Field trials on ethyl formate for fumigation of on-farm storage

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Abstract. CSIRO Stored Grain Research Laboratory (SGRL) has recently conducted several successful trials with ethyl formate on wheat (Harden, NSW), sorghum (milo) (Warwick, Queensland) and split faba bean (Two Wells, SA) in unsealed farm bins. The liquid ethyl formate was applied as a pulsed, or double, dose to the top of the grain through a polyvinyl chloride (PVC) probe (φ 4 cm x 1.2 m). This method of application was chosen to maintain ethyl formate concentrations below the flammable level, reduce vaporisation and maintain an effective concentration of ethyl formate for >20 hours, and to avoid liquid ethyl formate accumulating at the bottom of the bin. With wheat, the concentration of ethyl formate was maintained at effective levels for about 2 days, all insects at all stages were killed rapidly, and by 3–5 days after application, the residues were reduced to natural levels without aeration. Faba bean sorbed ethyl formate strongly and the residues persisted longer, but full control was achieved. Control was high but not 100% in the sorghum trials. Residues in the sorghum at 10°C persisted significantly longer than at 20°C. During application and fumigation, the levels of ethyl formate in the working environment did not exceed the worker safety level of 100 ppm (threshold limit value). Field trials have shown that ethyl formate has good potential as a fumigant in unsealed farm bins. Unlike phosphine, which takes days to kill insects, ethyl formate kills rapidly. Residues fall to natural levels without aeration. It also has the added advantage of degrading to non-poisonous, naturally occurring products (formic acid and ethanol).

Introduction

After 2005, methyl bromide will be phased out and carbon disulfide is now no longer registered for use as a fumigant in NSW. Hence, phosphine is set to become the only registered fumigant available for farm use in Australia. Fumigation with phosphine requires a long (>5 days) exposure in sealed bins at temperatures above 15°C. The majority of existing farm bins are unsealed, and are therefore unsuitable for effective fumigation as concentrations cannot be maintained for the time required for total insect control (Bridgeman 2002). The over-reliance on phosphine in unsealed bins in Australia has resulted in (1) a higher frequency of resistance (Collins et al. 2002, 2003), (2) dangerous practices, and (3) grain delivered to grain depots containing live insects and unreacted aluminium phosphide residues (Pratt and Desmarchelier 1998). There is an urgent requirement for the development of a multifunctional grain treatment for on-farm use which should ideally be inexpensive and easy to handle and administer.

For the past few years, the CSIRO Stored Grain Research Laboratory (SGRL) has been re-evaluating ethyl formate as an alternative fumigant for grain stored in unsealed farm bins (Annis 2002). Ethyl formate has a long history of use as a fumigant for stored products and is currently registered as a fumigant for dried fruit in Australia. It is a colourless liquid with a low boiling point (54.1°C) and a pleasant, aromatic odour. It occurs naturally in soil, the ocean, vegetation and a range of food products, including vegetables, fruits, grain, beer, and animal products such as milk and cheese (Desmarchelier et al. 1998; Ren and Desmarchelier 2001; Le and Ren 2004).

Field trial procedure

Protocol

The trials were conducted under an experimental permit from the National Registration Authority (NRA PER3366) (now known as the Australian Pesticides and Veterinary Medicines Authority, or APVMA). The current maximum residue limit (MRL) for ethyl formate in the dried fruit industry is 1 mg/kg (ppm). The APVMA does not specify an ethyl formate MRL for other stored commodities, as it considers the residues should be identical to those commonly found in untreated food and hence of no toxicological significance. Consequently, for this trial, it was required that the residue levels of the treated grains should be no more than the natural levels (0.6 mg/kg) occurring in the untreated control samples. The in-bin concentration of
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ethyl formate at outloading was required to be below the threshold limit value (TLV) of 100 ppm v/v. The trials are summarised in Table 1.

**Ambient conditions of temperature and humidity**

During fumigation, grain temperature, headsapce air temperature and relative humidity were monitored with two HOBO® data loggers (Onset Computer Corporation, MA 02532, USA). For measurement of grain temperature, one HOBO® was placed in the centre of the silo and 1 m below the grain peak (Figure 1). For measurement of headsapce air temperature and relative humidity, the other HOBO® was hung in the headspace of the silo and 0.5 m above the grain peak (Figure 1). The HOBO® data loggers had previously been calibrated in the laboratory against each other and an alcohol glass thermometer.

