VAPORMATE™, a formulation of ethyl formate with CO₂, for disinfestation of grain

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Abstract. Ethyl formate (EtF) is being evaluated as a fumigant for the rapid disinfestation of grain, including a cylinderised mixture of EtF in carbon dioxide (16.7% by weight), VAPORMATE™, formulated by BOC Ltd. Insect mortality achieved with a 3-hour dynamic (forced-flow) application versus a static application of the EtF formulation was investigated. In the dynamic application, gaseous VAPORMATE™ was pumped through grain in a 65 L model silo at 6 L/min. In the static application, 2.7 L desiccators were dosed with equivalent concentrations of the EtF formulation applied to the headspace. The mortality of mixed-age cultures of the most tolerant insect species, *Sitophilus oryzae* (L.) and *Tribolium castaneum* (Herbst), were compared in the two systems over a range of EtF concentrations. The mortality of *S. oryzae* was also compared after a 3 or 24 h dynamic application of this EtF formulation.

A high level of insect control is achievable with a 3-hour VAPORMATE™ fumigation, and extending the fumigation time to 24 hours improves the level of insect control. The 3-hour dynamic application gave higher mortality than the static application. An application of EtF at a rate of 147 g/t grain by forced-flow fumigation gave very high control (>99% mortality) of a phosphine-resistant strain of *Rhizopertha dominica* and the *T. castaneum* but lower control (82% mortality) of *S. oryzae*. Extending the fumigation time to 24 hours and reducing the applied amount of EtF to 100 g/t grain resulted in an improvement of the mortality of *S. oryzae* to 86%.

Introduction

Insect control in stored grain in Australia, in particular in on-farm storages, relies heavily on phosphine and dichlorvos. The wide-scale, convenient use of these materials continues to be threatened by the development of insect resistance. Alternative treatments are necessary and ethyl formate (EtF) has shown potential in this role, in particular for the rapid disinfestation of grain. EtF is currently used as a fumigant of dried fruit in Australia and elsewhere.

Extensive work has been carried out with EtF where it has been found to be effective against stored grain insects (Muthu et al. 1984; Hilton and Banks 1997). More recently, it has been reported that EtF can have a very rapid action against stored grain insects and this property may make it useful for rapid disinfestation of grain (Damcevski and Annis 2001, 2002). We have found that improved insect control is obtained when using gaseous carbon dioxide (CO₂) with EtF.

The aim of this work was to investigate VAPORMATE™, a cylinderised formulation of EtF (16.7% by weight) mixed with CO₂, formulated by BOC Ltd for control of stored grain insects. We investigated the mortality of fumigant-tolerant insect species in 3-hour dynamic (forced-flow) applications versus static applications of the EtF formulation. The insect species studied were laboratory strains of *Sitophilus oryzae* (L.) and *Tribolium castaneum* (Herbst), and a phosphine-resistant field strain of *Rhizopertha dominica* (F.). The *R. dominica* strain was selected because it represents a problem to the grain industry and, while cross-resistance to EtF has been shown not to occur (P.J. Collins and G.J. Daglish, pers. comm.), it is important to demonstrate the efficacy of EtF, as it could be a valuable treatment in resistance management where it is effective against phosphine-resistant insects. In addition, the mortality of *S. oryzae* achieved after a 3-hour dynamic application was also compared to the mortality after a 24-hour application.

The dynamic application was a model for farm-bin application via an aeration or drying system. The static application could represent an application into the headspace of a farm bin, with no assistance from air or gas flow; however, in this model, the slow and shallow penetration of EtF observed in desiccators (relative to the dynamic application) would perhaps be even more problematic in a farm bin.
Materials and methods

Culture preparation

Mixed-age cultures of *S. oryzae*, *T. castaneum* and *R. dominica* were set up by adding adults to media at 30°C and 55% relative humidity (RH). Cultures were left for 5 weeks, after which time they contained representative numbers from each stage; egg, larval, pupal and adult, based on knowledge of development rates (Howe 1952; Beckett et al. 1994). The insects were Stored Grain Research Laboratory (SGRL) strains CLS2 and CTC4, and a field strain QRD569, respectively. QRD569 is a phosphine-resistant field strain collected from southern Queensland (via Queensland Department of Primary Industries). Table 1 lists the experimental conditions of the cultures. The wheat used for all cultures and exposures was of the variety Rosella, Australian standard white (ASW) grade, 12% moisture content (wet basis).

The cultures were prepared for exposure by first passing them through a Börner divider three times to mix them thoroughly. Each culture was then split into replicates and weighed. Test insects were placed in stainless steel mesh discs or bags before being placed into the fumigation container. One replicate was kept as a control. This was done for each species and exposure detailed in Table 1.

