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DOES ETHYL FORMATE HAVE A ROLE AS A RAPID GRAIN FUMIGANT? PRELIMINARY FINDINGS

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ABSTRACT

Ethyl formate is a fumigant that is currently used in Australia on dried fruit. Recently, there has been a re-evaluation of the usefulness of this material in disinfesting stored commodities. We have discovered that ethyl formate has a very rapid action and this property could make it useful in rapid disinfestation. We studied the response of *Sitophilus oryzae* (L.) adults to ethyl formate in a sealed system in preliminary experiments. *S. oryzae* was chosen because it is generally one of the more difficult stored-product beetles to kill by fumigation. The insects were exposed to three different dosages of ethyl formate for a range of exposure times with a 50% filling ratio of wheat. Ethyl formate required a very short time to kill all adults for the range of concentrations and times studied. Exposures of 12 min and 2 h achieved 100% mortality for concentrations of 340 and 210 g m^{-3}, respectively. For 130 g m^{-3}, an exposure of 3 h. achieved 94% mortality. These results are consistent with the requirements for a potential replacement for methyl bromide as a rapid disinfestant. Ethyl formate has the advantages of rapid loss from the gaseous phase and breakdown by the grain. The high flammability of ethyl formate, which may make its application a potential problem, can be significantly reduced by its application in water. The rapid kill makes ethyl formate suitable for treatments in conditions where long-term gas retention is not possible, for example, the disinfestation of machinery, work-spaces and surfaces.

INTRODUCTION

Insect control in stored products continues to rely heavily on methyl bromide (MB) and phosphine (PH_3). The wide-scale convenient use of these materials continues to be threatened by the well-documented problems of the ozone depleting potential of MB and the risk of development of insect resistance in the case of PH_3. Some alternative treatments are necessary and ethyl formate (EF) has shown some potential in this role. Part of the investigations into this potential has been an examination of its toxicity to stored-product insects. However, a better understanding of the dosage requirement is needed before it is possible to define what role, if any, EF has as a grain fumigant. EF is currently used as a fumigant of dried fruit in Australia and elsewhere.

Extensive work has been carried out where EF was found to be generally effective against stored-product pests (Muthu *et al.,* 1984; Hilton and Banks, 1997). More recently it has been reported that EF can have a very rapid action against stored-product insects and this property may make it useful for rapid stored-product disinfestation (Damcevski and Annis, 1998).

Several workers have observed the rapid action of EF outside the stored-product area. For example: 1, 2, 5 and 10 h EF exposures applied respectively at 135, 68, 34 and 24 mg/L, at 10°C, killed 100% of body lice (Busvine and Vasuvat, 1966); tests done on western flower thrips, *Frankliniella occidentalis* (Pergande), on strawberries found that a concentration of 0.5% EF for 1 h at 30 mm Hg pressure gave 100% mortality (Aharoni *et al.,* 1980); vacuum fumigation for 2 h with a concentration of 1.0% EF killed 97% of the green peach aphid, *Myzus persicae* (Sulzer) (Stewart and Aharoni, 1983); and a 3 h fumigation of grapefruit infested with the California red scale, *Aonidiella aurantii* (Maskell), at a concentration of 1.5% EF resulted in 100% mortality of all stages of the insect (Aharoni *et al.,* 1987).

The aim of the current work was to investigate the response of *Sitophilus oryzae* adults and larvae to short exposures (<2 days) of EF in a sealed system. *S. oryzae* was chosen because it is one of the more difficult stored-product insects to kill by gaseous treatments in general (Damcevski and Annis, in press) and its response to a range of other fumigants is known. In an investigation of the response of mixed age cultures to EF vapour in a sealed system Damcevski and Annis (in press) showed that EF controlled *Tribolium castaneum* and *Rhyzopertha dominica* but not *S. oryzae*.

MATERIALS AND METHODS

The EF used was laboratory grade, at 920 g/L. The *S. oryzae* adults and larvae used were , CSIRO Entomology Stored Grain Research Laboratory strain LS2. Adults were 7 to 14 days post-emergent and larvae were 14 to 17 days old. The wheat used was from a single source (variety, Rosella; grade, ASW; harvest, 1996). The moisture content (m.c.) of the wheat was 12.0% (wet basis), determined using the standardised ISO oven-dried method (Anon., 1985).

