Seed store disinfection trials with VAPORMATE™
(ethyl formate + CO₂)

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Abstract. In December 2002, a semi-sealed seed store (260 m³ capacity), 30% loaded with newly harvested seeds of barley, wheat and sorghum, was treated with VAPORMATE™ (ethyl formate + CO₂). In total, 40 kg of VAPORMATE™ containing 6.85 kg of ethyl formate was applied in three doses (within 20 hours) to achieve a relatively constant ethyl formate concentration of about 19–23 g/m³ for slightly more than two days. Two hours after application, the ethyl formate was evenly distributed throughout the seed store and had penetrated into seed bags. All tested mixed-aged insects of Callosobruchus phaseoli, Tribolium castaneum, Rhyzopertha dominica, Sitophilus oryzae, Oryzaephilus spp., Cryptolestes spp. and psocids were controlled. During the fumigation period, the concentrations of ethyl formate in the adjacent stores and 1 m and 3 m from the front door of the fumigated store were 5–25 ppm lower than the threshold limit value (TLV) of 100 ppm. After fumigation, ethyl formate residues declined to natural levels in semi-sealed conditions. VAPORMATE™ had no effect on the germination of the stored seeds.

Introduction

Previous attempts have been made to disinfect seed stores with insecticides such as dichlorvos and pyrethrins. However, the insecticides resulted in detection of residues, particularly in oil-containing seeds. Moreover, these insecticides were unable to penetrate the seed bags completely—achieving only surface disinfection of the storage structure and bags. Phosphine is currently the only fumigant widely available for grain storage and structural treatment. However, it is not suitable for seed-store fumigation as it typically involves a 7–20 day fumigation in a sealed environment at temperatures above 15°C, and can have a phytotoxic effect on treated seeds. Thus, there is an urgent need to develop a seed and seed-store treatment which is safe and easy to apply.

Ethyl formate as a fumigant was successfully evaluated through the Grains Research and Development Corporation (GRDC) Project CSE164, Strategic toxicology in support of phosphine and alternative fumigants, and the GRDC Project CSE168, Development of application systems for the fumigants ethyl formate and carbon disulfide in unsealed farm bins. Allen and Desmarchelier (2002) reported that ethyl formate mixed with carbon dioxide was successful as a fast fumigant for disinfection of sampling systems at a grain-exporting terminal. Ethyl formate is a liquid fumigant that is currently registered in Australia for use on dried fruit. It has attributes of high volatility and rapid action against insects, indicating its suitability to fulfil some of the theoretical requirements for fumigants to be used in partially sealed structures (Banks and Desmarchelier 1979). Here, we report on the use of VAPORMATE™ (ethyl formate + CO₂), which was developed by BOC Australia, to treat the seed store and seeds at the Hermitage Research Station, Warwick, Queensland.

Seed-store fumigation procedure

Protocol

The current maximum residue limit (MRL) for ethyl formate in the dried fruit industry is 1 mg/kg. The Australian Pesticides and Veterinary Medicines Authority (APVMA; previously the National Registration Authority, or NRA) 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 seeds should be no more than the natural levels (0.6 mg/kg) occurring in the untreated control samples. The in-bin concentration of ethyl formate after ventilation was required to be below the threshold limit value (TLV)
of 100 ppm v/v. The trial permit (PER5060) is summarised as follows:
• ethyl formate (95 g/t); commodity; 200 t
• ethyl formate (2 g/t); structural; 10,000 m².

Seed-store ambient conditions

During fumigation, the seed-store ambient air temperature and relative humidity were monitored with HOBO® data loggers (Onset Computer Corporation, MA 02532, USA). For the measurement of ambient temperature, the HOBO® was placed in the centre of the seed store and 3 m above the ground. All the HOBO® data loggers had previously been calibrated in the laboratory against each other and an alcohol glass thermometer.

Materials and application

The seed store, of 260 m³ capacity, was semi-sealed and 30% loaded with newly harvested and bagged seeds of barley and wheat in 1 kg bags (40 bags in a crate) and sorghum in 20 kg bags (Figure 1).

The ethyl formate formulation used was VAPORMATE™ (16.7% by weight ethyl formate; 83.3% by weight carbon dioxide) supplied by BOC in a ‘G’-size cylinder. The VAPORMATE™ was applied into the store through a spray nozzle, with a calculated application rate of active ingredient (ethyl formate) of 25 g/m³. In total, 54 kg of VAPORMATE™ containing 8.4 kg of ethyl formate was applied in three doses (topped up at 2 hours and 20 hours after the first dose).

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

Before the application of the gas, the store was fitted with six gas-sampling lines (nylon, 3 mm i.d.), 1 and 3 m above floor level with three lines at each level. A monitoring station was set up 8 m from the store and outside a 3 m restricted area.

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-aged insects of a single species in metal mesh bags (10 cm × 15 cm × 1 cm) and small tubular cages (15 mL volume, metal sides and mesh ends) containing the standard laboratory culture medium for that species. These were placed around the room and in seed containers, at 1 and 3 m above the floor and at least 0.5 m from the walls. After 2 days of fumigation, the seed store was opened and all test insect cages were retrieved. Insects were counted, adults removed and the cultures incubated at 25°C and 55–60% relative humidity (RH). Subsequently, emerging
adult insects were counted weekly for a period of 8 weeks, and live and dead adults removed at each count. Laboratory cultures used in the bioassay were: Tribolium castaneum (Herbst), Rhyzopertha dominica (F.), Sitophilus oryzae (L.), Callosobruchus phaseoli (Gyll.), Oryzaephilus spp., Cryptolestes spp. and psocids.

