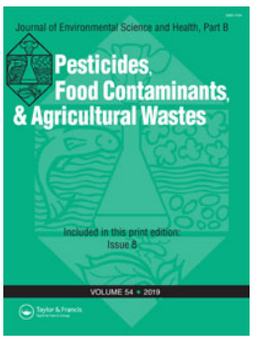

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Commercial trials evaluating the novel use of ethyl formate for in-transit fumigation of shipping containers

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ABSTRACT

The use of shipping containers for cargo transportation has the potential to transport insect pests from infested to non-infested areas. Therefore, fumigation is required as an appropriate biosecurity measure to exterminate these pests. In-transit fumigation trials were conducted in two 20 ft shipping containers during a two-day journey in both September and December 2017. Ethyl formate (90 g m^{-3}) was purged with nitrogen (EF + N_2) into the containers. Ethyl formate concentration inside containers and the surrounding environment were monitored at timed intervals throughout the journey. Fumigation achieved sufficient concentration × time (*Ct*) products in the containers during the journey, which can exterminate all stages of most common insect pests. The *Ct* products in-transit were greater than those in a shipping container being fumigated in a stationary position at a dose rate of 90 g m^{-3} for 24 hours exposure. Levels of EF in the environment between 1-15 m downwind from the containers and driver's cabin were less than 0.5 ppm at each of the timed intervals, 200 times below 100 ppm of EF Threshold Limit Value (TLV). Our study indicates that in-transit EF + N_2 technology has the potential to deliver cost savings in the fumigation process through reduction of the Labor cost, elimination of the time a container and cargo must remain stationary in a fumigation yard and a significant decrease in total supply chain time (between container packing and receival).

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In-transit fumigation; fumigant; ethyl formate; insect pest control; worker safety; environmental safety

Introduction

Globalization of agricultural trade has led to a worldwide increase in the use of shipping containers. More than 20 million international shipping containers are said to be involved in the transport of cargoes globally.[1] The containers may carry residues of previous cargoes and associated live insect infestations.

Infestation of containers and its cargo with exotic pest species has the potential to impact on industry and biosecurity management of cargo.[2] A shipping container, also referred to as a freight container, is "an article of transport equipment intended to facilitate the carriage of goods by one or more modes of transport, without intermediate loading".[3] Although shipping containers are convenient for shipping cargo, they are also high-risk carriers of insect pests. Insects may be present anywhere in the cargo, on packaging materials, pallets and in other parts of the container itself. Effective biosecurity measures are essential to decrease the risk of transporting insect pests in shipping containers from country to country and from infested to non-infested areas. Fumigation has been the primary method for treating commodities infested with insect pests since the advent of methyl bromide in the early 1940s and ethylene dibromide in the early 1950s. As these fumigants were inexpensive and easily used, there was a reduction in both the use of and research into physical or other nontoxic disinfestation methods until the 1980s.[4]

Methyl bromide became the fumigant of choice, as it is fast acting and eradicates insects in a short time period (less than 48 hours). Methyl bromide was, however, banned in 2006 under the Montreal Protocol in most developed countries, except for exceptional biosecurity purposes, because it depletes atmospheric ozone.[5] Numerous alternative insect treatments have been tested as replacements for methyl bromide, from physical control methods (e.g. heat, cold and sanitation), to fumigant replacements (e.g. phosphine, sulphuryl fluoride and carbonyl sulphide). In 2002, naturally occurring plant volatiles that could be used as potential fumigants for post-harvest treatment of insect pests, such as ethyl formate (EF) were considered a priority for investigation.[6]

Ethyl formate has a long history as a fumigant for stored food products and particularly for dried fruit. It has been used successfully to eliminate a broad range of insect pests.[7–10] EF occurs naturally in soil, water, vegetation and a range of raw and processed foods (e.g. vegetables, fruit, grain, beer, wine), and animal products (e.g. milk and cheese).[11,12] In recent years, EF has been re-evaluated as an

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alternative fumigant for grain stored in unsealed farm bins[13,14] and is currently registered as a fumigant for dried fruit in Australia and as a postharvest fumigant for some pests in fruits and vegetables.[15–18] EF is also a safe food additive.[19]

Using EF as a fumigant has the advantage of a very short fumigation period at the approved concentration (six hours), low toxicity to mammals and environments, and rapid breakdown with no residues.[20,21] The Threshold Limit Value (TLV) for EF is 100 ppm, whereas the TLV for methyl bromide and phosphine is 3 ppm and 0.3 ppm, respectively. As a result, methyl bromide and phosphine are 30 and 330 times more toxic than EF. Unlike most other fumigants, EF kills insects rapidly and breaks down to two naturally occurring products, formic acid and ethanol.[22]

The use of an effective yet safe fumigant to exterminate potential pest species within containers benefit both biosecurity and worker safety. Fumigation must be conducted efficiently, without risk to the operators and persons involved in unloading of the goods.[2] The process of container fumigation is typically completed pre-shipment, at the border on arrival and occasionally in-transit.

If disinfested properly before shipment, containerized transport eliminates the risk of cross-infestation of commodities during transport, facilitates in-transit fumigation and ensures delivery of the cargoes to the consignee in an insect-free condition.[2] Assessment of in-transit fumigation of containers carrying agricultural products and commercial cargo using fumigants such as methyl bromide, CO_2 [23,24] and phosphine [25,26] has been conducted in only a limited number of studies due to the toxicity of these two pesticides. There have been no studies, however, that have investigated the use of EF plus nitrogen for in-transit fumigation of shipping containers.

The aims of this study were (i) to determine whether shipping containers undergoing in-transit fumigation with EF plus nitrogen are able to maintain sufficient concentrations over a 48-hour exposure period and (ii) to determine the risk to the public/workers, which include transport and logistic personnel from exposure to EF whilst the containers are in-transit and at the journey's end when venting and unloading the containers.