Adult exposures

S. oryzae adults were treated at a rate of 168, 244 and 400 mg/L of EF for a range of exposure times between 3 and 360 min. (Table 1). These concentrations and times were initially selected on the basis of data from Muthu *et al.,* (1984) and Hilton and Banks (1997). Triplicate samples of 50 adults were used for each exposure concentration/time period.

Adults were exposed in 300 mL conical flasks sealed with glass stoppers containing a septum. Each flask had its volume calibrated gravimetrically according to the amount of water it could contain. The flasks also contained 123 g of wheat, which gave a 50% filling ratio (based on bulk density). EF was applied in an 8% (v/v) water mix to give initial concentrations of 168, 244 and 400 mg/L calculated using the gas volume of the flasks (the total volume minus the volume of the wheat calculated from the true density of 12% m.c. wheat). The flasks were shaken gently to mix the solution with the grain and insects. The exposures were carried out at 25°C.

TABLE 1
Applications used in ethyl formate (EF) exposures of *Sitophilus oryzae* (L.) adults

Applied EF (mg/L)	Exposure times (minutes)
168	8, 17, 35, 70, 100, 200, 360.
244	5, 10, 20, 40, 60, 120, 240.
400	3, 6, 12, 25, 38, 75, 150.

After each exposure period was complete, the flask was opened and the grain and insects placed inside a fresh jar. These were left to air for an hour. The insects were then sieved off each replicate, and placed on 80 g fresh wheat and incubated at 25°C and 60% r.h. Mortality was assessed at 7 days after fumigation.

Larval exposures

S. oryzae larvae, 14 to 17 days old, were obtained by adding adults to wheat, removing them after 3 days, and allowing the progeny to develop for another 2 weeks before treatment. At this time, the cultures of wheat plus larvae were thoroughly mixed by passing them through a Börner divider three times. This was then split into 300 g samples, some for tests and 4 for controls, and fresh wheat was added to give the total amount required as listed in Table 2. This procedure was used to give greater than 500 insects per sample.

Fumigation studies were carried out using 2.7 L glass desiccators with lids sealed with glass stoppers containing a septum. Each container had its volume calibrated gravimetrically according to the amount of water it could contain.

Various replicated combinations of different amounts of wheat (g) and numbers of *S. oryzae* larvae (Table 2) were sealed in the desiccators. EF was applied in an 8% (v/v) water mix into the grain mass to give initial concentrations of 109, 130 and 155 mg/L, calculated according to the gas volume of the desiccator. The desiccators were then rolled to mix the volatilised EF into the atmosphere. They were then kept at 25°C for 48 h. The cultures were then removed from the desiccators and placed inside a fume-hood to aerate for another 24 h before being placed at 25°C and 60% r.h. The application rates were selected on the basis of data from Hilton and Banks (1997).

TABLE 2
Experimental conditions used in ethyl formate (EF) exposures of *Sitophilus oryzae* (L.) larvae

Applied EF (mg/L)	Wheat (g)	Number of insects per replicate *	Number of desiccators
109	500	1060	4
130	1000	1060	4
155	1500	1060	4

*Approximation based on control emergence

Survival was assessed by counting emerged adults 6 and 7 weeks after the start of the exposures. Speed of kill was assessed by measuring the CO_2 production as it declined during the exposures. Carbon dioxide (CO_2) production in controls of wheat plus EF, as well as wheat alone, was also measured. The CO_2 produced from wheat respiration and EF breakdown was subtracted from the total CO_2 produced in the exposures to obtain the CO_2 produced by the larvae.

CO_2 and oxygen (O_2) concentrations were measured with a Fisher model 1200 Gas Partitioner. This was fitted with 80-100 mesh Columpak™ PQ (6.5 feet _ 1/8 inch) and 60-80 mesh molecular sieve 13X (11 feet _ 3/16 inch) columns in series and a thermal conductivity detector operated at 50°C.

EF concentrations for the larval exposures and for desiccators filled with wheat at a 50% filling ratio (same conditions as for adult exposures) were measured against standard concentrations. A Shimadzu GC-6AM gas chromatograph was used fitted with a flame ionisation detector and a 20% OV101 on Gas Chrom Q column (1 m _ 3 mm i.d.), run at 110°C.

RESULTS AND DISCUSSION

The experimental results showed that ethyl formate applied at high concentrations in a sealed system provided a rapid kill of *S. oryzae* adults and larvae.

