34±1.06	3.95	24.21 (23.06 - 25.29)	44.04 (41.29 - 47.68)
	4h	14,242	9.47±1.04	4.19	22.24 (21.15 -23.26)	40.45 (37.89 - 43.84)

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

Table 5: Summary of *Helicoverpa punctigera* trials (1999 – 2006). Estimated response (LD_{50} & LD_{99}) of eggs, young larvae and adults exposed for 2-4 hours at 15°C to ethyl formate + 10% CO_2.

Stage tested	Time treated	Number treated	Slope ±SE	Chi-sq (df= 6) [1]	LD_{50} (g/m^3) ±95% CL	LD_{99} (g/m^3) ±95% CL
Eggs	2h	12,248	5.66±0.59	4.30	22.82 (21.40 - 24.15)	52.66 (48.55 - 58.17)
	3h	13,110	6.26±0.70	4.33	20.27 (18.97 - 21.49)	46.78 (43.22 - 51.51)
	4h	12,209	6.08±0.65	1.06	17.27 (16.10 - 18.36)	39.85 (36.87 - 43.80)
Early larval instars	2h	13,756	7.09±0.88	3.31	25.85 (24.23 - 27.36)	53.74 (49.37 - 59.86)
	3h	14,640	7.53±1.00	2.78	22.9 (21.41 - 24.26)	47.61 (43.93 - 52.70)
	4h	12,821	6.56±0.81	1.60	18.61 (17.31 - 19.79)	38.69 (35.72 - 42.75)
Late larval instars	2h	15,410	6.71±0.70	2.86	22.44 (21.00 - 23.78)	46.08 (42.69 - 50.68)
	3h	16,268	7.98±1.05	3.59	19.00 (17.72 - 20.18)	39.02 (36.18 - 42.83)
	4h	16,772	6.33±0.78	5.10	16.65 (15.48 - 17.73)	34.20 (31.72 - 37.50)

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

Table 6: Summary of *Helicoverpa punctigera* trials (1999 – 2006). Estimated response (LD_{50} & LD_{99}) of eggs, young larvae and adults exposed for 2-4 hours at 20°C to ethyl formate + 10% CO_2.

Stage tested	Time treated	Number treated	Slope ±SE	Chi-sq (df= 6) [1]	LD_{50} (g/m^3) ±95% CL	LD_{99} (g/m^3) ±95% CL
Eggs	2h	12,128	5.97±0.63	4.49	22.96 (21.58 - 24.25)	49.41 (45.77 - 54.25)
	3h	14,096	7.33±0.83	3.42	19.72 (18.48 - 20.87)	42.43 (39.36 - 46.48)
	4h	14,665	6.07±0.69	4.55	17.35 (16.21 - 18.40)	37.33 (34.68 - 40.80)
Early larval instars	2h	15,290	8.61±1.08	4.61	24.96 (23.51 - 26.30)	46.55 (43.22 - 51.14)
	3h	11,100	10.261.41	2.93	21.61 (20.30 - 22.80)	40.30 (37.51 - 44.11)
	4h	12,292	6.51±0.80	1.38	19.27 (18.05 - 20.37)	35.93 (33.48 - 39.26)
Late larval instars	2h	14,215	6.820.73	2.49	22.00 (20.52 - 23.36)	45.64 (42.23 - 50.26)
	3h	13,225	7.45±0.96	1.97	18.83 (17.50 - 20.05)	39.08 (36.23 - 42.90)
	4h	13,772	6.21±0.79	5.49	16.36 (15.17 - 17.46)	33.95 (31.45 - 37.30)

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

5.1.3 Two-spotted spider mite *Tetranychus urticae* (Koch) (Acarina: Tetranychidae)

The results (Tables 7-9) show that in general at all temperatures eggs are the most tolerant stage, followed by adults. The most susceptible stage is nymphs.

Table 7: Summary of Two-spotted spider mite trials (2000 – 2005). Estimated response (LD_{50} & LD_{99}) of eggs, nymphs and adults exposed for 1-4 hours at 10°C to ethyl formate (+10% CO_2).

Stage tested	Time treated	Number treated	Slope ±SE	Chi-sq (df= 6) [1]	LD_{50} (g/m^3) ±95% CL	LD_{99} (g/m^3) ±95% CL
Eggs	1h	9,081	4.33±0.41	2.53	16.54 (14.99 - 18.09)	65.75 (57.52 - 77.22)
	2h	10,115	4.77±0.51	1.90	11.28 (10.12 - 12.43)	44.84 (39.58 - 52.03)
	3h	12,264	4.07±0.48	1.81	8.20 (7.27 - 9.11)	32.59 (28.83 - 37.65)
	4h	11,289	2.86±0.45	1.31	4.85 (4.24 - 5.46)	19.26 (16.91 - 22.40)
Nymphs	1h	12,398	3.59±0.51	2.16	11.57 (10.24 - 12.87)	45.41 (39.83 - 53.18)
	2h	11,275	3.54±0.52	2.57	9.19 (8.07 - 10.29)	36.07 (31.72 - 42.05)
	3h	10,291	2.88±0.50	2.19	6.65 (5.77 - 7.52)	26.09 (22.94 - 30.37)
	4h	11,882	2.59±0.54	2.88	4.75 (4.08 - 5.41)	18.63 (16.21 - 21.92)
Adults	1h	12,949	3.33±0.52	0.80	15.82 (14.47 - 17.15)	48.84 (43.55 - 56.09)
	2h	12,006	3.80±0.57	1.95	13.25 (12.07 - 14.41)	40.90 (36.60 - 46.74)
	3h	13,226	3.34±0.50	2.11	9.64 (8.68 - 10.57)	29.75 (26.71 - 33.80)
	4h	11,870	3.40±0.52	0.75	6.98 (6.22 - 7.74)	21.56 (19.24 - 24.61)

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

Table 8: Summary of Two-spotted spider mite trials (2000 – 2005). Estimated response (LD_{50} & LD_{99}) of eggs, nymphs and adults exposed for 1-4 hours at 15°C to ethyl formate (+10% CO_2).

Stage tested	Time treated	Number treated	Slope ±SE	Chi-sq (df= 6) [1]	LD_{50} (g/m^3) ±95% CL	LD_{99} (g/m^3) ±95% CL
Eggs	1h	10,987	2.95±0.47	0.65	15.82 (14.32 - 17.30)	52.25 (46.09 - 60.92)
	2h	11,799	4.48±0.65	2.96	11.21 (10.04 - 12.35)	37.01 (32.83 - 42.78)
	3h	10,541	4.55±0.69	1.28	7.99 (7.06 - 8.91)	26.39 (23.36 - 30.51)
	4h	12,040	3.13±0.59	1.00	5.18 (4.51 - 5.84)	17.10 (15.05 - 19.85)
Nymphs	1h	12,672	2.80±0.61	1.83	11.76 (10.23 - 13.23)	45.95 (39.89 - 54.73)
	2h	13,218	2.35±0.59	0.92	8.76 (7.52 - 9.96)	34.25 (29.84 - 40.50)
	3h	12,055	2.28±0.64	1.34	6.28 (5.32 - 7.22)	24.55 (21.30 - 29.09)
	4h	11,238	2.79±0.67	0.72	4.45 (3.73 - 5.16)	17.38 (14.90 - 20.85)
Adults	1h	12,472	2.35±0.57	1.70	12.76 (11.33 - 14.15)	46.64 (40.92 - 54.72)
	2h	11,065	2.55±0.55	2.11	9.30 (8.15 - 10.41)	33.99 (29.96 - 39.55)
	3h	12,174	2.61±0.56	1.16	6.35 (5.48 - 7.20)	23.21 (20.33 - 27.14)
	4h	13,002	2.88±0.63	1.18	4.41 (3.77 - 5.05)	16.11 (13.96 - 19.06)

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

Table 9: Summary of Two-spotted spider mite trials (2000 – 2005). Estimated response (LD_{50} & LD_{99}) of eggs, nymphs and adults exposed for 1-4 hours at 20°C to ethyl formate (+10% CO_2).