Dynamic exposures

Exposures were carried out at 25°C in a sealed model silo (Figure 1) for 3 or 24 hours (Table 1). The model silo held 50 kg wheat and was 65 L in volume, giving a fill ratio of 95%. Three stainless steel mesh discs were used to each hold 350 or 400 g of insect culture. These cages were of a diameter to give a tight fit inside the model silo, so that no EtF could flow around their outside edges. Sample ports above each cage were used to take gas samples for analysis by gas chromatography.

VAPORMATE™ formulation was obtained by adding liquid EtF and gaseous CO$_2$ to a total of 65 L in a 100 L Tedlar® bag, and allowing the EtF to volatilise and mix with the CO$_2$.

![Figure 1. The 65 L model silo used for the dynamic applications of VAPORMATE™.](image)

<table>
<thead>
<tr>
<th>Species (strain)</th>
<th>Application</th>
<th>Media composition + insect numbers</th>
<th>Culture age at exposure (days)</th>
<th>Sample</th>
<th>Average sample weight (g)</th>
<th>Exposure time (hours)</th>
<th>Ethyl formate (g/t wheat)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. oryzae</em> (CLS2)</td>
<td>Dynamic</td>
<td>1.6 kg wheat + 800 adults</td>
<td>35</td>
<td>Test Control</td>
<td>400 x 3 300</td>
<td>3 24</td>
<td>147 + 209 100</td>
</tr>
<tr>
<td></td>
<td>Static</td>
<td>2 kg wheat + 1000 adults</td>
<td>35</td>
<td>Test Control</td>
<td>100 400</td>
<td>3</td>
<td>48–214</td>
</tr>
<tr>
<td><em>T. castaneum</em> (CTC4)</td>
<td>Dynamic</td>
<td>1.4 kg flour + 120 g yeast + 800 adults</td>
<td>37</td>
<td>Test Control</td>
<td>350 x 3 250</td>
<td>3</td>
<td>147</td>
</tr>
<tr>
<td></td>
<td>Static</td>
<td>1.4 kg flour + 120 g yeast + 800 adults</td>
<td>37</td>
<td>Test Control</td>
<td>100 340</td>
<td>3</td>
<td>107–214</td>
</tr>
<tr>
<td><em>R. dominica</em> (QRD569)</td>
<td>Dynamic</td>
<td>1.6 kg wheat + 260 g flour + 800 adults</td>
<td>36</td>
<td>Test Control</td>
<td>400 x 3 600</td>
<td>3</td>
<td>147</td>
</tr>
</tbody>
</table>
A range of EtF and CO₂ concentrations was bioasayed (Table 1). The EtF formulation was applied to the model silo at a rate of 6 L/min using a peristaltic pump and monitored with a flow meter. This flow rate was selected to achieve an even distribution of EtF in the quickest time possible. The 65 L of EtF formulation were pumped into the model silo, taking approximately 12 minutes, and representing one gas exchange. This was the forced-flow component of the application. The model silo was then left sealed for 3 or 24 hours. It was then opened, aired and the cultures removed and placed inside a fumehood to air for a further 24 hours before being placed in a controlled temperature room at 25°C and 55% RH.

Static exposures

Exposures were carried out at 25°C in gastight 2.7 L glass desiccators for 3 hours. Either 1 or 1.6 kg wheat was placed in each desiccator, giving a fill ratio of 50% or 75%, respectively. Stainless-steel mesh bags were used to hold 100 g of insect culture which were then placed inside the wheat. The desiccators were sealed with glass stoppers containing a septum through which fumigant was dosed and gas samples were taken for analysis by gas chromatography. EtF was applied by dosing as a liquid onto a filter paper inside the stopper and allowing it to volatilise into the headspace; gaseous CO₂, equivalent to the VAPORMATE™ mixture, was dosed straight into the headspace. A range of EtF concentrations was bioassayed (Table 1). The desiccators were left sealed for 3 hours and the cultures then aired as for the dynamic exposures.