Materials and methods

Shipping containers

Two in-transit fumigation trials with ethyl formate (EF) and nitrogen (N_2) mixture ($EF + N_2$) were conducted in shipping containers in-transit during September 2017 and December 2017 during a two-day journey by truck from Perth (31°55′292″S, 115°59′44″E) to Dampier (20°37′23″S, 116°45′58″E). Each trial used two replicate rated 20 ft general-purpose (GP) steel containers (32 m^3 capacity) with wooden floors and hinged doors with rubber-lined seals, which increase air tightness.[27] During both trials, containers contained general cargo, which included wood, plastic, steel and cardboard. The condition of the containers was inspected prior to fumigation. The rubber seals and wooden floors were in good condition.

Measurement of temperature

Temperature and relative humidity (r.h.) were automatically recorded during the entire fumigation period using two HOBO® data logger units (Model number H08-004-02, Onset Computer Corporation, MA 02532, USA, www.onset-comp.com) in each of the containers (Fig. 1). Recorded data were analysed with the software BoxCar® Version 3.6+ for Windows (Onset Computer Corporation, MA 02532, USA, www.onsetcomp.com). The HOBO®s were calibrated in the laboratory prior to commencing the trials.

Fumigant and fumigation

Gas-tightness of containers is important for a successful fumigation,[2] with leakage the main cause of fumigation failure.[24,27] As such, prior to fumigation, containers were inspected for any holes and doors that did not seal properly. Rubber seals around doors and wooden floors were assessed as in good condition. Vents in the top corner castings were taped off. A stainless-steel manifold that maintained the integrity of door seals was placed between the container doors to inject the EF plus nitrogen mix.

The dose rate of 90 g m^{-3} of EF was achieved by vaporizing three litres of EF with heated high purity nitrogen (99.5%) and applied to the 20 ft container via a manifold inserted between the doors using a purpose build vaporizer. The fumigant used was an analytical grade (99.9%) liquid formulation of EF supplied by Aldrich, Sydney, Australia. Heated high purity nitrogen (99.5%) was generated by a Membrane Nitrogen Separator and applied to act as a carrier gas for the vaporized EF and generated a non-flammable $EF + N_2$ formulation.

The stationary container was fumigated in a fumigation yard for 24 hours exposure and the in-transit containers were fumigated in the fumigation yard and loaded onto a truck directly after injection of $EF + N_2$. These containers were mobile an hour after injection took place.

Gas sampling from container and environment

During both September and December trials, EF samples were taken in various locations in the containers (Fig. 1). Six 4.76 mm (i.d.) monitoring tubes were installed in each of the two 20 ft GP containers in various defined locations within the container space, and also one monitoring tube inserted into the cargo present (Fig. 1). Sampling tubes were run to the front of the containers to enable gas samples to be drawn at scheduled intervals.

To compare the stationary and in-transit container fumigation, EF concentrations in the stationary container were monitored over a period of 24 hours. The containers undergoing in-transit fumigation were monitored for 48 hours at timed intervals. For in-transit fumigation, ethyl formate gas concentration levels were determined *in situ* at set periods inside the container (0, 1, 3, 4, 6, 10 and 48 hours post-fumigation). Samples for 0 hour were taken directly after fumigation at the Perth fumigation yard. Samples for 1 hour

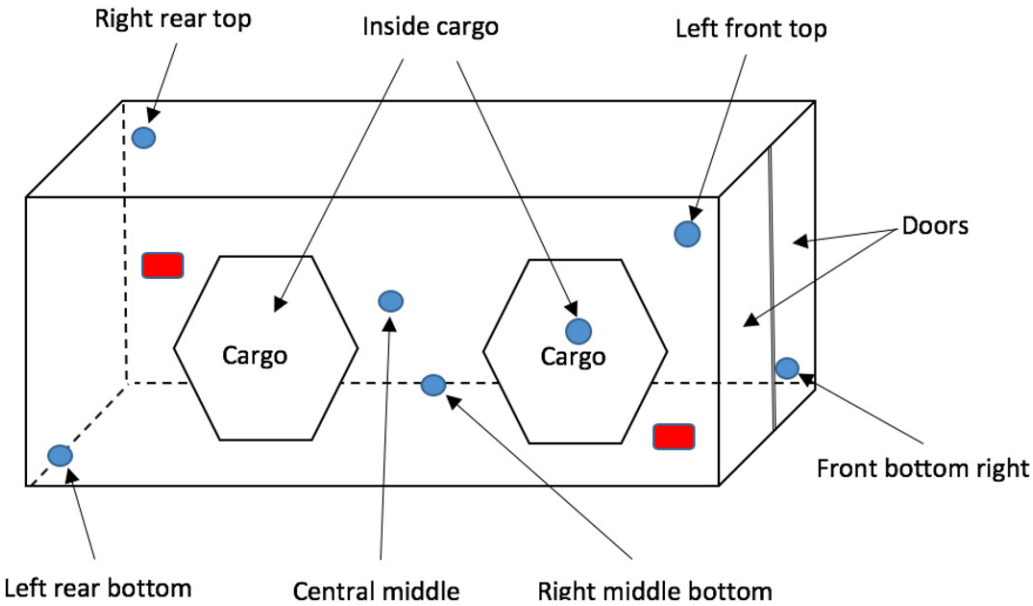

Figure 1. Placement of fumigant monitoring ports (-●-), and temperature and relative humidity monitors (-▬-) in containers.

were taken after loading of the containers on the truck at the fumigation yard. Samples for 3, 4, 6 and 10 hours were taken at the specified times at different truck rest stops. The final samples (48 hours) were taken just before venting at the end destination (Dampier).

Due to the time taken to collect gas concentration measurements from the containers and environment, and to load containers onto their trailer, the one-hour gas concentration samples were taken at the fumigation site prior to transit. Once at the destination, containers were unloaded into a secure area and vented. Ventilation screens and temperature loggers were retrieved from inside the containers once EF gas concentrations fell below the TLV of 100 ppm in the monitoring tubes.