Stage tested	Time treated	Number treated	Slope ±SE	Chi-sq (df= 6) [1]	LD_{50} (g/m^3) ±95% CL	LD_{99} (g/m^3) ±95% CL
Eggs	1h	10,460	2.56±0.56	1.07	13.60 (12.00 - 15.14)	47.47 (41.41 - 56.27)
	2h	12,442	4.49±0.85	1.47	10.12 (8.83 - 11.37)	35.34 (30.88 - 41.70)
	3h	11,686	2.64±0.60	1.91	6.98 (5.99 - 7.94)	24.36 (21.25 - 28.73)
	4h	10,788	2.65±0.64	2.19	4.13 (3.49 - 4.76)	14.41 (12.44 - 17.16)
Nymphs	1h	12,275	4.06±0.79	2.25	13.37 (11.94 - 14.71)	32.57 (28.97 - 37.85)
	2h	11,498	5.60±0.96	2.24	9.75 (8.61 - 10.83)	23.74 (21.00 - 27.71)
	3h	12,391	3.51±0.65	2.44	7.46 (6.50 - 8.37)	18.16 (16.03 - 21.17)
	4h	12,882	2.59±0.58	0.76	4.71 (4.05 - 5.37)	11.47 (9.96 - 13.64)
Adults	1h	10,486	2.56±0.54	1.19	13.92 (12.44 - 15.34)	41.96 (37.00 - 49.10)
	2h	12,723	3.19±0.59	2.64	10.59 (9.37 - 11.76)	31.93 (28.20 - 37.21)
	3h	11,486	2.71±0.61	2.19	7.79 (6.81 - 8.75)	23.50 (20.71 - 27.37)
	4h	10,273	3.17±0.62	1.30	5.22 (4.50 - 5.93)	15.72 (13.72 - 18.50)

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

5.1.4 Western Flower Thrips (WFT) *Frankliniella occidentalis* (Pergande) (Thripidae: Thysanoptera)

The results (Tables 10-12) show that in general at all temperatures adults are more tolerant than larvae.

Table 10: Summary of Western Flower Thrips trials (2000 – 2007). Estimated response (LD_{50} & LD_{99}) of nymphs and adults exposed for 1-4 hours at 10°C to ethyl formate (+10% CO_2).

Stage tested	Time treated	Number treated	Slope ±SE	Chi-sq (df= 6) [1]	LD_{50} (g/m^3) ±95% CL	LD_{99} (g/m^3) ±95% CL
Adults	1h	26,140	3.08±0.56	0.25	6.97 (6.47 - 7.44)	17.65 (16.17 - 19.59)
	2h	28,218	3.07±0.61	0.73	5.40 (4.98 - 5.79)	13.68 (12.55 - 15.14)
	3h	24,282	4.24±0.66	1.12	4.43 (4.07 - 4.77)	11.22 (10.29 - 12.42)
	4h	24,226	5.72±0.85	0.70	3.08 (2.80 - 3.36)	7.81 (7.11 - 8.70)
Larvae	1h	25,724	2.49±0.69	1.71	6.06 (5.59 - 6.50)	11.99 (11.04 - 13.29)
	2h	26,218	3.01±0.72	2.98	4.94 (4.53 - 5.32)	9.78 (8.97 - 10.88)
	3h	26,442	4.57±0.77	0.98	3.94 (3.59 - 4.26)	7.79 (7.14 - 8.67)
	4h	28,316	4.03±0.84	0.96	2.96 (2.68 - 3.23)	5.87 (5.33 - 6.59)

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

Table 11: Summary of Western Flower Thrips trials (2000 – 2007). Estimated response (LD_{50} & LD_{99}) of nymphs and adults exposed for 1-4 hours at 15°C to ethyl formate (+10% CO_2).

Stage tested	Time treated	Number treated	Slope ±SE	Chi-sq (df= 6) [1]	LD_{50} (g/m^3) ±95% CL	LD_{99} (g/m^3) ±95% CL
Adults	1h	18,518	4.40±0.67	2.24	6.71 (6.21 - 7.19)	15.92 (14.61 - 17.66)
	2h	19,224	4.81±0.74	1.04	5.38 (4.95 - 5.79)	12.75 (11.70 - 14.13)
	3h	16,862	6.31±0.96	1.16	4.15 (3.79 - 4.49)	9.84 (8.99 - 10.95)
	4h	18,748	4.41±0.79	2.45	3.21 (2.91 - 3.50)	7.60 (6.93 - 8.47)
Larvae	1h	18,248	6.49±1.00	2.06	6.08 (5.56 - 6.56)	12.89 (11.74 - 14.49)
	2h	16,442	5.33±0.86	1.17	4.80 (4.37 - 5.21)	10.19 (9.28 - 11.44)
	3h	18,270	4.28±0.81	1.29	3.88 (3.51 - 4.23)	8.24 (7.49 - 9.25)
	4h	20,400	3.45±0.89	2.91	3.04 (2.73 - 3.34)	6.45 (5.81 - 7.31)

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

Table 12: Summary of Western Flower Thrips trials (2000 – 2007). Estimated response (LD_{50} & LD_{99}) of nymphs and adults exposed for 1-4 hours at 20°C to ethyl formate (+10% CO_2).

Stage tested	Time treated	Number treated	Slope ±SE	Chi-sq (df= 6) [1]	LD_{50} (g/m^3) ±95% CL	LD_{99} (g/m^3) ±95% CL
Adults	1h	23,448	4.53±0.69	2.67	5.99 (5.47 - 6.47)	14.08 (12.83 - 15.77)
	2h	24,226	5.73±0.91	3.14	4.83 (4.39 - 5.25)	11.36 (10.34 - 12.73)
	3h	23,442	5.08±0.84	1.66	3.76 (3.40 - 4.11)	8.84 (8.03 - 9.93)
	4h	22,220	3.51±0.84	1.51	3.06 (2.74 - 3.36)	7.18 (6.49 - 8.10)
Larvae	1h	20,228	5.16±0.93	1.03	6.22 (5.64 - 6.75)	12.61 (11.43 - 14.32)
	2h	19,482	4.45±0.78	2.52	4.99 (4.49 - 5.45)	10.12 (9.16 - 11.49)
	3h	22,618	3.84±0.80	1.85	3.92 (3.50 - 4.30)	7.94 (7.16 - 9.06)
	4h	24,900	3.11±0.90	0.42	2.89 (2.56 - 3.21)	5.86 (5.24 - 6.73)

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

5.1.5 Plague thrips *Thrips imaginis* (Bagnall) (Thripidae: Thysanoptera)

The results (Tables 13-15) show that in general at all temperatures adults are more tolerant than larvae.

Table 13: Summary of Plague thrips (*Thrips imaginis*) trials (2000 – 2007). Estimated response (LD_{50} & LD_{99}) of nymphs and adults exposed for 1-4 hours at 10°C to ethyl formate (+10% CO_2).

Stage tested	Time treated	Number treated	Slope ±SE		Chi-sq (df= 6) [1]	LD_{50} (g/m^3) ±95% CL	LD_{99} (g/m^3) ±95% CL
Adults	1h	14,240	3.52	0.64	0.64	7.74 (7.22 - 8.23)	18.02 (16.54 - 19.97)
	2h	13,290	3.90	0.71	1.46	6.42 (5.97 - 6.86)	14.96 (13.77 - 16.53)
	3h	14,287	4.59	0.78	2.20	5.42 (5.02 - 5.81)	12.63 (11.63 - 13.92)
	4h	12,725	6.46	1.02	1.12	3.95 (3.63 - 4.26)	9.19 (8.43 - 10.18)
Larvae	1h	20,753	4.43	0.65	2.36	6.56 (6.04 - 7.05)	15.63 (14.29 - 17.43)
	2h	22,118	5.64	0.89	1.20	5.20 (4.76 - 5.62)	12.38 (11.31 - 13.81)
	3h	20,471	5.50	0.88	1.32	4.04 (3.68 - 4.39)	9.64 (8.78 - 10.76)
	4h	18,395	4.88	0.85	0.53	3.07 (2.76 - 3.37)	7.32 (6.64 - 8.20)

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

Table 14: Summary of Plague thrips (*Thrips imaginis*) trials (2000 – 2007). Estimated response (LD_{50} & LD_{99}) of nymphs and adults exposed for 1-4 hours at 15°C to ethyl formate (+10% CO_2).