Fumigant measurement

Ethyl formate concentrates were monitored by measuring against standard concentrations using a Varian 3800 gas chromatograph fitted with a flame ionisation detector and a Zebron ZB/1 column (15 m × 0.53 mm id), run isothermally at 100°C. CO₂ concentrations were monitored by measuring with a Fisher model 1200 gas chromatograph fitted with a flame ionisation detector and a Zebron ZB/1 column (15 m × 0.53 mm id), run isothermally at 100°C. CO₂ concentrations were monitored by measuring with a Fisher model 1200 gas chromatograph fitted with a flame ionisation detector and a Zebron ZB/1 column (15 m × 0.53 mm id), run isothermally at 100°C. CO₂ concentrations were monitored by measuring with a Fisher model 1200 gas chromatograph fitted with a flame ionisation detector and a Zebron ZB/1 column (15 m × 0.53 mm id), run isothermally at 100°C. CO₂ concentrations were monitored by measuring with a Fisher model 1200 gas chromatograph fitted with a flame ionisation detector and a Zebron ZB/1 column (15 m × 0.53 mm id), run isothermally at 100°C. CO₂ concentrations were monitored by measuring with a Fisher model 1200 gas chromatograph fitted with a flame ionisation detector and a Zebron ZB/1 column (15 m × 0.53 mm id), run isothermally at 100°C. CO₂ concentrations were monitored by measuring with a Fisher model 1200 gas chromatograph fitted with a flame ionisation detector and a Zebron ZB/1 column (15 m × 0.53 mm id), run isothermally at 100°C. CO₂ concentrations were monitored by measuring with a Fisher model 1200 gas chromatograph fitted with a flame ionisation detector and a Zebron ZB/1 column (15 m × 0.53 mm id), run isothermally at 100°C. CO₂ concentrations were monitored by measuring with a Fisher model 1200 gas chromatograph fitted with a flame ionisation detector and a Zebron ZB/1 column (15 m × 0.53 mm id), run isothermally at 100°C. CO₂ concentrations were monitored by measuring with a Fisher model 1200 gas chromatograph fitted with a flame ionisation detector and a Zebron ZB/1 column (15 m × 0.53 mm id), run isothermally at 100°C. CO₂ concentrations were monitored by measuring with a Fisher model 1200 gas chromatograph fitted with a flame ionisation detector and a Zebron ZB/1 column (15 m × 0.53 mm id), run isothermally at 100°C. CO₂ concentrations were monitored by measuring with a Fisher model 1200 gas chromatograph fitted with a flame ionisation detector and a Zebron ZB/1 column (15 m × 0.53 mm id), run isothermally at 100°C. CO₂ concentrations were monitored by measuring with a Fisher model 1200 gas chromatograph fitted with a flame ionisation detector and a Zebron ZB/1 column (15 m × 0.53 mm id), run isothermally at 100°C. CO₂ concentrations were monitored by measuring with a Fisher model 1200 gas chromatograph fitted with a flame ionisation detector and a Zebron ZB/1 column (15 m × 0.53 mm id), run isothermally at 100°C. CO₂ concentrations were monitored by measuring with a Fisher model 1200 gas chromatograph fitted with a flame ionisation detector and a Zebron ZB/1 column (15 m × 0.53 mm id), run isothermally at 100°C.

Survivorship assessment

The survivorship of each species was recorded at fixed times and compared to the controls held at the same exposure temperatures. Adults were removed from the cultures, including the control replicate, 1 day after exposure and mortality assessed after 5–6 days. Subsequently, adults from the cultures were removed and counted weekly for 5–6 weeks. In this way, adults at each weekly count represented an arbitrary immature stage at the time of treatment. Adults at the first weekly count were likely to have been in-grain adults (for CLS2 and QRD569) or pupae at the time of treatment, and those at the last weekly count were likely to have been eggs or early larvae. After each count, the adults were discarded. During the post-treatment period, all cultures were kept at 25°C and 55% RH.

Results and discussion

The mortality of the whole cultures of S. oryzae, T. castaneum, and R. dominica is shown in Figures 2 and 3. As mentioned above, these cultures were made up of all developmental stages; egg, larvae, pupae and adults. In Figure 2, the mortality obtained for S. oryzae exposed to 3-hour dynamic and static applications of VAPORMATE™ is given. The dynamic application gave better control of the whole culture of insects than the static application for equivalent concentrations of EtF. This was most likely due to the improved distribution of EtF through the grain, resulting in the fumigant reaching the insects faster.

For 24-hour exposure of mixed-age cultures of S. oryzae, the dose of EtF was lowered by a third to 100 g/t grain (based on an observation where improved insect control in a static system was obtained for 24-hour over 3-hour exposure). A slightly higher level of control of the insects was obtained at 100 g/t for 24 hours than was obtained at 147 g/t for 3 hours, indicating that extended exposure times contributed a great deal to the mortality observed, even with the high level of sorption of EtF that occurs on wheat (Desmarchelier et al. 1998; Damecevski and Annis 2001). This also suggests that leaving the fumigant with the grain for even longer, e.g. 48 hours, may achieve a higher mortality rate.