**Table 1.** Details of farm bins fumigated with ethyl formate using three commodities.

<table>
<thead>
<tr>
<th>Conditions</th>
<th>Bin no.</th>
<th>Wheat (Harden, NSW)</th>
<th>Faba bean splits (Two Wells, SA)</th>
<th>Sorghum (Warwick, Qld)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bin capacity (t)</td>
<td>1</td>
<td>125</td>
<td>75</td>
<td>145</td>
</tr>
<tr>
<td>Amount of grain (t)</td>
<td>1</td>
<td>125</td>
<td>75</td>
<td>140</td>
</tr>
<tr>
<td>Bin capacity (t)</td>
<td>2</td>
<td>125</td>
<td>75</td>
<td>135</td>
</tr>
<tr>
<td>Amount of grain (t)</td>
<td>2</td>
<td>125</td>
<td>75</td>
<td>15.2</td>
</tr>
<tr>
<td>Moisture content (%)</td>
<td>1</td>
<td>11.1</td>
<td>11.8</td>
<td>15.2</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>11.3</td>
<td>11.8</td>
<td>15.6</td>
</tr>
<tr>
<td>Grain temperature (°C)</td>
<td>1</td>
<td>34</td>
<td>27</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>34</td>
<td>27</td>
<td>10</td>
</tr>
<tr>
<td>Double dose of ethyl formate</td>
<td></td>
<td>85 g/t × 2</td>
<td>85 g/t × 2</td>
<td>85 g/t × 2</td>
</tr>
</tbody>
</table>

**Figure 1.** Schematic representation of an unsealed farm bin, flat or cone bottom silo, gas sampling ports (1–16) and ethyl formate application system.

- Gas sample ports 3, 6, 9 and 12 were located at 1 m below the grain surface and 0.4–0.5 m from the silo wall.
- Gas sample ports 2, 5, 8 and 11 were located at 2.5 m below the grain surface and 0.4–0.5 m from the silo wall.
- Gas sample ports 1, 4, 7 and 10 were located at 4 m below the grain surface and 0.4–0.5 m from the silo wall.
- Gas sample ports 13, 14 and 15 were located at 1, 2.5 and 4 m below the grain surface in the centre of the silo.
- Gas sample port 16 was located at 0.5 m above the grain surface in the central headspace.
Materials and application

The ethyl formate formulation used was Eranol® supplied by Orica. The calculated application rate of the Eranol® active ingredient (ethyl formate) was 85 g/t. The liquid ethyl formate was applied as a pulsed, or double, dose to the top of the grain through a perforated polyvinyl (PVC) probe (φ 4 cm × 1.2 m) (made by CSIRO Entomology workshop). In this method, application through the probe takes about 10 minutes, and the second dose is applied 2–4 hours after the first. This method of application was chosen to: (1) maintain ethyl formate concentrations below the flammable level; (2) reduce vaporisation and maintain an effective concentration of ethyl formate for >20 hours; and (3) avoid liquid ethyl formate accumulating at the bottom of the bin.

Measuring in-bin concentration of ethyl formate during fumigation and airing

The silo was fitted with 16 gas-sampling lines (nylon, 3 mm i.d.) which led to a sampling position outside the hazard area (8 m from the silo). The location of gas-sampling ports is shown in Figure 1.

During application, exposure and aeration, in-bin gas samples from each sampling point were withdrawn at timed intervals using an electric pump (2 L/min). They were stored in Tedlar® gas-sampling bags (Air Met Scientific Australia) until analysed, usually within 1 hour of sampling. Fumigant concentrations were determined on-site using a portable gas chromatograph (GC), the Photovac 10S Portable Air Analyzer.