Stage tested	Time treated	Number treated	Slope ±SE		Chi-sq (df= 6) [1]	LD_{50} (g/m^3) ±95% CL	LD_{99} (g/m^3) ±95% CL
Adults	1h	24,336	4.27	0.67	1.31	6.75 (6.25 - 7.22)	15.25 (14.01 - 16.90)
	2h	19,825	4.84	0.75	1.57	5.42 (5.00 - 5.83)	12.26 (11.25 - 13.58)
	3h	25,246	5.82	0.92	2.52	4.41 (4.04 - 4.76)	9.97 (9.13 - 11.07)
	4h	22,118	4.69	0.81	1.56	3.45 (3.14 - 3.74)	7.79 (7.10 - 8.68)
Larvae	1h	20,840	4.70	0.78	1.15	6.72 (6.18 - 7.22)	14.98 (13.69 - 16.73)
	2h	24,186	5.21	0.85	1.07	5.25 (4.80 - 5.68)	11.71 (10.70 - 13.06)
	3h	23,082	5.32	0.88	1.11	4.30 (3.91 - 4.67)	9.59 (8.74 - 10.72)
	4h	20,550	3.90	0.81	1.33	3.37 (3.04 - 3.68)	7.52 (6.82 - 8.44)

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

Table 15: Summary of Plague thrips (*Thrips imaginis*) trials (2000 – 2007). Estimated response (LD_{50} & LD_{99}) of nymphs and adults exposed for 1-4 hours at 20°C to ethyl formate (+10% CO_2).

Stage tested	Time treated	Number treated	Slope ±SE		Chi-sq (df= 6) [1]	LD_{50} (g/m^3) ±95% CL	LD_{99} (g/m^3) ±95% CL
Adults	1h	26,740	5.08	0.94	2.54	5.84 (5.28 - 6.35)	12.35 (11.19 - 14.00)
	2h	24,935	5.27	0.83	1.31	4.58 (4.11 - 5.01)	9.69 (8.77 - 10.99)
	3h	27,290	4.21	0.82	1.34	3.47 (3.10 - 3.83)	7.35 (6.61 - 8.39)
	4h	28,400	2.84	0.93	0.80	2.87 (2.54 - 3.18)	6.07 (5.43 - 6.96)
Larvae	1h	24,316	5.18	0.86	2.39	6.46 (5.89 - 6.98)	13.26 (12.05 - 14.99)
	2h	22,990	6.38	0.97	1.78	5.06 (4.58 - 5.49)	10.37 (9.44 - 11.70)
	3h	26,446	5.21	0.74	2.99	3.80 (3.42 - 4.16)	7.80 (7.05 - 8.85)
	4h	24,218	3.96	0.82	0.63	2.81 (2.50 - 3.11)	5.77 (5.18 - 6.58)

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

5.1.6 Green peach aphid *Myzus persicae* (Sulzer) (Hemiptera: Aphididae)

The results (Tables 16-18) show that in general at all temperatures adults are more tolerant than nymphs.

Table 16: Summary of green aphid trials (1999 – 2006). Estimated response (LD_{50} & LD_{99}) of adults and nymphs exposed for 0.5-1.5 hours at 10°C to ethyl formate + 10% CO_2.

Stage tested	Time treated	Number treated	Slope ±SE	Chi-sq (df= 6) [1]	LD_{50} (g/m^3) ±95% CL	LD_{99} (g/m^3) ±95% CL
Adults	0.5h	12,200	6.47±0.65	3.98	9.89 (9.28 - 10.46)	21.55 (19.86 - 23.82)
	1h	11,980	6.88±0.77	3.12	8.58 (8.03 - 9.10)	18.71(17.28 - 20.62)
	1.5h	11,615	5.97±0.66	2.11	7.35 (6.85 - 7.81)	16.01 (14.78 - 17.65)
Nymphs	0.5h	14,293	5.84±0.67	3.80	9.45 (8.76 - 10.09)	21.33 (19.50 - 23.86)
	1h	11,448	6.38±0.78	1.70	7.26 (6.68 - 7.80)	16.39 (15.01 - 18.26)
	1.5h	11,633	5.07±0.58	5.12	6.12 (5.60 - 6.60)	13.81 (12.61 - 15.44)

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

Table 17: Summary of green aphid trials (1999 – 2006). Estimated response (LD_{50} & LD_{99}) of adults and nymphs exposed for 0.5-1.5 hours at 15°C to ethyl formate + 10% CO_2.

Stage tested	Time treated	Number treated	Slope ±SE	Chi-sq (df= 6) [1]	LD_{50} (g/m^3) ±95% CL	LD_{99} (g/m^3) ±95% CL
Adults	0.5h	11,222	4.17±0.43	2.31	7.01 (6.26 - 7.71)	23.93 (21.35 - 27.52)
	1h	14,319	4.32±0.50	2.07	5.86 (5.21 - 6.48)	20.02 (17.92 - 22.92)
	1.5h	14,339	3.72±0.51	4.12	4.69 (4.13 - 5.22)	16.03 (14.35 - 18.32)
Nymphs	0.5h	15,275	5.81±0.70	3.18	8.54 (7.82 - 9.2)	19.2 (17.46 - 21.66)
	1h	15,680	5.88±0.81	2.38	7.14 (6.5 - 7.72)	16.05 (14.62 - 18.06)
	1.5h	15,100	4.62±0.58	1.13	5.46 (4.93 - 5.94)	12.26 (11.10 - 13.89)

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

Table 18: Summary of green aphid trials (1999 – 2006). Estimated response (LD_{50} & LD_{99}) of adults and nymphs exposed for 0.5-1.5 hours at 20°C to ethyl formate + 10% CO_2.

Stage tested	Time treated	Number treated	Slope ±SE	Chi-sq (df= 6) [1]	LD_{50} (g/m^3) ±95% CL	LD_{99} (g/m^3) ±95% CL
Adults	0.5h	13,241	5.73±0.61	2.28	8.69 (8.00 - 9.34)	21.32 (19.42 - 23.92)
	1h	13,860	6.20±0.76	1.26	6.77 (6.19 - 7.32)	16.62 (15.16 - 18.58)
	1.5h	13,492	4.22±0.54	2.99	5.28 (4.79 - 5.74)	12.96 (11.81 - 14.49)
Nymphs	0.5h	13,255	6.20±0.76	1.39	7.48 (6.79 - 8.10)	15.94 (14.50 - 18.00)
	1h	14,502	6.85±1.01	3.51	6.04 (5.45 - 6.58)	12.88 (11.67 - 14.59)
	1.5h	13,880	3.36±0.62	1.06	4.61 (4.13 - 5.05)	9.82 (8.87 - 11.16)

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

4.1.7 Corn aphid *Rhopalosiphum maidis* (Homoptera: Aphididae)

The results (Tables 19-21) show that in general at all temperatures adults are more tolerant than nymphs.

Table 19: Summary of corn aphid trials (1999 – 2006). Estimated response (LD_{50} & LD_{99}) of adults and nymphs exposed for 0.5-1.5 hours at 10°C to ethyl formate + 10% CO_2.