To monitor environmental levels of EF, a pump attached G460 (GFG Instrumentation, USA) gas detection device was used to draw a gas sample from the monitored location to the sensor at distances downwind 1, 3, 5 and 15 m from the container, as well as inside the cab of the truck and 1 m outside the cab at the same time periods as for inside the container (0, 1, 3, 4, 6, 10 and 48 hours post fumigation). The environmental air samples were also simultaneously collected at every sampling location with a 1-liter gas syringe (SGE Jumbo syringe, Australia) and stored in 1-L Tedlar gas sample bags (Air Met Scientific Australia). A vacuum pump was connected between the monitoring tubes and Tedlar® bags to draw the gas samples. All gas sample bags were labelled and stored in an enclosed trailer prior to analysis the same day with Potable gas chromatography (GC) and later analysis with HP 5890 gas chromatograph in the laboratory.

Monitoring ethyl formate in container and environment

For monitoring concentration of EF in the containers, a pump attached the G460 EF gas detection device was used to draw a gas sample through air sample tubing from the monitored location to the sensor. The G460 gas detection device fitted with an Infrared (NDIR) sensor specifically calibrated for EF detection (range of 0–5%) were used to take measurements in both the containers and environment.

A DPS portable GC was used to compare the accuracy of the G469 EF monitor for monitoring EF concentrations as well as the interference of other chemicals such as ethanol and formic acid. The DPS portable GC (Companion 600; DPS Instruments, Rancho Cucamonga, CA) installed with a flame ionization detector (FID) and fitted with Zebron capillary column 30 m × 0.53 mm (i.d.) 0.5 μm model ZB-WAX (B13844, Part no. 7HK-G007-17, Phenomenex, Inc.) was operated at an oven temperature of 95 °C. Nitrogen (N_2) was used as a carrier gas at a flow rate of 6 mL min^{-1} at 103 kPa for analysis of environmental air sample stored in Tedlar® bags.

Preparation of gas standard for DPS portable GC

Gas standards were prepared by injection of a known volume of liquid EF with a 5-μL syringe (SGE, Melbourne, Australia; Cat. No. 5 R-GT) into 250 mL flasks each containing five glass beads (2-3 mm o.d.) and equipped with a Mininert valve (Alltech Associates, Deerfield, IL; Cat. No. 9535). The exact volume of each bottle was measured by the mass of water it held at 25 °C. A fumigant sample volume of 60 µL was injected manually into the DPS portable GC and the concentrations were calculated based on peak areas against the calibrated gas standards.

Comparison of the different equipment used for analysis of ethyl formate

To understand the sensitivity and accuracy of the G460 EF monitor and DPS portable GC, the series of EF gas standards (Table 1) were prepared by injection of known

Table 1. Comparison of sensitivity and working range for different instruments for the analysis of EF gas.

and working range Ethyl formate standard gas		Lab. Desktop HP5890 GC	DPS Portable GC	G460 ethyl formate monitor
g m^{-3}	ppm	ppm	ppm	ppm
0	0	0	0	0
0.0001	0.05	0.03 ± 0.02	0	0
0.0003	0.1	0.11 ± 0.03	0.04 ± 0.03	0
0.0015	0.5	0.49 ± 0.00	0.52 ± 0.08	0
0.003	1	1.0 ± 0.05	1.14 ± 0.05	0
0.0149	5	5.22 ± 0.07	4.93 ± 0.01	0
0.0299	10	10.41 ± 0.06	10.65 ± 0.09	8.5 ± 1.0
0.2985	100	102.64 ± 1.12	105.01 ± 4.20	103.5 ± 2.0
2.9851	1000	995.41 ± 3.08	1006.17 ± 5.1	1005.0 ± 2.5
29.8507	10000	10007.19 ± 5.26	10010 ± 9.26	10008.5 ± 7.5
59.7015	20000	20008.46 ± 7.91	19986 ± 8.54	19985.5 ± 3.0
119.4030	40000	40015.25 ± 8.22	40021.28 ± 11.60	40010.0 ± 10.0

volume of EF liquid into 1 L Tedlar® bags (Air Met Scientific Australia).

The G460 EF monitor sample inlet was directly connected with the Tedlar® bag sampling port and taken two readings from each bag. A Hewlett Packard 5890 (HP5890) Desktop gas chromatograph (GC) equipped with a flame ionization detector (FID) after isothermal separation on a 30 m × 0.53mm (internal diameter) megabore capillary column ZBWAX (B13844) at an oven temperature of 95 °C and carrier flow (N_2) of 6 ml min^{-1} at 1320 mm Hg was used to calibrate G460 EF monitor and DPS portable GC. A 100-μL syringe (SGE, Melbourne, Australia, Cat. No. 005250) was used for the injection of gas samples into both the gas chromatographs, and duplicated injections. Ethyl formate concentrations were calculated based on peak areas against external EF gas standards.

Determination of concentration × time (Ct)

The concentrations of fumigant were monitored at timed intervals of 0, 1, 3, 4, 6, 10 and 48 h respectively, and were used to calculate the *Ct* product. The *Ct* products were calculated according to Eq. (1).

$$Ct = \sum (C_i + C_{i+1})(t_{i+1} - t_i)/2. \quad (1)$$

where: C is fumigant concentration (g m^{-3}),

t is time of exposure (h),

i is the order of measurement,

Ct is concentration × time product (g h m^{-3}).

Statistical analysis

The concentrations of EF were calculated on the basis of peak areas which were calibrated periodically using the gas standards, and data were recorded in Microsoft Excel. The *Ct* products were calculated from the arithmetic average of EF concentration readings during the 6-hour exposure period. The variations (Standard Deviation) of fumigant concentrations and injections in comparison with average readings were analysed within Microsoft Excel. The T-test was used to determine how the two containers compare to each other.

Results

Sensitivity and accuracy of the G460 EF monitor and the DPS portable GC

The comparison study showed that both G460 EF monitor and DPS portable GC readings were consistent with laboratory Hewlett Packard 5890 (HP5890) Desktop GC (Table 1). However, the limits of detection (LOD) were different, for example, 0.1, 0.5 and 10 ppm which much lower than that Threshold Limit Value (TLV) of 100 ppm for HP5890 GC, DPS portable GC and G460 EF monitor, respectively. The G460 EF monitor is sensitive and accurate enough for monitoring EF in the containers accurately and ensure worker's safety during in-transit EF fumigation.