Figure 3 shows the mortality obtained for T. castaneum exposed to 3-hour dynamic and static applications of VAPORMATE™. Again, the dynamic application gave better control of the insects than static application for equivalent concentrations of EtF.

For the dynamic exposure of the phosphine-resistant R. dominica, a very high mortality of over 99.95% was obtained after a 3-hour exposure after application of 147 g/t (Figure 3). This value was obtained from exposing more than 22,000 insects to the EtF formulation with only 12 survivors. It has been shown that there is no cross-resistance between phosphine and EtF in R. dominica (P.J. Collins and G.J. Daglish, pers. comm.). However, it was desirable to show that this short VAPORMATE™ application could disinfect grain heavily infested with a phosphine-resistant strain which represents a problem to the grain industry, and confirms that VAPORMATE™ may be useful in a phosphine resistance management strategy. An application rate of 100 g/t for 24 hours will be bioassayed for T. castaneum and the phosphine-resistant R. dominica to investigate the effect of longer exposure times at lower doses.

Figure 4 gives an example of the mortality obtained for S. oryzae stages exposed to a 3-hour dynamic application of VAPORMATE™ at 209 g EtF/t grain. Most of the survivors emerged as adults 7–21 days after exposure, and
most likely were pupae when fumigated, based on knowledge of development rates (Howe 1952).

The mortality data for the 3-hour dynamic applications of mixed stages of *S. oryzae*, *T. castaneum*, and *R. dominica* with the EtF formulation at 147 g/t grain are summarised in Figure 5. They show the very good control of large numbers of insects of each species that is achievable with a very short application. However, *S. oryzae* pupae remain the most tolerant of the common stored-product insects and further investigation is needed to determine how to control them successfully.

These mortality data compare very well to those published for dichlorvos. Dichlorvos is relatively fast acting and behaves as a fumigant in a similar manner to EtF, even though it is less volatile (Strong and Sbur 1964; Champ et al. 1969; McGaughey 1970). It is also expected that VAPORMATE™ could replace dichlorvos as an option for quick disinfestation of stored grains. Various publications show the mortality of *S. oryzae* juveniles achieved when dichlorvos is applied as a spray to grain. Strong and Sbur (1964) found that an application of 10 g/t gave 90% mortality, Champ et al. (1969) found that 5 and
10 g/t gave approximately 50 and 70% mortality, respectively, and McGaughey (1970) found that 5, 10 and 15 g/t gave 82, 89 and 93% mortality, respectively. The variability in the data possibly derives from different proportions of juveniles present in the exposures. Champ et al. (1969) found that *S. oryzae* pupae and later-stage larvae were very tolerant of dichlorvos.

It is also known that resistance to dichlorvos is found in *R. dominica* in the field (P.J. Collins, pers. comm.), and is suspected to be widespread. VAPORMATE™ would be a better option for control of this resistant strain, as well as the phosphine-resistant strains, and would give comparable control of *S. oryzae* juveniles.

**Conclusions**

It was found that a dynamic (forced-flow) application of VAPORMATE™, ethyl formate in carbon dioxide, gave better control of stored grain insects than a static application. A very high level of mortality (>99%) was achieved in dynamic applications of EtF of 147 g/t grain for 3 hours for the phosphine-resistant field strain of *R. dominica* and the laboratory strain of *T. castaneum*, but lower control (82% mortality) of *S. oryzae*. Extending the fumigation time to 24 hours and reducing the applied amount of EtF to 100 g/t grain resulted in an improvement in the mortality (86%) of *S. oryzae*. Extending the fumigation

![Insect stage at time of treatment](image)

**Figure 4.** Mortality of *Sitophilus oryzae* (L.) stages exposed to a 3-hour dynamic application of VAPORMATE™, at the dose rate of ethyl formate of 209 g/t grain.

![Mortality of mixed-age cultures](image)

**Figure 5.** Mortality of mixed-age cultures of the most tolerant stored grain insect species exposed to a 3-hour VAPORMATE™ fumigation with a dose of ethyl formate of 147 g/t grain.
time improves the level of insect control, and would allow the application rate to be lowered, if desired.

Further work is needed before VAPORMATE™ is trialled in farm bins to determine:

- the application rate for control of *T. castaneum* and *R. dominica* in a 24-hour fumigation
- a higher EtF dose and longer exposure time for better control of *S. oryzae* juveniles, with the possibility of using a double dose
- the limits of grain temperature and moisture for fumigation.

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References