Stage tested	Time treated	Number treated	Slope ±SE	Chi-sq (df= 6) [1]	LD_{50} (g/m^3) ±95% CL	LD_{99} (g/m^3) ±95% CL
Adults	0.5h	16,306	5.65±0.56	2.75	9.23 (8.60 - 9.83)	21.89 (20.06 - 24.34)
	1h	14,822	6.31±0.71	2.49	7.67 (7.11 - 8.20)	18.18 (16.69 - 20.17)
	1.5h	14,335	5.25±0.58	1.96	6.51 (6.01 - 6.98)	15.44 (14.17 - 17.13)
Nymphs	0.5h	14,866	5.93±0.64	1.89	8.74 (8.06 - 9.38)	20.67 (18.86 -23.15)
	1h	14,220	6.20±0.76	1.76	7.19 (6.59 - 7.75)	17.00 (15.53 - 18.99)
	1.5h	13,274	4.87±0.59	1.93	6.10 (5.57 - 6.59)	14.42 (13.16 - 16.12)

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

Table 20: Summary of corn aphid trials (1999 – 2006). Estimated response (LD_{50} & LD_{99}) of adults and nymphs exposed for 0.5-1.5 hours at 15°C to ethyl formate + 10% CO_2.

Stage tested	Time treated	Number treated	Slope ±SE	Chi-sq (df= 6) [1]	LD_{50} (g/m^3) ±95% CL	LD_{99} (g/m^3) ±95% CL
Adults	0.5h	14,406	5.15±0.56	3.00	8.74 (8.04 - 9.39)	21.74 (19.77 - 24.48)
	1h	13,272	6.19±0.77	3.19	7.10 (6.5 - 7.67)	17.67 (16.06 - 19.86)
	1.5h	13,755	4.60±0.54	4.07	5.80 (5.28 - 6.29)	14.43 (13.1 - 16.23)
Nymphs	0.5h	14,650	6.63±0.85	2.50	9.30 (8.51 - 10.03)	20.81 (18.87 - 23.60)
	1h	14,772	6.91±1.00	1.93	7.74 (7.04 - 8.37)	17.31 (15.74 - 19.54)
	1.5h	14,729	4.69±0.64	4.57	6.28 (5.68 - 6.83)	14.06 (12.77 - 15.87)

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

Table 21: Summary of corn aphid trials (1999 – 2006). Estimated response (LD_{50} & LD_{99}) of adults and nymphs exposed for 0.5-1.5 hours at 20°C to ethyl formate + 10% CO_2.

Stage tested	Time treated	Number treated	Slope ±SE	Chi-sq (df= 6) [1]	LD_{50} (g/m^3) ±95% CL	LD_{99} (g/m^3) ±95% CL
Adults	0.5h	13,410	6.07±0.66	1.96	8.68 (7.99 - 9.31)	20.15 (18.39 - 22.57)
	1h	13,224	6.48±0.83	1.83	6.84 (6.25 - 7.38)	15.87 (14.49 - 17.77)
	1.5h	13,488	4.39±0.55	1.62	5.46 (4.97 - 5.92)	12.68 (11.55 - 14.21)
Nymphs	0.5h	13,741	6.49±0.84	2.01	8.21 (7.46 - 8.88)	17.89 (16.23 - 20.28)
	1h	14,552	6.60±1.01	1.43	6.81 (6.15 - 7.40)	14.83 (13.47 - 16.78)
	1.5h	13,724	4.28±0.60	1.70	5.54 (4.98 - 6.06)	12.08 (10.92 - 13.71)

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

5.2 RESULTS OF LARGE SCALE FUMIGATION TRIALS

Large scale fumigations were done in refrigerated shipping containers from 2000 to 2006.

5.2.1 TEST INSECTS

Test insects: corn earworm, *Helicoverpa armigera*, Australian bollworm, *H. punctigera*, two spotted spider mite *Tetranychus urticae*, plague thrips *Thrips imaginis*, western flower thrips *Frankliniella occidentalis*, green peach aphid *Myzus persicae* and Corn aphid (CA) *Rhopalosiphum maidis*; were inserted into the front, middle and rear of the container using vials placed inside the sweet corn harvesting tubs. In the majority of trials more than 3,000 insects of all stages were tested in large scale trials. These insects were examined after an appropriate period (depending on life stage tested) to assess for survivors. In the trials reported below no live insects were found after treatment.

5.2 REFRIGERATED CONTAINER FUMIGATIONS (2000 – 2006)

A series of large scale trials were conducted in South Perth over a number of years as test insects and produce became available. Trials were conducted in a 20 ft / 6m (27.6 m^3) refrigerated shipping container (AMSU 3000185) under export conditions. Fruit was loaded in tubs >50% of volume in separate trials. Ethyl formate was supplied by BOC Special Gases in 'G' size cylinders containing Vapormate® (Gas Code 279): a mixture consisting of 16.7% Ethyl Formate and 83.3% CO_2.

The approach was to maintain required dosage of ethyl formate and carbon dioxide in the air space for trials where produce was held in 30 litre plastic bins and fumigated at temperatures in the harvest temperature range >10, 15 and 20°C. The objective was to discover the lower limit of dosage and exposure period at each temperature.

To account for absorption and adsorption, applied doses ranged from 46 to 72g/m^3 ethyl formate and 14-22 % carbon dioxide for 4 hour exposure periods.

The results of these trials are summarised in Tables 22-27 and Figures 1-6.

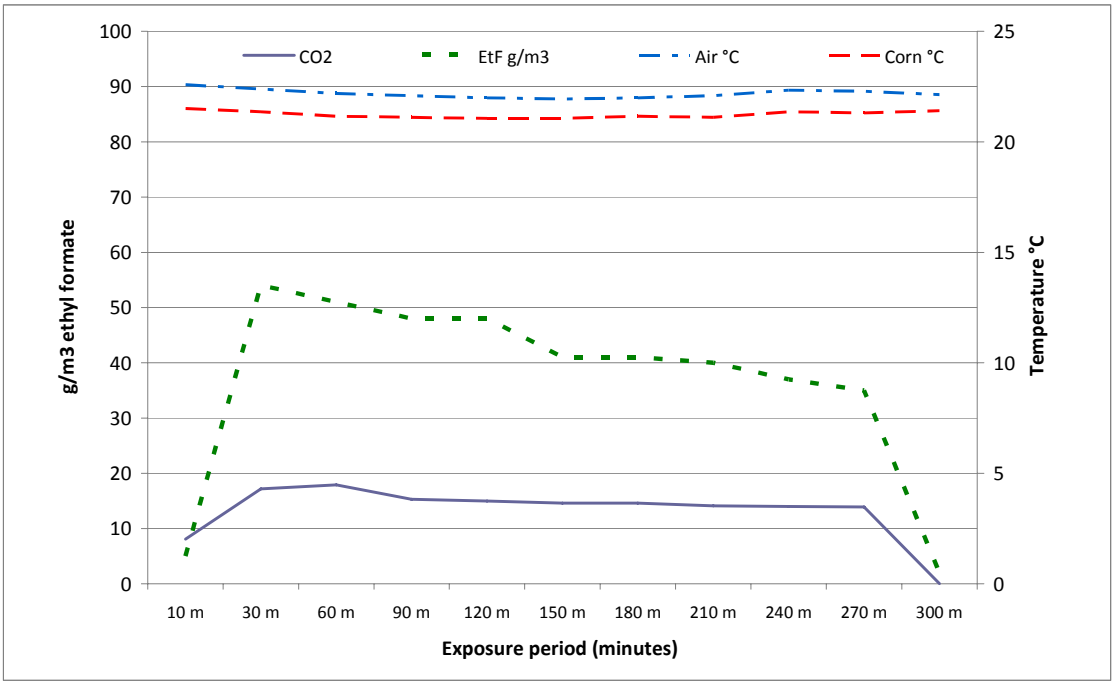

Figure 1: Large scale fumigation summary data for sweet corn for 4 h at >20°C: (LHS) ethyl formate (g/m^3) and $\%CO_2$; (RHS) air and fruit temperature. Equilibration 30 min; venting 30 min.