Variation of temperature and relative humidity

There was variation in temperature and relative humidity (r.h.) in the containers during the six-hour exposure period between the two trials (Fig. 2). The temperature and relative humidity varied from 9–32 °C and 55–76% r.h., in both containers during the September trial and from 20–39 °C and 45–69% r.h. in both containers, during the December trials.

Mean temperature and r.h. for both containers during the December trial were 28.05 °C and 56.1%, respectively, during the 48-hour trial period. Mean temperature and r.h. for both containers during the September trial were 21.05 °C and 67.5%, respectively, during the 48 hours.

Safety of ethyl formate fumigation during the in-transit journey

For both the September and December trials, environmental gas concentration measurements taken at the fumigation site after application and throughout the journey indicated nil or below the instrument detection limits of EF in the immediate surroundings of the containers, up to 15 m downwind, or inside and outside of the truck cab. Environmental samples were below the LOD for the handheld G460 gas detection device (10 ppm), the DPS portable GC (0.5 ppm) and

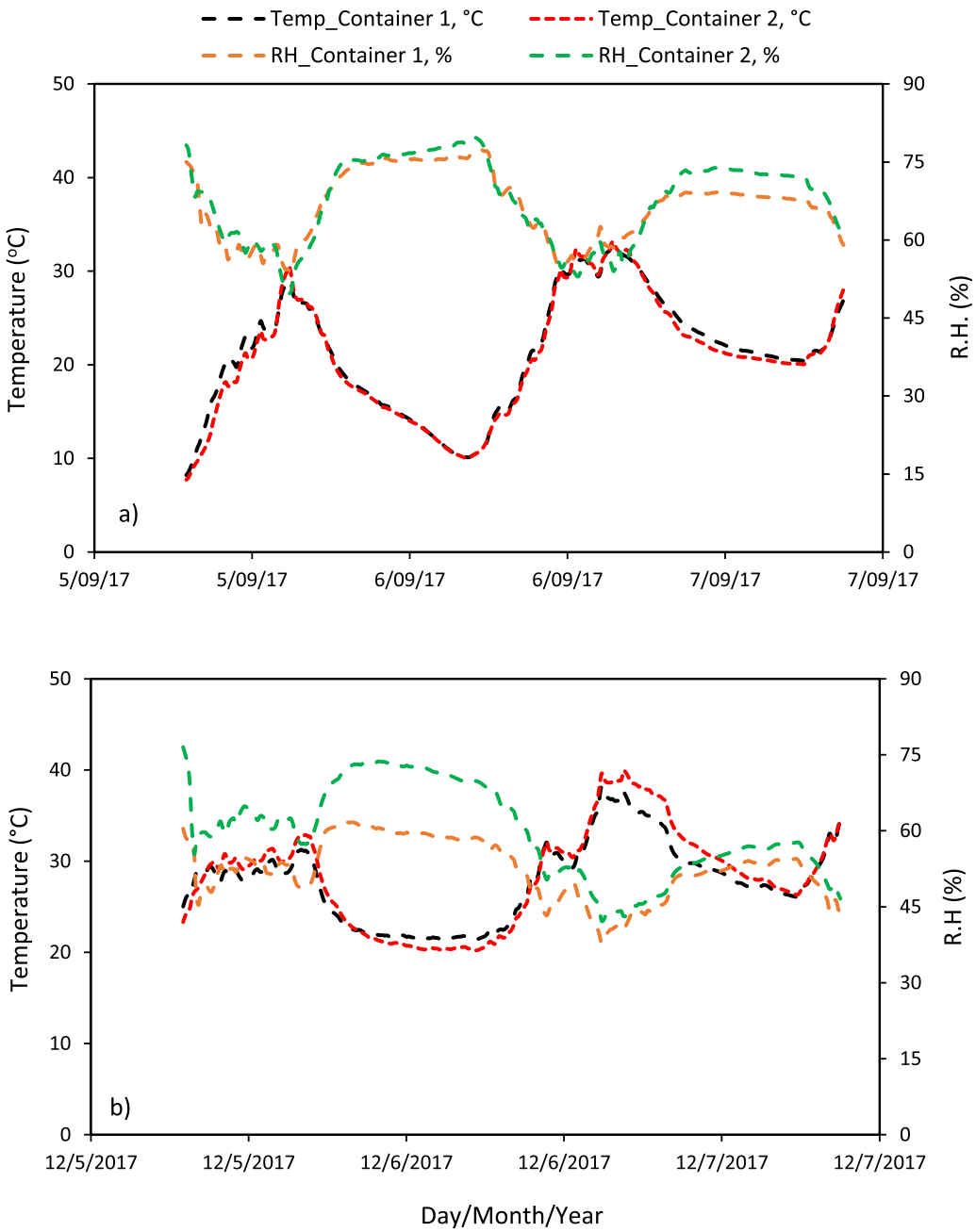

Figure 2. Temperature (˚C) and relative humidity (R.H.) data from containers 1 and 2 during the a) September and b) December trials.

HP5809 GC (0.1 ppm) which is much lower than the EF TLV of 100 ppm (Tables 2 and 3).

Concentration of ethyl formate in the stationary container and containers during the in-transit journey

Ethyl formate concentrations were monitored during both September and December trials using the handheld G460 gas detection device. The disappearance of EF was consistent between two containers during both September and December trials (Figs. 3 and 4, and Table 4). The results indicated that 57-64% ($P < 0.001$) and 20–28% ($P < 0.001$) of EF was present in the containers during the September trial at 10 and 48 hours in-transit fumigation, and 46–58% ($P < 0.001$) and 27–33% ($P < 0.001$) of EF present in the December trial containers at 10 and 48 hours in-transit fumigation (Figs. 3 and 4, and Table 4).

The variation of EF concentrations were 2–12% ($P < 0.05$) from different locations in containers, 7–11% ($P < 0.05$) between two containers during same trip in both September and December trials and 9% ($P < 0.05$) between September and December trials (Table 4). However, EF concentrations in cargo in all trials were lower than other locations within the first hour of fumigation. This is because EF penetrate packed cargo slower (Figs. 3 and 4).