Measuring environmental levels of fumigant during application and airing

During application, fumigation and airing, environmental gas samples were taken with a 1 L syringe at head height around the building (the adjacent stores) at distances of 1.5 m, 3 m and 6 m from the front door of the fumigated store. The samples were stored in Tedlar® bags, and the levels of fumigant were determined by GC at the Stored Grain Research Laboratory (SGRL).

Ethyl formate levels were determined on a Varian 3600 GC, after isothermal separation on DB FFAP (J&W 125-3212), 30 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 for analysis of ethyl formate residues in grain, whose concentration was calculated on the basis of peak areas. The peak areas were calibrated periodically using gas standards.

Analysis of fumigant residues in grain

Before and after fumigation, eight lots of seed samples (each sample 100 g) were taken from different positions in the store to determine the distribution of levels of natural and fumigant residues in the grain. All samples were analysed by the SGRL laboratory by a solvent extraction method as described by Ren and Desmarchelier (2001) and Le and Ren (2004).

Germination test

Germination rates and plumule lengths were determined on representative samples taken before and after fumigation. Germination tests on the grain use the procedure of Ghaly and Van Der Touw (1982), based on the rules of the International Seed Testing Association (1976).

Results and discussion

A semi-sealed seed store (260 m³ capacity), 30% loaded with newly harvested seeds of barley, wheat and sorghum was treated with VAPORMATE™ at temperatures of 24–28°C and 52–58% RH (Figure 2) in December 2002. In total, 54 kg of VAPORMATE™ containing 8.4 kg of ethyl formate was applied in three doses (the second and third doses were applied 2 hours and 20 hours after first dosing, respectively). Two hours after applying VAPORMATE™, the ethyl formate was evenly distributed throughout the seed-store space and had penetrated into seed bags. An effective ethyl formate concentration of 16–28 g/m³ was maintained for about two days (Figure 3).

The results from the bioassays show that all mixed-aged insects of Callosobruchus phaseoli, Tribolium castaneum, Rhyzopertha dominica, Oryzaephilus spp. and Cryptolestes spp. were controlled, but the egg stage of Sitophilus oryzae was not (Table 1). Complete control might be achieved with
an extended exposure time of 3 days, as the concentration of ethyl formate was still maintained at the effective level (16 g/m³) until the end of the trial on day 2. The results are consistent with those reported by Allen and Desmarchelier (2002). They used ethyl formate mixed with carbon dioxide to control insects in grain-sampling systems at grain-export terminals.

Ethyl formate did not affect either germination (7-day count) or plumule length of barley, wheat and sorghum. These results are consistent with data reported after a trial with ethyl formate on wheat, barley, oats and canola (Ren et al. 2003) and Dun field peas (Mahon et al. 2003), where ethyl formate had no effect on the viability of peas.

During the fumigation period, the concentrations of ethyl formate in the adjacent stores and 1 m and 3 m from the front door of the fumigated store were 5–25 ppm lower than the TLV of 100 ppm. Ethyl formate residues declined to background levels without aeration.

Conclusions

The results indicate that VAPORMATE™ is a highly effective formulation of ethyl formate very suitable for seed-store disinfection, as it had a fast application, was evenly distributed within the storage space and seed bags, and had no effect on germination or the working environment.

Table 1. Insects emerging after treatment with VAPORMATE™.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Insect species</th>
<th>Live insects before fumigation</th>
<th>Live insects after fumigation</th>
<th>Further live insects emerging after holding for 7, 28 or 56 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>QOS301</td>
<td><em>Oryzaephilus</em> spp.</td>
<td>124</td>
<td>0</td>
<td>0 0 0</td>
</tr>
<tr>
<td>QRD569</td>
<td><em>Rhyzopertha dominica</em></td>
<td>102</td>
<td>0</td>
<td>0 0 0</td>
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<tr>
<td>QRD369</td>
<td><em>R. dominica</em></td>
<td>67</td>
<td>0</td>
<td>0 0 0</td>
</tr>
<tr>
<td>QTC931</td>
<td><em>Tribolium castaneum</em></td>
<td>54</td>
<td>0</td>
<td>0 0 0</td>
</tr>
<tr>
<td>QNCF037</td>
<td><em>Cryptolestes</em> spp.</td>
<td>45</td>
<td>0</td>
<td>0 0 0</td>
</tr>
<tr>
<td>QSO335</td>
<td><em>Sitophilus oryzae</em></td>
<td>14</td>
<td>0</td>
<td>0 5 7</td>
</tr>
<tr>
<td>Bruchids</td>
<td><em>Callosobruchus phaseoli</em></td>
<td>224</td>
<td>0</td>
<td>0 0 0</td>
</tr>
<tr>
<td>Psocids</td>
<td></td>
<td>&gt;500</td>
<td>0</td>
<td>0 0 0</td>
</tr>
</tbody>
</table>

Figure 3. The concentration of ethyl formate (EtF) at different locations in the seed store and inside a seed (sorghum) bag.
Acknowledgments

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References