The applied ethyl formate dose was $46 g/m^3$ and at the end of the trial 65.4% remained; cumulative gas concentration over 4h was 164.0 g.h/ m^3. CO_2 was 13.2±2.6%. Temperatures were maintained evenly: air 22.1±0.2°C; produce 21.2±0.1 °C. There were no survivors in all stages of the test insects. Estimated mortality after correction for control mortality was >99%.

Table 22: Large scale fumigation (2000 – 2004) in 20 ft refrigerated containers. Insect data from >3 replicated tests. 7.6 kg Vapormate® sweet corn for 4 h; >20°C (*estimate based on > 150 vials in >15 replicate tests; S = survivors)

Species Tested	Stages tested											
	Eggs (early + late)			Early larvae / nymphs			Late larvae / nymphs			Adults		
	Number of insects*	S	Estimated mortality 95% CL	Number of insects*	S	Estimated mortality 95% CL	Number of insects*	S	Estimated mortality 95% CL	Number of insects*	S	Estimated mortality 95% CL
H. armigera	34,298	0	99.91	26,840	0	99.89	18,200	0	99.84			
H. punctigera	42,040	0	99.93	24,400	0	99.88	15,280	0	99.80			
T. urticae	48,240	0	99.94	32,582	0	99.91				29,800	0	99.90
T. imaginis							85,720	0	99.97	32,400	0	99.91
F. occidentalis							75,220	0	99.96	44,000	0	99.93
M. persicae							90,116	0	99.97	68,200	0	99.96
R. maidis							41,480	0	99.93	32,008	0	99.91

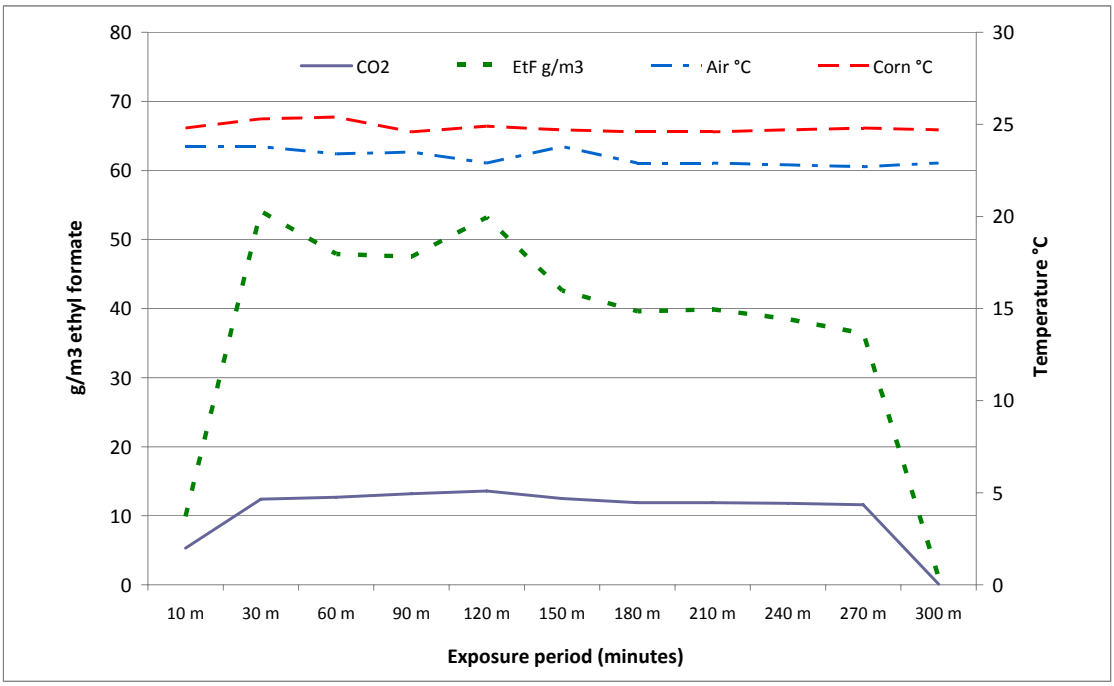

Figure 2: Large scale fumigation summary data for sweet corn for 4 h at >20°C: (LHS) ethyl formate (g/m^3) and $\%CO_2$; (RHS) air and fruit temperature. Equilibration 30 min; venting 30 min.

The applied ethyl formate dose was 42g/m3 and at the end of the trial 88% remained; cumulative gas concentration over 4h was 177.2 g.h/ m3. CO2 was 11.7±2.3%. Temperatures were maintained evenly: air 23.3±0.5°C; produce 24.8±0.3°C. There were no survivors in all stages of the test insects. Estimated mortality after correction for control mortality was >99%.

Table 23: Large scale fumigation (2000– 2004) in 20 ft refrigerated containers. Insect data from >3 replicated tests. 7 kg Vapormate® sweet corn for 4 h; >20°C (*estimate based on > 120 vials in >12 replicate tests; S = survivors)

Species Tested	Stages tested											
	Eggs (early + late)			Early larvae / nymphs			Late larvae / nymphs			Adults		
	Number of insects*	S	Estimated mortality 95% CL	Number of insects*	S	Estimated mortality 95% CL	Number of insects*	S	Estimated mortality 95% CL	Number of insects*	S	Estimated mortality 95% CL
H. armigera	42,200	0	99.94	23,560	0	99.87	18,600	0	99.83			
H. punctigera	44,680	0	99.94	34,500	0	99.91	20,420	0	99.84			
T. urticae	31,480	0	99.91	28,440	0	99.89				27,900	0	99.89
T. imaginis							46,370	0	99.94	35,500	0	99.91
F. occidentalis							39,300	0	99.92	36,600	0	99.91
M. persicae							34,200	0	99.91	28,400	0	99.89
R. maidis							18,000	0	99.83	26,000	0	99.88

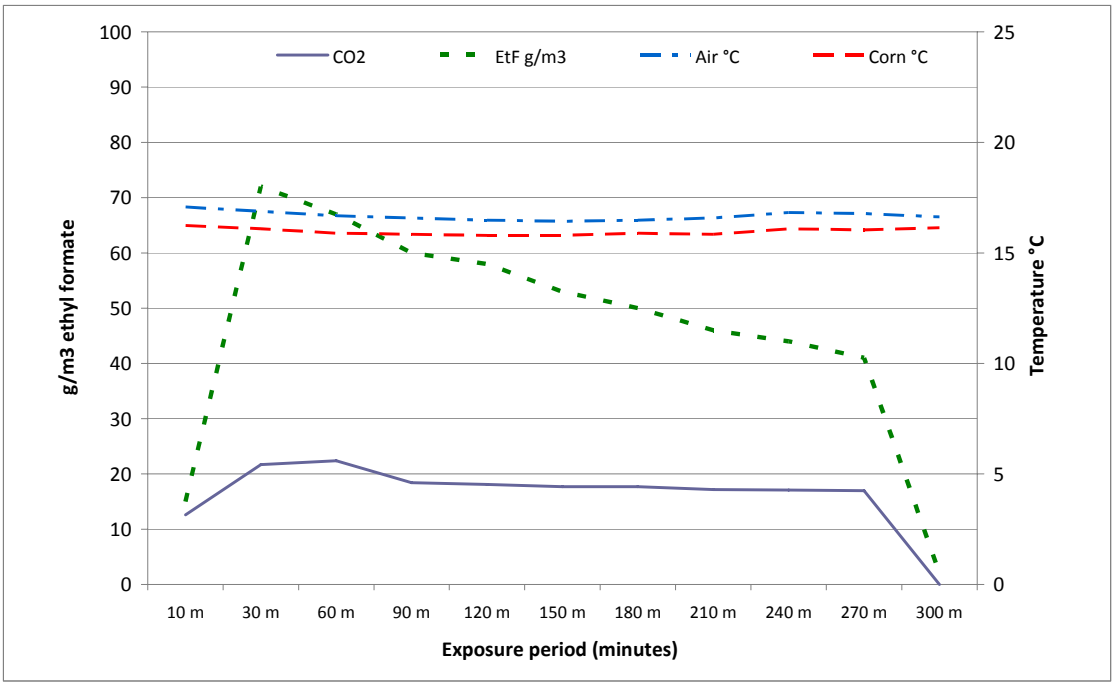

Figure 3: Large scale fumigation summary data for sweet corn for 4 h at >15°C: (LHS) ethyl formate (g/m^3) and $\%CO_2$; (RHS) air and fruit temperature. Equilibration 30 min; venting 30 min.