Adult exposures

The mortality of *S. oryzae* adults exposed to applications of 168, 244 and 400 mg/L of EF with a 50% filling ratio of wheat for a range of exposure times is shown in Fig. 1. The 100% mortality line shows those replicates where 100% mortality was achieved. The points for the exposures at 400 mg/L for 12, 25, 75 and 150 min represent 2 replicates and the point for the exposure at 244 mg/L for 240 min represents 3 replicates.

The exposures at 400 mg/L achieved up to 100% mortality at 12 min. The exposures at 244 mg/L achieved 100% mortality at 240 min and those at 168 mg/L achieved an average of 94% mortality (the highest being 98% for one replicate) at 360 min.

Loss of ethyl formate from the headspace with 50% filling ratio: The EF concentrations during the adult exposures were not measured by gas chromatography. However, the loss of EF from the headspace of 2.7 L desiccators with a 50% filling ratio of wheat was measured by gas chromatography. The EF loss from the headspace following applications at concentrations of 160, 234 and 300 mg/L is shown in Fig. 2.

Larval exposures

All *S. oryzae* larvae were killed when exposed to applications of 109, 130 and 155 mg/L of EF on 500, 1,000 and 1,500 g wheat for 48 h, based on the criterion that no adults had emerged after 6 and 7 weeks following the EF exposures.

The CO_2 produced by the larvae during the exposures is shown in Fig. 3 and reveals the speed of kill. Each series of points represents respiration levels of the insects. The exposures to EF at 155 mg/L resulted in little increase in CO_2 after

1.6 h, indicating that all larvae were killed within 1.6 h. The exposures at 130 mg/L EF resulted in a slightly higher increase in CO_2 after 1.5 h and a little higher again for the exposures at 109 mg/L EF. (The higher concentrations of CO_2 at 1.3 to 1.6 h for the exposures at 155 and 130 mg/L EF were due to the different headspace volumes with the different amounts of wheat). The control larvae produced CO_2 concentrations of 0.7% at 2 h and 15% at 48 h. This data was not graphed with that for the larvae exposed to EF as it was much greater than the maximum of 0.4% at 48 h obtained for the EF exposures.

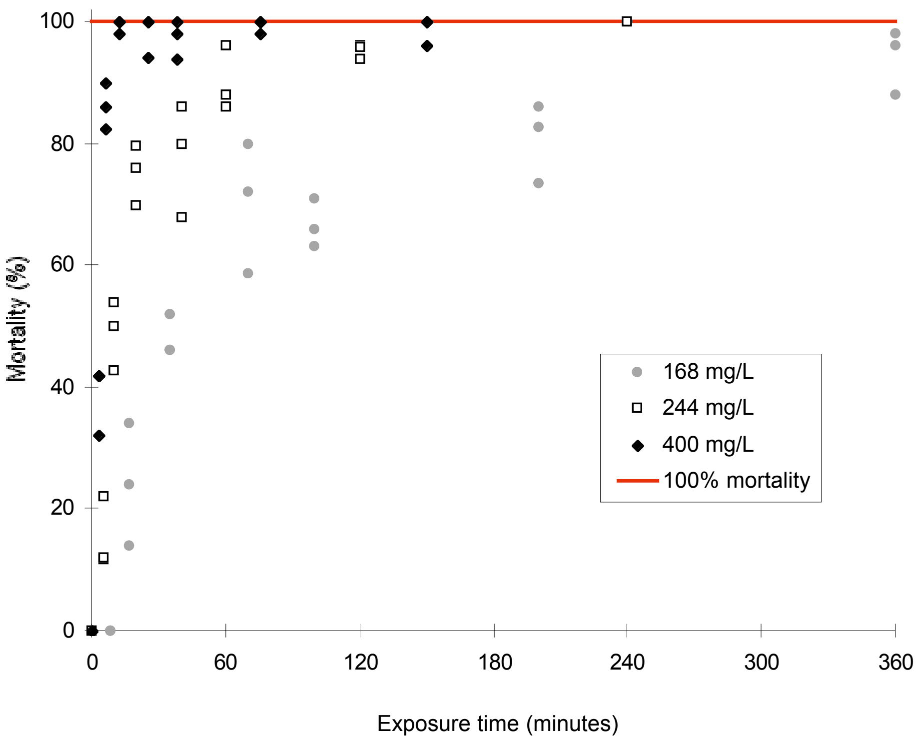

Fig. 1. Mortality of adult *Sitophilus oryzae* exposed to applications of 168, 244 and 400 mg/L of ethyl formate for different exposure times.