Bioassays

Bioassays were conducted by placing mixed-age insects of a single species in either metal cones (2.5 cm × 3 cm o.d.) or metal mesh bags (10 cm × 15 cm × 1 cm) containing the standard laboratory culture medium for that species. These were inserted into the grain bulk at least 0.5 m from the walls and at a depth of 1–3 m (Figure 1). The bioassay samples were retrieved at the end of the fumigation period. The adult insects were counted, removed and the remaining mixed-age cultures incubated at 25°C and 55–60% relative humidity (RH). Subsequently, emerging adult insects were counted weekly for 6 weeks, and live and dead adults removed at each count. Laboratory cultures used in the bioassay were Callosobruchus phaseoli Gyll., Tribolium castaneum (Herbst), Rhyzopertha dominica (F.) and Sitophilus oryzae (L.).

Measuring environmental levels of fumigant during application and airing

During application and airing, environmental gas samples were taken with a 1 L syringe, at head height and down-wind at distances of 1.5 m, 3 m, 6 m and 15 m from the base of the bin. The samples were stored in Tedlar® bags, and the levels of fumigant were determined by GC. Before taking samples back to the laboratory, we prepared spiked standards on-site, by injecting 10 mL of diluted fumigant (4 mL of fumigant in 250 mL of glass bottle) into a Tedlar® bag (1 L), giving 44 ppm v/v of ethyl formate.

Ethyl formate was detected using a Varian 3600 Gas Chromatograph (GC), after isothermal separation on DB FFAP (J&W 125-3212), 15 m long, i.d. of 0.53 mm, with the oven temperature set at 70°C. A sample volume of 100 μL was injected manually into the GC. The levels of ethyl formate were calculated on the basis of peak areas which were calibrated periodically using gas standards.

Analysis of fumigant residues in grain

Before and after fumigation, 10–20 commodity samples of 200 mL (150 g) were taken via a probe from different positions in the bin to determine the distribution of levels of natural and fumigant residues of ethyl formate in the grain. Samples were analysed at the SGRL and the Queensland Department of Primary Industries (QDPI) laboratories. Residues were analysed by a solvent extraction method as described by Ren and Desmarchelier (2001) and Vu and Ren (2004).

Results and discussion

Ambient conditions of temperature and humidity

Figure 2 shows the changes in temperature and relative humidity in the headspace of the silo, and grain temperature during the fumigation. The temperature and relative humidity in the headspace of the silos varied between 18–48°C and 25–50% RH during the wheat trial at Harden, NSW, 3–32°C and 10–30% RH during the sorghum trial at Warwick, Queensland, and 17–34°C and 20–30% RH during the faba bean trial at Two Wells, SA.

During fumigation, the grain temperature was stable and was not affected by ambient temperature around each silo, i.e. 33.5 ± 0.7°C in wheat (bins 1 and 2, Harden, NSW), 20 ± 0.5°C (bin 1) and 10 ± 0.5°C (bin 2) in sorghum (Warwick, Queensland), and 26.5 ± 0.6°C in faba bean splits (bins 1 and 2, Two Wells, SA). The change in grain temperature was <0.7°C between day and night (Figure 2).

In-bin ethyl formate concentration during fumigation and airing

Concentrations of ethyl formate in the silos were measured at 16 in-bin sample ports at timed intervals during the fumigation of the wheat, sorghum and faba bean splits. The average of these concentrations after 1, 24 and 48 hours of application are shown in Table 2. The in-bin concentrations declined rapidly within the first day for sorghum and faba bean splits. The distribution of ethyl formate became relatively even 4 hours after the first dosing, in all but the low-temperature sorghum bin. With wheat and faba bean, the concentration of ethyl formate was maintained at effective levels for about 2 days. Faba bean and sorghum sorbed ethyl formate more
strongly than wheat. After 2 days, the concentration of ethyl formate declined to 8.5–8.8 g/m$^3$ in wheat bins, 3.4–3.6 g/m$^3$ in faba bean bins and 1.6–3.8 g/m$^3$ in sorghum bins. From days 2 to 5, the average readings fell from 8.8 g/m$^3$ to 0.1 g/m$^3$ for wheat at 33.5°C, from 3.5 g/m$^3$ to 0.05 g/m$^3$ for faba bean at 26.5°C, and from 3.8 g/m$^3$ to 0.1 g/m$^3$ for sorghum at 20°C and 10°C. Before opening the inloading hatch, concentrations were well below the safety workspace level of 100 ppm of ethyl formate v/v and further declined once the grain was aerated. This result is consistent with results from the previous trials with ethyl formate on wheat (Desmarchelier et al. 1998; Wright et al. 2002; Ren et al. 2003). The ethyl formate sorption profiles for freshly harvested sorghum and the older split faba bean were also examined in the laboratory. Both commodities showed rapid sorption of ethyl formate when added at single and double doses and ethyl formate declined to 10 g/t in the headspace within 24 h.