The applied ethyl formate dose was 60g/m3 and at the end of the trial 68% remained; cumulative gas concentration over 4h was 217.3 g.h/ m3. CO2 was 18.0±2.7%. Temperatures were maintained evenly: air 16.7±0.2°C; produce 16.0±0.2°C. There were no survivors in all stages of the test insects. Estimated mortality after correction for control mortality was >99%.

Table 24: Large scale fumigation (2000– 2004) in 20 ft refrigerated containers. Insect data from >3 replicated tests. 10 kg Vapormate® sweet corn for 4 h; >15°C (*estimate based on > 150 vials in >15 replicate tests; S = survivors)

Species Tested	Stages tested											
	Eggs (early + late)			Early larvae / nymphs			Late larvae / nymphs			Adults		
	Number of insects*	S	Estimated mortality 95% CL	Number of insects*	S	Estimated mortality 95% CL	Number of insects*	S	Estimated mortality 95% CL	Number of insects*	S	Estimated mortality 95% CL
H. armigera	46,400	0	99.94	32,480	0	99.90	25,400	0	99.86			
H. punctigera	48,540	0	99.94	35,000	0	99.91	24,280	0	99.86			
T. urticae	72,300	0	99.96	47,600	0	99.94				46,600	0	99.94
T. imaginis							72,000	0	99.96	44,200	0	99.94
F. occidentalis							78,000	0	99.96	62,000	0	99.95
M. persicae							87,000	0	99.97	75,400	0	99.96
R. maidis							32,800	0	99.90	36,420	0	99.92

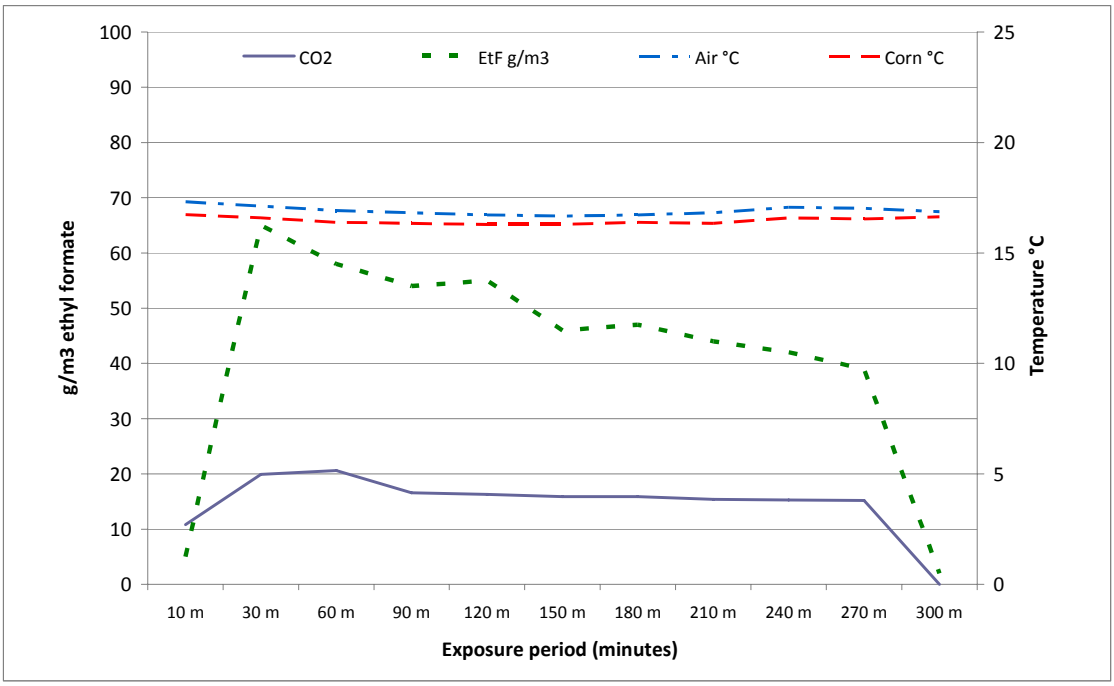

Figure 4: Large scale fumigation summary data for sweet corn for 4 h at >15°C: (LHS) ethyl formate (g/m^3) and $\%CO_2$; (RHS) air and fruit temperature. Equilibration 30 min; venting 30 min.

The applied ethyl formate dose was 54g/m3 and at the end of the trial 65.8% remained; cumulative gas concentration over 4h was 186.0 g.h/ m3. CO2 was 14.7±2.7%. Temperatures were maintained evenly: air 16.9±0.2°C; produce 16.4±0.1 °C. There were no survivors in all stages of the test insects. Estimated mortality after correction for control mortality was >99%.

Table 25: Large scale fumigation (2000– 2004) in 20 ft refrigerated containers. Insect data from >3 replicated tests. 9 kg Vapormate® sweet corn for 4 h; >15°C (*estimate based on > 120 vials in >12 replicate tests; S = survivors)

Species Tested	Stages tested											
	Eggs (early + late)			Early larvae / nymphs			Late larvae / nymphs			Adults		
	Number of insects*	S	Estimated mortality 95% CL	Number of insects*	S	Estimated mortality 95% CL	Number of insects*	S	Estimated mortality 95% CL	Number of insects*	S	Estimated mortality 95% CL
H. armigera	27,320	0	99.89	21,200	0	99.86	14,420	0	99.79			
H. punctigera	40,600	0	99.93	17,840	0	99.83	12,880	0	99.77			
T. urticae	38,200	0	99.92	28,900	0	99.90				18,400	0	99.84
T. imaginis							58,000	0	99.95	24,280	0	99.86
F. occidentalis							62,000	0	99.95	36,540	0	99.92
M. persicae							81,000	0	99.96	52,360	0	99.94
R. maidis							24,000	0	99.86	28,800	0	99.90