Ethyl formate concentrations in a stationary container were compared with the EF concentrations in the containers undergoing in-transit fumigation (Fig. 5). There are no significant different concentrations of EF between the stationary and in-transit containers (Fig. 4).

Table 2. Environmental levels of EF at different locations downwind during truck stops for September 2017 trials.

Hours and Locations	Containers	Location of meters and sampling ports	Lab. Desktop Chromatograph (ppm)	Portable Chromatograph (ppm)	G460 (ppm)
0		1, 3, 5 and 15 m	<0.1[a]	<0.5[b]	0.0
At fumigation site	1	Inside cab	<0.1	<0.5	0.0
(After application)		Outside cab	<0.1	<0.5	0.0
		1, 3, 5 and 15 m	<0.1	<0.5	0.0
	2	Inside cab	<0.1	<0.5	0.0
		Outside cab	<0.1	<0.5	0.0
1		1, 3, 5 and 15 m	<0.1	<0.5	0.0
At fumigation site	1	Inside cab	<0.1	<0.5	0.0
(After loading of containers on truck)		Outside cab	<0.1	<0.5	0.0
		1, 3, 5 and 15 m	<0.1	<0.5	0.0
	2	Inside cab	<0.1	<0.5	0.0
		Outside cab	<0.1	<0.5	0.0
3		1, 3, 5 and 15 m	<0.1	<0.5	0.0
Truck stop	1	Inside cab	<0.1	<0.5	0.0
		Outside cab	<0.1	<0.5	0.0
		1, 3, 5 and 15 m	<0.1	<0.5	0.0
	2	Inside cab	<0.1	<0.5	0.0
		Outside cab	<0.1	<0.5	0.0
4		1, 3, 5 and 15 m	<0.1	<0.5	0.0
Truck stop	1	Inside cab	<0.1	<0.5	0.0
		Outside cab	<0.1	<0.5	0.0
		1, 3, 5 and 15 m	<0.1	<0.5	0.0
	2	Inside cab	<0.1	<0.5	0.0
		Outside cab	<0.1	<0.5	0.0
6		1, 3, 5 and 15 m	<0.1	<0.5	0.0
Truck stop	1	Inside cab	<0.1	<0.5	0.0
		Outside cab	<0.1	<0.5	0.0
		1, 3, 5 and 15 m	<0.1	<0.5	0.0
	2	Inside cab	<0.1	<0.5	0.0
		Outside cab	<0.1	<0.5	0.0
10		1, 3, 5 and 15 m	<0.1	<0.5	0.0
Truck stop	1	Inside cab	<0.1	<0.5	0.0
		Outside cab	<0.1	<0.5	0.0
		1, 3, 5 and 15 m	<0.1	<0.5	0.0
	2	Inside cab	<0.1	<0.5	0.0
		Outside cab	<0.1	<0.5	0.0
48		1, 3, 5 and 15 m	<0.1	<0.5	0.0
Truck stop	1	Inside cab	<0.1	<0.5	0.0
(Before venting)		Outside cab	<0.1	<0.5	0.0
		1, 3, 5 and 15 m	<0.1	<0.5	0.0
	2	Inside cab	<0.1	<0.5	0.0
		Outside cab	<0.1	<0.5	0.0

[a]Lower than detection level of 0.1 ppm.
[b]Lower than detection level of 0.5 ppm.

Efficacy of ethyl formate fumigation during the in-transit journey

The concentration × time products were calculated from the arithmetic EF concentration readings during in transit fumigation exposure period. In the September trial, the C*t* products were achieved at ranges of 346.84–354.40, 499.70–557.38, 763.08–746.96 and 2290.34–2327.64 g h m^{-3} ($P < 0.07$) after 3, 4, 6, 10 and 48 hours in transit fumigation, respectively (Table 5). In the December trial, the range of *Ct* products were 283.88–289.28, 371.53–386.78 g h m^{-3}, 538.68–557.38, 734.48–813.31 and 2247.20–2391.54 g h m^{-3} ($P < 0.05$) after 3, 4, 6, 10 and 48 hours in transit fumigation, respectively (Table 5).

Discussion

Efficacy of in-transit fumigation on EF concentration and its Ct products

This study is the first to test the efficacy of ethyl formate (EF) as an in-transit fumigant for use in shipping containers. Most studies investigating fumigation of insects (mainly stored grain insects) with EF were conducted in laboratory or stationary container conditions, but none on in-transit container fumigation. Environmental forces can cause fumigant loss from the container during in-transit fumigation. These include wind and transport velocity, rate of ascent and descent, internal (headspace) changes in temperature and aeration in barometric pressure.[24] If there is inadequate maintenance of the containers, leakage in plank-floored containers can also be extensive because of gaps opening up between the planks due to shrinkage.[29] Therefore, it is important to use high quality containers and the containers are properly inspected to ensure there is limited potential for gas leakage. Gas-tightness of containers is important for effective fumigation. Gas tightness has been determined as dependent on container construction rather than age.[27] Ill-fitting door seals, gaps between the floorboards of planked floors, nail-holes in the floor and gaps between the panels of plywood floors have been identified as the sources of gas leakage in containers[2]. Gas tightness can

Table 3. Environmental levels of EF at different locations downwind during truck stops for December 2017 trials.