Loss of ethyl formate from the headspace: The loss of EF from the headspace is shown in Fig. 4. Ethyl formate loss was more rapid for the exposures at 155 mg/L EF with 1,500 g wheat than for 130 mg/L EF with 1,000 g wheat, which was in turn more rapid than for 109 mg/L EF with 500 g wheat. After 1 h exposure, the EF concentration in all exposures had dropped below the lower explosive limit (LEL) of EF, which is 2.8% v/v in air (85 mg/L).

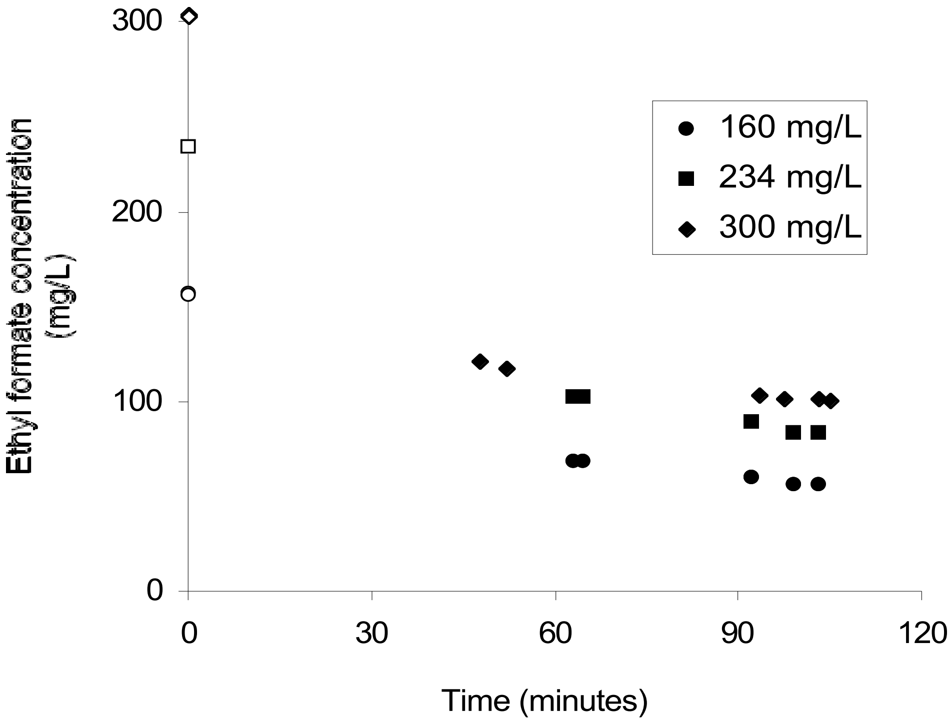

Fig. 2. Loss of ethyl formate from the headspace dosed at 160, 234 and 300 mg/L with 50% filling ratio of wheat: closed symbols - observations; open symbols - calculated from dosage.

The presence of wheat reduced the EF headspace concentration during the exposure periods. When more wheat was present in the larval exposures, more EF was lost from the headspace. These observations are consistent with the observation that EF is rapidly lost from the headspace and broken down by grain (Desmarchelier *et al.,* 1998; Damcevski and Annis, 1998).

In these experiments EF was applied in an 8% (v/v) water mix to simulate a possible field application in which an aqueous solution would be used to reduce the risks associated with flammability. In a previous field trial by this laboratory, Desmarchelier *et al.,* (1998) also used aqueous solutions of EF as a grain treatment. The application of EF in aqueous solution would increase the relative humidity (r.h.) and the grain m.c.; in these exposures the grain m.c. would have increased by approximately 0.3–0.8%.

On the basis of the rapid kill observed with adults and larvae of *S. oryzae* it is worth extending similar mortality studies to other species and stages. The results so far are encouraging enough to warrant other biological studies designed to determine to what extent, and over what period, high EF concentrations are needed for a high degree of control. Preliminary physical, chemical and engineering studies will also be required to determine how the concentrations needed for rapid disinfestations can be generated and maintained.