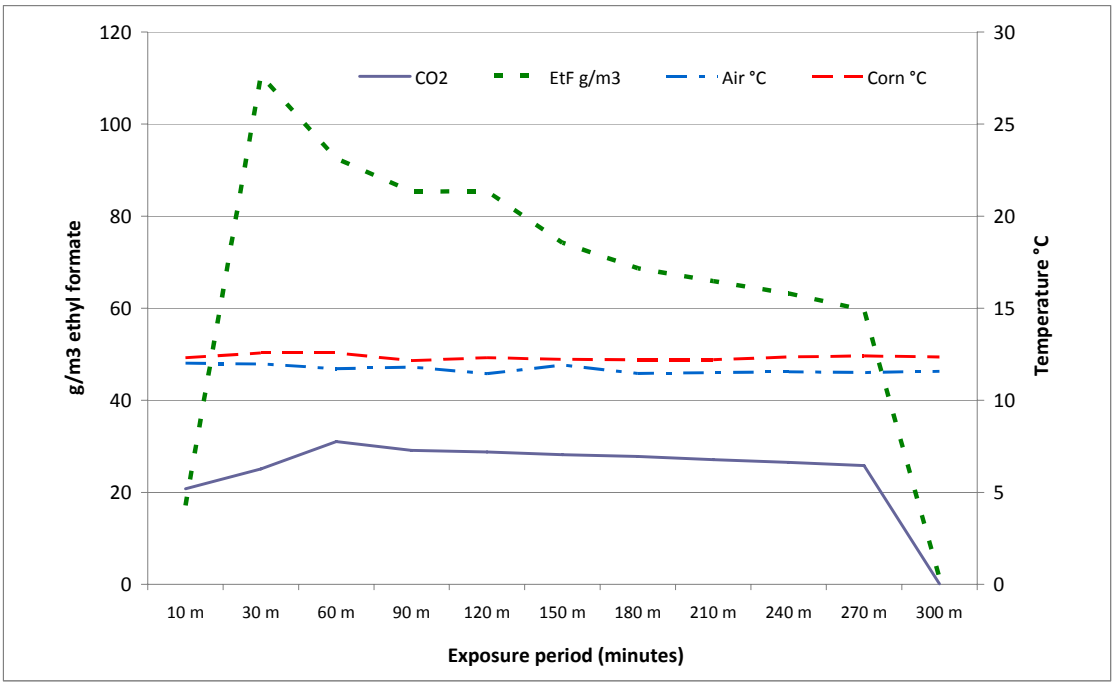

Figure 5: Large scale fumigation summary data for sweet corn for 4 h at >10°C: (LHS) ethyl formate (g/m^3) and $\%CO_2$; (RHS) air and fruit temperature. Equilibration 30 min; venting 30 min.

The applied ethyl formate dose was 75g/m3 and at the end of the trial 81.3% remained; cumulative gas concentration over 4h was 310.2 g.h/ m3. CO2 was 27.0±2.8%. Temperatures were maintained evenly: air 11.7±0.2°C; produce 12.3±0.1°C. There were no survivors in all stages of the test insects. Estimated mortality after correction for control mortality was >99%.

Table 26: Large scale fumigation (2000– 2004) in 20 ft refrigerated containers. Insect data from >3 replicated tests. 12.5 kg Vapormate® sweet corn for 4 h; >10°C (*estimate based on > 120 vials in >12 replicate tests; S = survivors)

Species Tested	Stages tested											
	Eggs (early + late)			Early larvae / nymphs			Late larvae / nymphs			Adults		
	Number of insects*	S	Estimated mortality 95% CL	Number of insects*	S	Estimated mortality 95% CL	Number of insects*	S	Estimated mortality 95% CL	Number of insects*	S	Estimated mortality 95% CL
H. armigera	45,700	0	99.94	24,000	0	99.87	19,800	0	99.85			
H. punctigera	43,800	0	99.94	32,200	0	99.91	22,200	0	99.86			
T. urticae	32,200	0	99.91	30,000	0	99.90				26,100	0	99.88
T. imaginis							48,000	0	99.95	36,600	0	99.91
F. occidentalis							46,700	0	99.94	35,600	0	99.91
M. persicae							34,600	0	99.91	28,800	0	99.89
R. maidis							18,200	0	99.83	26,400	0	99.88

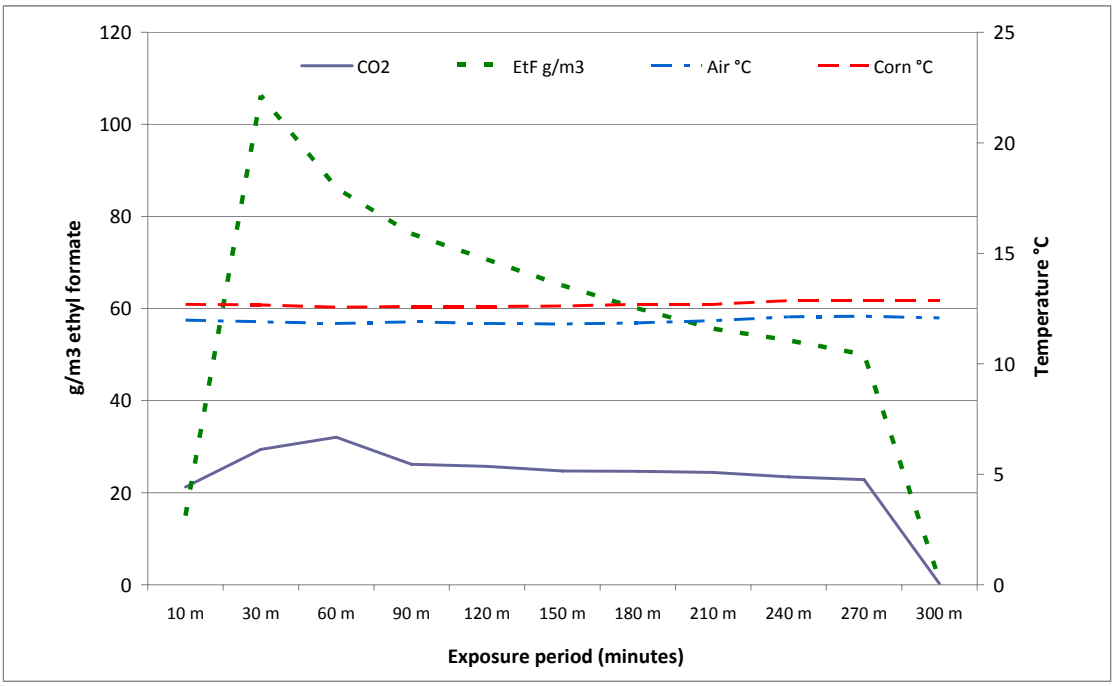

Figure 6: Large scale fumigation summary data for sweet corn for 4 h at >10°C: (LHS) ethyl formate (g/m^3) and $\%CO_2$; (RHS) air and fruit temperature. Equilibration 30 min; venting 30 min.

The applied ethyl formate dose was 70g/m3 and at the end of the trial 71% remained; cumulative gas concentration over 4h was 272.5 g.h/ m3. CO2 was 25.5±3.2%. Temperatures were maintained evenly: air 11.9±0.1°C; produce 12.7±0.1°C. There were no survivors in all stages of the test insects. Estimated mortality after correction for control mortality was >99%.

Table 27: Large scale fumigation (2000– 2004) in 20 ft refrigerated containers. Insect data from >3 replicated tests. 11.5 kg Vapormate® sweet corn for 4 h; >10°C (*estimate based on > 180 vials in >18 replicate tests; S = survivors)

Species Tested	Stages tested											
	Eggs (early + late)			Early larvae / nymphs			Late larvae / nymphs			Adults		
	Number of insects*	S	Estimated mortality 95% CL	Number of insects*	S	Estimated mortality 95% CL	Number of insects*	S	Estimated mortality 95% CL	Number of insects*	S	Estimated mortality 95% CL
H. armigera	70,400	0	99.96	44,220	0	99.94	32,320	0	99.91			
H. punctigera	62,200	0	99.95	74,200	0	99.96	36,420	0	99.92			
T. urticae	96,480	0	99.97	60,960	0	99.96				54,000	0	99.95
T. imaginis							120,000	0	99.98	63,900	0	99.95
F. occidentalis							98,000	0	99.97	72,000	0	99.96
M. persicae							104,000	0	99.97	96,000	0	99.97
R. maidis							48,900	0	99.94	48,000	0	99.94

Table 28: Large scale fumigation (2000– 2004) in 20 ft refrigerated containers. Combined mortality data from above 5 trials (S = survivors). Quarantine level of mortality >Probit 9 is achieved for all insects as shown below.