Hours and locations	Containers	Location of meters and sampling ports	Lab. Desktop Chromatograph (ppm)	Portable Chromatograph (ppm)	G460 (ppm)
0		1, 3, 5 and 15 m	<0.1[a]	<0.5[b]	0.0
At fumigation site	1	Inside cab	<0.1	<0.5	0.0
(After application)		Outside cab	<0.1	<0.5	0.0
		1, 3, 5 and 15 m	<0.1	<0.5	0.0
	2	Inside cab	<0.1	<0.5	0.0
		Outside cab	<0.1	<0.5	0.0
1		1, 3, 5 and 15 m	<0.1	<0.5	0.0
At fumigation site	1	Inside cab	<0.1	<0.5	0.0
(After loading of containers on truck)		Outside cab	<0.1	<0.5	0.0
		1, 3, 5 and 15 m	<0.1	<0.5	0.0
	2	Inside cab	<0.1	<0.5	0.0
		Outside cab	<0.1	<0.5	0.0
3		1, 3, 5 and 15 m	<0.1	<0.5	0.0
Truck stop	1	Inside cab	<0.1	<0.5	0.0
		Outside cab	<0.1	<0.5	0.0
		1, 3, 5 and 15 m	<0.1	<0.5	0.0
	2	Inside cab	<0.1	<0.5	0.0
		Outside cab	<0.1	<0.5	0.0
4		1, 3, 5 and 15 m	<0.1	<0.5	0.0
Truck stop	1	Inside cab	<0.1	<0.5	0.0
		Outside cab	<0.1	<0.5	0.0
		1, 3, 5 and 15 m	<0.1	<0.5	0.0
	2	Inside cab	<0.1	<0.5	0.0
		Outside cab	<0.1	<0.5	0.0
6		1, 3, 5 and 15 m	<0.1	<0.5	0.0
Truck stop	1	Inside cab	<0.1	<0.5	0.0
		Outside cab	<0.1	<0.5	0.0
		1, 3, 5 and 15 m	<0.1	<0.5	0.0
	2	Inside cab	<0.1	<0.5	0.0
		Outside cab	<0.1	<0.5	0.0
10		1, 3, 5 and 15 m	<0.1	<0.5	0.0
Truck stop	1	Inside cab	<0.1	<0.5	0.0
		Outside cab	<0.1	<0.5	0.0
		1, 3, 5 and 15 m	<0.1	<0.5	0.0
	2	Inside cab	<0.1	<0.5	0.0
		Outside cab	<0.1	<0.5	0.0
48		1, 3, 5 and 15 m	<0.1	<0.5	0.0
Truck stop	1	Inside cab	<0.1	<0.5	0.0
(Before venting)		Outside cab	<0.1	<0.5	0.0
		1, 3, 5 and 15 m	<0.1	<0.5	0.0
	2	Inside cab	<0.1	<0.5	0.0
		Outside cab	<0.1	<0.5	0.0

[a]Lower than detection level of 0.1 ppm.
[b]Lower than detection level of 0.5 ppm.

be improved by replacing door seal gaskets and by treating the floor with a suitable sealant. Gas-tightness of a container is best determined by a pressure test and not merely by visual inspection.[2] These are all important factors to consider when using EF for in-transit fumigation.

The comparison study showed that the EF concentration within in-transit containers are consistent with that of a stationary container undergoing fumigation for 24 hours (Fig. 5), which demonstrated that environmental influences on a moving container under fumigation were negligible in reducing the efficacy of the treatment. This study showed that the EF exposure period required for successful insect pest extermination was successfully achieved during the in-transit trials. More than half of the applied EF concentration was maintained over the ten-hour exposure period (Figs. 3 and 4, and Table 4), demonstrating that concentrations of EF are suitable for exterminating insect pests and can be maintained during a 10-hour exposure period. There were limited seasonal effects on the *Ct* product of EF associated with temperature variation. The mean C*t* product recorded in the containers at the different times of the year were consistent, despite seasonal temperature variation (Table 5). High levels of EF were maintained during the first 10-hour exposure period for both September and December trials. This is important as air tightness and overloading in containers can be a major hindrance to good distribution of fumigant. To our knowledge, there are no other studies that have investigated the influence of seasonal temperature variations on the efficacy of EF.

Efficacy of Ct products achieved during in-transit fumigation

High mortality $LD_{99.5}$ was achieved for for EF fumigation in previous studies of common adult stored grain insect pests, such as *Sitophilus oryzae, Tribolium castaneum, Rhyzopertha dominica* and *Plodia interpunctella* with a maximum *Ct* product of 207.4 g h m^{-3}.[28,29] which was less than that the C*t* product range of 260.18–267.87 g h m^{-3} and

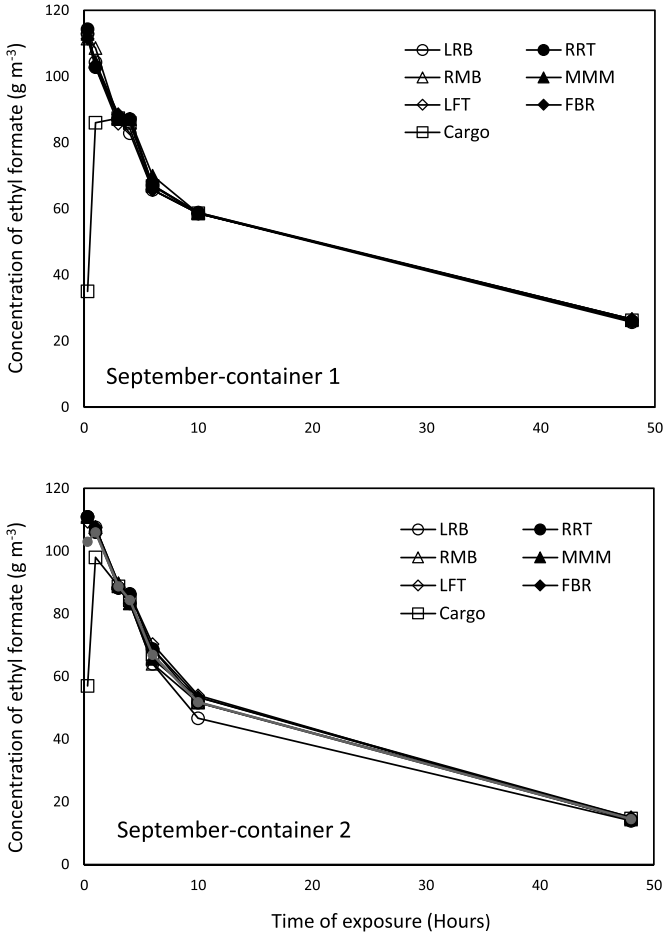

Figure 3. In transit fumigation of cargo container using EF showing concentrations (g m^{-3}) in various parts of the container during the September trial in containers 1 and 2 (LRB = Left rear bottom, RRT = Right rear top, RMB = Right middle bottom, MMM = Middle middle middle, LFT = Left front top, FBR = Front bottom right, Cargo = cargo).