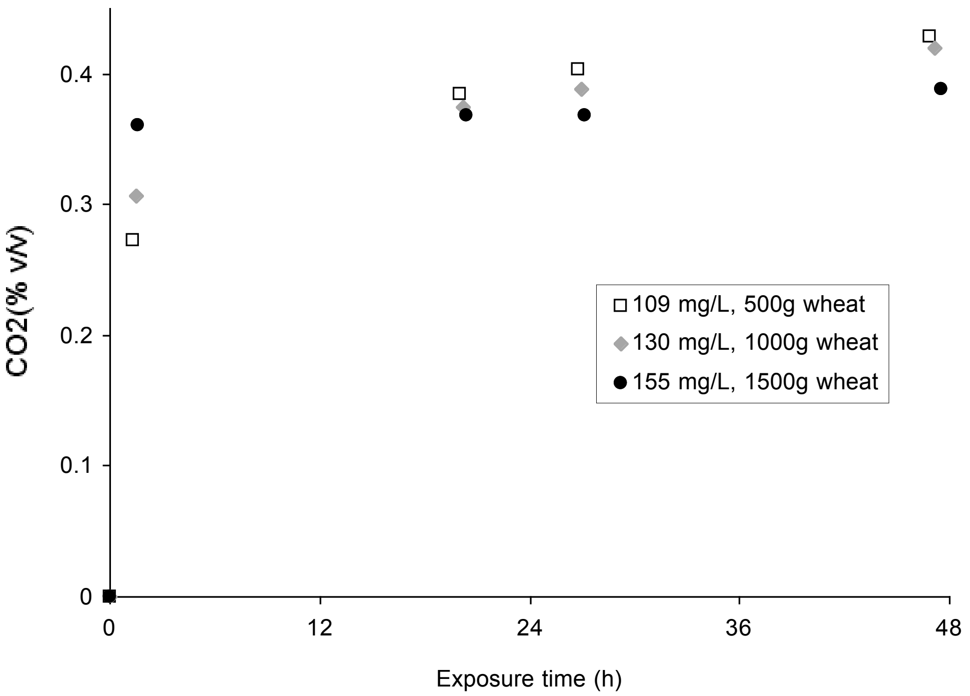

Fig. 3. Carbon dioxide produced by *Sitophilus oryzae* larvae exposed to applications of 109, 130 and 155 mg/L ethyl formate for 2 days on 500, 1000, and 1500 g wheat, respectively, giving 100% mortality.

CONCLUSIONS

This work shows that EF can give rapid disinfestation of grain and helps support the continuing investigation of EF as a potential fumigant for stored-products. Many further biological and application issues need to be resolved before EF could be used routinely for large-scale commodity fumigation. These include: the effects of EF on other stages of *S. oryzae* and other species; the effects of temperature on the effectiveness of EF; the necessity of maintaining a high concentration of EF during a fumigation; the maintenance of an effective concentration of EF with a large amount of grain.

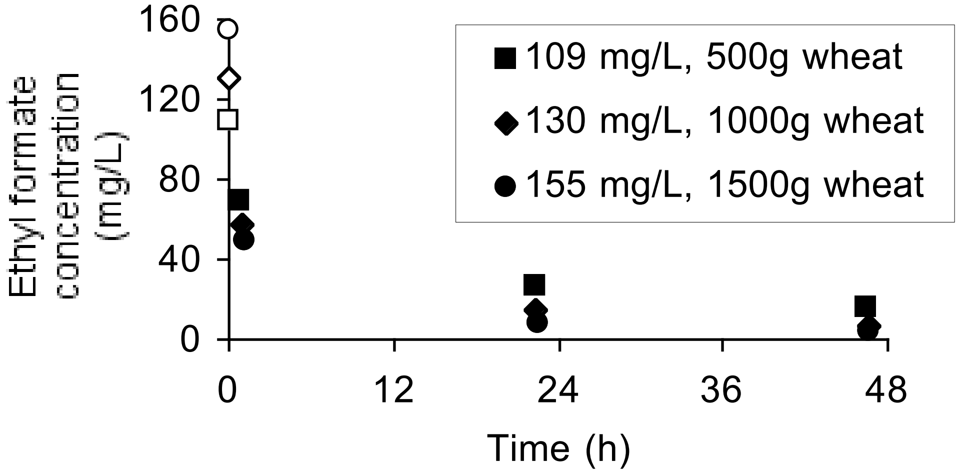

Fig. 4. Loss of ethyl formate from the headspace dosed at 109, 130 and 155 mg/L, during exposures of *Sitophilus oryzae* larvae: closed symbols - observations; open symbols - calculated from dosage.

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