Species Tested	Stages tested							
	Eggs (early + late)		Early larvae / nymphs		Late larvae / nymphs		Adults	
	Number of insects	S	Number of insects	S	Number of insects	S	Number of insects	S
H. armigera	266,318	0	172,300	0	128,740	0		
H. punctigera	281,860	0	218,140	0	131,480	0		
T. urticae	318,900	0	228,482	0			202,800	0
T. imaginis					430,090	0	236,880	0
F. occidentalis					399,220	0	286,740	0
M. persicae					430,916	0	349,160	0
R. maidis					183,380	0	197,628	0

From the data presented in this report no survivors were found in large scale container trials. The treatments were 100% effective. To assess what this means for confidence in the use of Vapormate® as a quarantine treatment an analysis of the data was made using the equations of Couey and Chew (1986). The data given in Tables 22-27 above confirms with 95% confidence that quarantine control >99% was achieved. In the combined data shown above, the estimate improves to 99.99% control with 95% confidence. When the data from all 6 trials are combined (Table 28), > Probit 9 level of control is achieved in aggregate with >95% confidence for every insect species. In summary therefore the results show that effective doses are:

(1) >20°C applied dose 42g/m^3 achieves 60% ethyl formate (25g/m^3) + >10%CO_2 x 4h = 100 g.h/m^3

(2) >15°C applied dose 54g/m^3 achieves 60% ethyl formate (32g/m^3) + >10%CO_2 x 4h = 128 g.h/m^3

(3) >10°C applied dose 70g/m^3 achieves 60% ethyl formate (42g/m^3) + >10%CO_2 x 4h = 168 g.h/m^3

The results of this comprehensive data package are summarised as quarantine treatments in Appendix1. The dosage rates in Appendix 1 are greater than the final effective treatment doses at each of the specified temperatures. Therefore the dosage recommended in Appendix 1 provides a very high level of confidence in the success of the treatments.

Importance of carbon dioxide in effectiveness of ethyl formate fumigations

Carbon dioxide has a considerable potentiating effect on ethyl formate enabling successful fumigations to be achieved at relatively low doses. No successful ethyl formate fumigation can be done without at least 8-10% CO2 being present in the fumigation space for the duration of the treatment. Vapormate® is made up of a mixture consisting of 16.7% ethyl formate and 83.3% CO2. The volatilised ratio of the 2 gases in air is not the same as in the liquid formulation in the cylinder. 1 kg of pure liquid CO2 gives 0.534 m3 on volatilisation to gas. Theoretically, 1kg of liquid Vapormate® will give 0.44 m3 of CO2 which when diluted by air in the fumigation chamber will give the expected % CO2 in the treatment relative to the volume of the chamber. In all the fumigations reported above the carbon dioxide level was maintained

above 10%. Because CO2 requires a higher level of gas-tightness than ethyl formate, the mixture of the 2 gases can only be successful if the container meets the sealing standard described above.

Ethyl formate residues in sweet corn

Samples of sweet corn from fumigation trials were submitted to NATA accredited Chemistry Centre, Department of Industry & Resources, 125 Hay Street, East Perth, WA 6004 for residue analysis. In an attempt to determine at what level residues would be found, treatments up to 100/gm3 for 6 hours exposure at 20°C were conducted to give maximum possibility for detecting residues. Fumigated samples (3 reps x 1kg) were taken after 10 minute venting and frozen before being taken for residue analysis. No residues of ethyl formate were detected but analysis of formate levels gave some comparisons as shown below. This data is referenced by the Chemistry Centre test report (05/1567 - 04E0452; 3.1.1 dated 15/10/2004).

Table: Large scale fumigation (2004) in 20 ft refrigerated containers. Residue analysis

Dose treated	Exposure period	Residue level Formate mg/kg	Difference from control mg/kg (ppm)	Date of treatment
Control	0	<5		20/09/2004
100g/m^3	6 hours	<5	0	20/09/2004

No residues were found demonstrating that ethyl formate rapidly volatilises from treated sweet corn. Since recommended Vapormate® treatments will not exceed 60 g/m^3 ethyl formate for 4 hour exposure periods, the expected residue levels in treated produce are effectively zero.

Quality and taste tests
The quality of sweet corn after treatment was evaluated. Selected treatments at 82 g/m^3 for 4 and 6 hours exposure periods at 20°C were applied to fresh sweet corn, which was then submitted for examination of qualitative effects by Dr S C Tan (Principal Post-harvest Scientist, South Perth) and Mr Jim Trandos (of Trandos Farms, Wanneroo). Both treatments had no phytotoxic effect on the sweet corn even when stored for one week at 8°C. There was no adverse effect on the green sheath leaves and no adverse effect on the taste of sweet corn.

6. DISCUSSION

Fumigation using ethyl formate + CO2 is a very effective method of disinfesting insects in fresh sweet corn destined for export overseas. The treatment fits in well with the cool chain process and does not have any adverse effect on the quality of sweet corn. No residues of ethyl formate were found in treated produce. Ethyl formate is a safe product and occurs naturally in Brassica vegetables and in food grains. It is also safe for use and because it is a natural product it has no harmful effect on the environment.

When the commercial formulation of a combination of ethyl formate + CO2, Vapormate® was first registered the data available did not cover the 7 species of insects tested in this report and the very high rates registered are not justified for these pests. This report covers temperatures in the range of 10 – 20°C which are not available in the registration. Thus, new data is available to the vegetable industry for market access to use the fumigant in a more cost effective way that fits in with the cool chain handling of produce from harvest to export.

7. TECHNOLOGY TRANSFER

Demonstration of the fumigation methods in the refrigerated shipping container in South Perth were made to three exporters in WA. A demonstration trial was held on site at one property in Wanneroo. Liaison was maintained and information was provided on fumigation methods to exporters in Queensland and Victoria over several years in preparation for export shipments. However the strong A$ above 60 cents US made exports unprofitable and there were no industry funds to pay for export shipments. Articles were presented in print media and at the Australian Postharvest Conference in Brisbane.

The results of the 6 years of research have enabled the project to provide the data to support a quarantine treatment schedule for use by AQIS (Appendix 1). This schedule has a lower dose rate than the label rate for Vapormate® and considerably reduces treatment costs while being completely effective.

8. RECOMMENDATIONS

Further trials using partial pressure need to be conducted to aid ingress of ethyl formate & CO2 into the produce and shorten treatment time to 2 hours. Alternatively, more densely packed produce can be treated within 4 hours. These are practical commercial issues, which will be solved according to the practice that industry wishes to develop. It will be necessary to test at lower temperatures possibly with other fumigant gases as well, that are more active at lower temperatures, because exporters prefer the fumigation temperature to be as low as possible to preserve sweet corn shelf life during and after export.

There is good potential that ethyl formate and/or propylene oxide can be combined with carbon dioxide and applied using partial pressure fumigation techniques to be effective against fruit flies in vegetables as a replacement for dimethoate and fenthion dips.

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Appendix 1: Recommended schedule for Quarantine Treatments using ethyl formate + >10%CO2 commercially as Vapormate®

On the basis of the large body of laboratory and large scale trial data the treatment schedule recommended for disinfestation of quarantine pests is specified below.

A. Tolerant species:

1. Cotton bollworm or corn earworm ***Helicoverpa*** *armigera* (Hübner)
2. Native budworm or Australian bollworm ***Helicoverpa*** *punctigera* (Wallengren)

>21 °C cooling to 20 °C
Ethyl formate (30g /m^3) or Vapormate® (180g /m^3) for 4 hours

20 °C cooling to >15 °C
Ethyl formate (40g /m^3) or Vapormate® (240g /m^3) for 4 hour

15 °C cooling to >10 °C
Ethyl formate (44g /m^3) or Vapormate® (264g /m^3) for 4 hour

B. Susceptible species:

1. Two-spotted spider mite *Tetranychus urticae* (Koch)
2. Western flower thrips *Frankliniella occidentalis* (Pergande)
3. Plague thrips *Thrips imaginis* (Bagnall)
4. Green peach aphid *Myzus persicae* (Sulzer)
5. Corn aphid *Rhopalosiphum maidis* (Fitch)

>21 °C cooling to 20 °C
Ethyl formate (20g /m^3) or Vapormate® (120g /m^3) for 2 hours

20 °C cooling to >15 °C
Ethyl formate (24g /m^3) or Vapormate® (144g /m^3) for 2 hours

15 °C cooling to >10 °C
Ethyl formate (28g /m^3) or Vapormate® (168g /m^3) for 2 hours