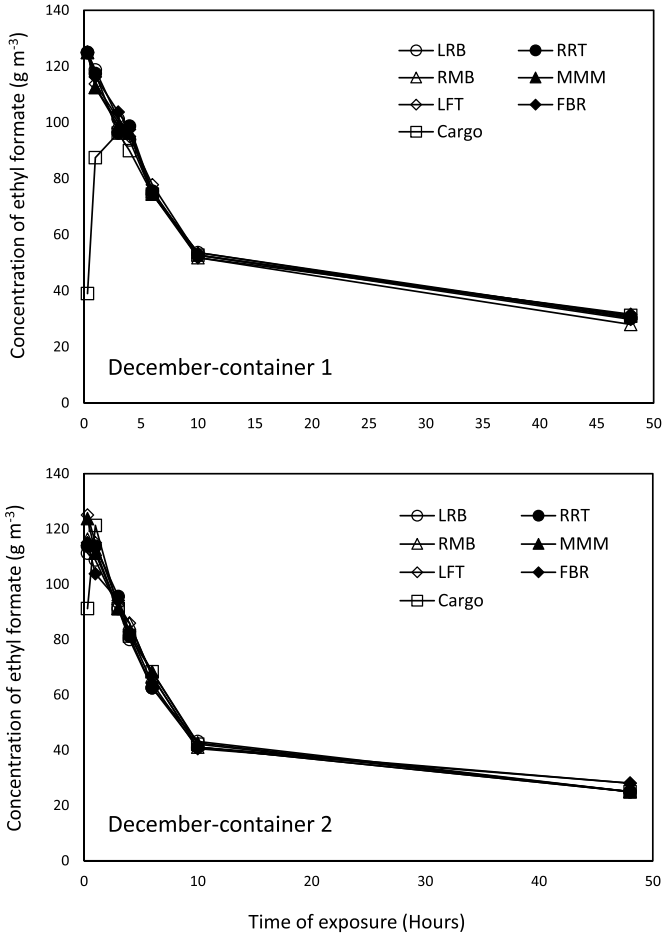

Figure 4. In transit fumigation of cargo container using EF showing concentrations (g m^{-3}) in various parts of the container during the December trial in containers 1 and 2 (LRB = Left rear bottom, RRT = Right rear top, RMB = Right middle bottom, MMM = Middle middle middle, LFT = Left front top, FBR = Front bottom right, Cargo = cargo).

Table 4. The variation of ethyl formate concentrations compared at different location in containers, between two containers during the same trip in September and December trials and between September and December trials.

Trials	Containers	Time of measurement during In-transit fumigation (hours)						
		0.3	1	3	4	6	10	48
September	Container 1	101.53[a] ± 0.64[b]	101.67 ± 3.57	87.38 ± 0.69	85.95 ± 1.43	66.90 ± 2.46	58.67 ± 2.45	26.29 ± 0.61
	Container 2	103.01 ± 1.08	105.99 ± 7.20	88.73 ± 0.98	84.34 ± 1.73	66.93 ± 1.52	51.87 ± 0.13	19.13 ± 0.33
	Between containers 1 & 2	102.27 ± 24.26	103.83 ± 5.90	88.05 ± 1.07	85.15 ± 1.74	66.92 ± 1.96	55.22 ± 3.94	22.71 ± 3.74
	Between containers 1 & 2	0.915[c]	0.018	0.012	0.042	0.019	0.001	0.001
December	Container 1	112.71 ± 1.08	111.25 ± 7.20	99.64 ± 0.98	95.36 ± 1.73	75.24 ± 1.52	52.73 ± 0.13	30.34 ± 0.33
	Container 2	113.75 ± 0.64	112.32 ± 3.57	92.44 ± 0.69	82.86 ± 1.43	65.60 ± 2.46	41.65 ± 2.45	25.86 ± 0.61
	Between containers 1 & 2	113.23 ± 23.35	111.79 ± 8.20	96.04 ± 4.36	89.11 ± 6.95	70.42 ± 5.36	47.19 ± 5.81	28.10 ± 2.66
	Between containers 1 & 2	0.938	0.018	0.001	0.001	0.001	0.001	0.001
September & December	Between four containers	104.96 ± 23.01	105.41 ± 7.22	88.98 ± 2.38	84.78 ± 2.17	66.58 ± 2.03	52.69 ± 7.20	24.39 ± 3.19
	Between September & December trials	0.377	0.049	0.001	0.028	0.016	0.001	0.001

[a]Average of ethyl formate concentrations from different gas sampling locations in container.
[b]standard deviation.
[c]the *P* value from T-test.

283.88–289.28 g h m^{-3} were achieved after 3 hours in transit fumigation in September and December trials (Table 5). It is clear that high mortality $LD_{99.5}$ for common adult stored grain insect pests can be achieved within three hours with in-transit fumigation.

In this study, after four hours exposure in both September and December trial, the C*t* protects achieved at arrange of 346.84–354.40 g h m^{-3} and 371.53–386.78 g h m^{-3} much higher than a *Ct* product of 300 g h m^{-3} (Table 5) which offered 100% killed adult of most common stored insect pests.[10,14,22,28,30]

This study demonstrated that an adequate C*t* product for EF could be achieved during in-transit fumigation to kill egg stage of common stored product pests. In the trials, after six hours exposure, the *Ct* products were recorded at range of 499.70–557.38 g h m^{-3} in both containers during the

September and December trials (Table 5). Previously reported bioassays suggest that a *Ct* product for EF of approximately 450 g h m^{-3} offered to kill egg stage insects.[14,22,28–30]

Ren and Mahon (2006) reported a 100% kill of all stages of most common stored product insect pests with a *Ct* product of 2200 g h m^{-3} [14], the mean C*t* product of 2327.64 and 2364.94 g h m^{-3} achieved in September and December trials (Table 5), indicated that during 48 hours in-transit fumigation, all stages of most common stored product insects can be completely controlled.