**Bioassays**

The results for liquid ethyl formate efficacy toward insects from all three farm-scale trials are shown in Table 3. With wheat and faba bean, the intra-bin concentration of ethyl formate was maintained at effective levels for about 2 days at grain temperature above 25°C. The concentration $\times$ time (Ct) products of 1100–2100 mg h/L obtained were sufficient to kill all insect stages rapidly (Tables 2–3). With sorghum, the temperature was below 20°C in bin 1 and below 10°C in bin 2, a relatively low Ct product of 850 mg h/L was obtained, and insects were controlled to a high level but not giving 100% mortality.

![Figure 2](image_url)

**Figure 2.** Temperature and relative humidity (RH) in the headspace of silo, and grain temperature, during the period of fumigation in Harden (NSW), Warwick (Queensland) and Two Wells (SA).
This is probably due to liquid ethyl formate more slowly vaporising to ethyl formate gas at these lower temperatures, and the grain also strongly absorbing the ethyl formate.

During the Harden, NSW trials, we found the natural insect population was >20 insect/kg after 6–8 weeks storage of the newly harvested wheat. The insects were *Tribolium* spp., *Sitophilus* spp., psocids and wasps. More than 2% of the wheat was damaged by the insects, causing loss of dry mass and quality (commercial value) of wheat. During the Two Wells, SA and Warwick, Queensland trials, we found similar damage on the sorghum and faba bean commodities. This indicates the loss of dry mass and commercial value due to insect damage is a common issue in on-farm storage.

**Workspace and environmental levels of ethyl formate**

During the three field applications, a total of 60 samples were taken in the working environment at distances of 3 m, 6 m and 15 m from the silos. The levels of ethyl formate in six air samples was lower than 15 ppm and did not exceed the worker safety level of 100 ppm (TLV). No ethyl formate was detected in the other 54 air samples, with a limit of detection of 0.5 ppm, v/v which is 0.5% of the TLV of 100 ppm v/v. Two to three days after ethyl formate application, 58 air samples were taken and the level of ethyl formate was below the limit of detection (0.5 ppm v/v) in all air samples. All readings taken to determine workspace and environmental levels of ethyl formate during application to the grain and during exposure were below the TLV limit of 100 ppm v/v. This result is consistent with results from the previous trials with ethyl formate on wheat (Desmarchelier et al. 1998; Wright et al. 2002; Ren et al. 2003).

**Residues and natural levels of ethyl formate in grain**

At the end of the fumigation, the top hatch was opened and grain samples were taken via a probe at depths of 0.5 m, 2 m and 3.5 m below the grain surface. The results of ethyl formate residues were obtained from two laboratories (SGRL and QDPI) and the data are shown in Table 4. More than 120 samples (including controls) were analysed by each laboratory. The data are compatible between two the laboratories. This inter-laboratory collaboration was successful with respect to sample organisation (preparation and delivery), communication, establishment of analytical methods and calculations. This experience could be used as a model for other fumigants and samples from future trials. The inter-laboratory work provided validation and reliable data, which is very important for registration of new fumigants or the re-evaluation of old fumigants.

Residues were even and declined uniformly and rapidly (Table 4). For wheat and sorghum, after 7 days fumigation, residues were reduced to natural levels (levels of ethyl formate in unfumigated samples) without aeration. For faba bean, the residues had declined to natural levels 3–4 weeks after fumigation. The ethyl formate residue profiles for freshly harvested sorghum and the older split faba bean were also examined in the laboratory. In freshly harvested sorghum, ethyl formate residues rapidly disappeared and were reduced to natural levels but faba bean took more than 26 days for residues to return to near background levels. These results support previous studies indicating that ethyl formate is rapidly sorbed and degraded by most commodities, especially where they have high moisture content or are warm. However, the residues in faba bean were slower to breakdown than expected. Residues in the sorghum at 10°C also persisted significantly longer than at 20°C.