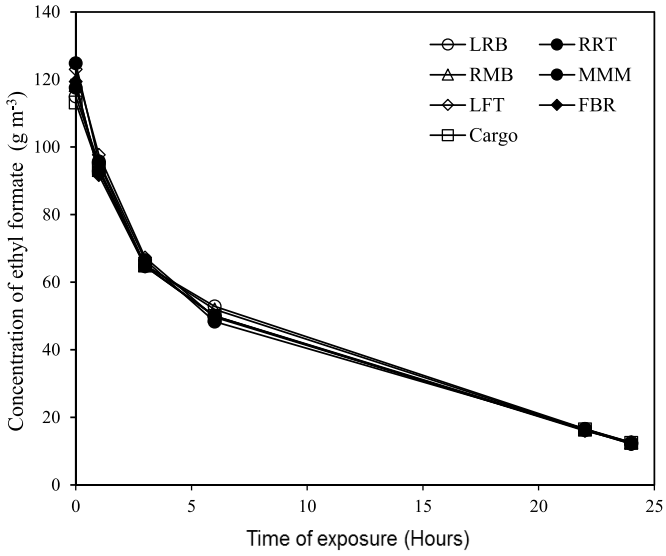

Figure 5. Non in-transit stationary container fumigation during the December 2017 trial.

Effect of in-transit fumigation with EF on environment and worker safety

This study indicates that there was no detectable risk to the public, truck driver or workers from exposure to EF throughout the two-day journey. Ethyl formate was not detected in the environment outside the containers up to 15 meters downwind or inside the cab of the truck throughout the 48-hour trial period journey for both the September and December trials. These results further suggest that there would be minimal risk to workers if the containers were to continue their journey or be vented after two days. During in-transit fumigation, containers do not have to be vented after six hours, but only before unloading, which in the case of the trip from Perth to Dampier, will be at least 18 hours. In this study, after 48 hours the EF concentration in the containers were above TLV of 100 ppm, necessitating venting.

This study also confirmed that the G460 gas detection device is accurate, reliable and easy to be used for monitoring EF in containers and the environment, in comparison with the DPS portable GC and lab based HP5809 GC.

The advantages of using in-transit EF + N_2 fumigation

Our results indicate that in-transit EF + N_2 fumigation will deliver cost savings by eliminating the time a container must be stationary in a fumigation yard, while also maintaining worker safety. The total time for a pre-shipment fumigation consists of preparation time, which include startup and fitting the insect screen and manifold (±30 minutes), fumigation time (six hours) and venting time, which includes removal of insect screen and manifold

Table 5. Comparison of Concentration × time (*Ct*) product (g h m^{-3}) for different exposure times (hours) and mortality of common stored product insect pests in our study and previous studies.

	Achieved Concentration × time (*Ct*) product (g h m^{-3}) for different exposure time (hours)				
Trials	3	4	6	10	48
Container 1, December	283.88[a] ± 12.45[b]	371.53 ± 2.22	538.68 ± 3.30	734.48 ± 2.64	2247.20 ± 29.68
Container 2, December	289.28 ± 5.25	386.78 ± 1.39	557.38 ± 4.14	813.31 ± 5.61	2391.54 ± 22.29
Mean December	286.58 ± 3.82[c]	379.16 ± 10.78	538.68 ± 26.44	773.89 ± 55.74	2319.37 ± 72.17
Container 1, September	260.18 ± 7.27	346.84 ± 1.21	499.70 ± 2.86	750.84 ± 3.09	2364.94 ± 6.77
Container 2, September	267.87 ± 3.67	354.40 ± 0.62	505.67 ± 3.65	763.08 ± 8.88	2290.34 ± 39.12
Mean September	264.02 ± 5.44	350.62 ± 5.34	502.69 ± 4.22	746.96 ± 5.49	2327.64 ± 37.30
Reported $LD_{99.5}$ (adult *Sitophilus oryzae*)[29]	207.4				
Reported $LD_{99.5}$ (adult *Tribolium castaneum*)[29]	167.1				
Reported $LD_{99.5}$ (adult *Rhyzopertha dominica*)[29]	122.2				
Reported $LD_{99.5}$ (adult *Plodia interpunctella*[28]	98.7				
Reported 100% kill (adults) of common stored product insects[10,14,22,28,30]		>300			
Reported 100% kill (eggs) of common stored product insects[10,14,22,28,30]			>450		
Reported 100% kill (larvae) of common stored product insects[10,14,22,28,30]				>500	
Reported 100% kill (all stages) all common stored product insects[14]					>2200

[a]Average of *Ct* products from different gas sampling locations in container.
[b]standard deviation.
[c]the mean of two same trip containers.

(±30 minutes). In total, the entire fumigation process will be approximately seven hours. During the six-hour fumigation period, two fumigators (head fumigator and technician) must be present. However, for in-transit fumigation, containers can be dispatched directly after EF + N_2 injection and will therefore reduce Labour by at least six hours. This means that there will also be no staging of containers, which occupy space in a fumigation yard. There is also a decrease in the demurrage costs. Therefore, use of in-transit fumigation will have significant cost and time savings.

Conclusion

The use of containers for international trade has been steadily increasing over the past decades and as such, the risk of introducing invertebrate pests through the containers will continue to rise. While this research has demonstrated that in-transit fumigation with EF is achievable, both from an efficacy and human health perspective, its successful implementation into supply chains will be highly dependent on using the quality of containers for in-transit EF fumigation. This research demonstrated that the C*t* product within the container was sufficient to kill all life stages of a range of common stored product pests, as determined by previous research. In-transit fumigation using EF should only be conducted after containers have passed inspections, in order to ensure minimal potential for gas leakage during a journey. Further research should be completed to verify required C*t* product for other insect species/groups that have the potential to be transported in shipping containers.

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