**Table 2.** Mean intra-bin ethyl formate (EtF) concentrations after 1, 24 and 48 hours after application and contraction × time (Ct) product.

<table>
<thead>
<tr>
<th>Commodity</th>
<th>Bin no.</th>
<th>EtF concentration (g/m³)</th>
<th>Ct product (mg h/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Wheat</td>
<td>1</td>
<td>82.3</td>
<td>2140</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>80.2</td>
<td>2100</td>
</tr>
<tr>
<td>Faba bean</td>
<td>1</td>
<td>67.1</td>
<td>3.6</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>58.9</td>
<td>100%</td>
</tr>
<tr>
<td>Sorghum</td>
<td>1</td>
<td>57.7</td>
<td>3.8</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>58.9</td>
<td>1.6</td>
</tr>
</tbody>
</table>

**Table 3.** Control of insects using ethyl formate in farm bins containing different commodities.

<table>
<thead>
<tr>
<th>Commodity</th>
<th>Bin no.</th>
<th><em>Sitophilus oryzae</em></th>
<th><em>Rhyzopertha dominica</em></th>
<th><em>Tribolium castaneum</em></th>
<th><em>Callosobruchus phaseoli</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Wheat</td>
<td>1</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
<td>–</td>
</tr>
<tr>
<td>Faba bean</td>
<td>1</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>Sorghum</td>
<td>1</td>
<td>84(68–100)%</td>
<td>99(97–100)%</td>
<td>100%</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>96(84–100)%</td>
<td>100%</td>
<td>100%</td>
<td>–</td>
</tr>
</tbody>
</table>

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Table 4. The levels of ethyl formate (EtF) residues in fumigated commodities. The samples were collected 3–5 days after application of ethyl formate. The residues were analysed by the Stored Grain Research Laboratory (SGRL) and Queensland Department of Primary Industries (QDPI) (n = 4).

<table>
<thead>
<tr>
<th>Sampling location (m from top)</th>
<th>Levels of EtF residues in grains (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Wheat (Harden, NSW)</td>
</tr>
<tr>
<td></td>
<td>SGRL</td>
</tr>
<tr>
<td>Centre (0.5)</td>
<td>4.4 ± 0.3</td>
</tr>
<tr>
<td>Centre (2)</td>
<td>2.8 ± 0.2</td>
</tr>
<tr>
<td>Centre (3.5)</td>
<td>5.3 ± 0.5</td>
</tr>
<tr>
<td>North (0.5)</td>
<td>4.2 ± 0.4</td>
</tr>
<tr>
<td>North (2)</td>
<td>0.9 ± 0.1</td>
</tr>
<tr>
<td>North (3.5)</td>
<td>2.1 ± 0.2</td>
</tr>
<tr>
<td>South (0.5)</td>
<td>0.7 ± 0.08</td>
</tr>
<tr>
<td>South (2)</td>
<td>0.3 ± 0.05</td>
</tr>
<tr>
<td>South (3.5)</td>
<td>0.3 ± 0.04</td>
</tr>
<tr>
<td>Control</td>
<td>0.05 ± 0.01</td>
</tr>
</tbody>
</table>

Conclusions

Field trials have shown that ethyl formate has good potential as a fumigant in unsealed farm bins. Unlike phosphine, which takes days to kill insects, ethyl formate kills rapidly (in about 20 hours). At the dosage of 85 g/t × 2, ethyl formate gives a high level of control of the all stages of most of the tested insects. However, it is very hard to achieve complete control of insects when the grain temperature is lower than 15°C. Therefore, we suggest that the marginal grain temperature is 15°C for use of ethyl formate as a grain fumigant. Ethyl formate, as shown in these and other trials, has advantages in terms of worker and environment safety. During application and fumigation, the levels of ethyl formate in the working environmental did not exceed the worker safety level of 100 ppm (TLV). Ethyl formate residues can be reduced to natural levels without aeration.

Acknowledgments

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References


