From: E.J. Wright, M.C. Webb and E. Highley, ed., Stored grain in Australia 2003.
Proceedings of the Australian Postharvest Technical Conference, Canberra,
25–27 June 2003. CSIRO Stored Grain Research Laboratory, Canberra.

# The potential of 1,8-cineole as a fumigant for stored wheat

*Byung-Ho Lee*
Stored Grain Research Laboratory, CSIRO Entomology, GPO Box 1700, Canberra, ACT 2601;
University of Newcastle, School of Applied Science, PO Box 127, Ourimbah, NSW 2258

*Peter C. Annis*
Stored Grain Research Laboratory, CSIRO Entomology, GPO Box 1700, Canberra, ACT 2601

*Fa'ale Tumaalii*
University of Newcastle, School of Applied Science, PO Box 127, Ourimbah, NSW 2258

**Abstract.** 1,8-cineole, a monoterpene identified in aromatic plants, was tested for its potential as an alternative fumigant in terms of behaviour of this chemical on wheat as well as its fumigant toxicity to three major stored-grain insects. In wheat fumigation, 1,8-cineole vapour is very stable with no chemical change, even though it showed a high level of sorption on wheat. After 24 hours aeration, about 95% of the absorbed 1,8-cineole was removed from wheat. The 1,8-cineole residues in fumigated wheat were consistent with the amount absorbed. In fumigation at different fill rates, 1,8-cineole showed low mortality with high filling ratios and high mortality with low filling ratios for *Sitophilus oryzae*. 1,8-cineole showed potential fumigant toxicity at 47.0, 18.8 and 21.6 μL/L air for 95% kill of *S. oryzae*, *Tribolium castaneum* and *Rhyzopertha dominica*, respectively. While 1,8-cineole is a material with potential for use as a fumigant, further research to develop a patentable device, formulations or application methods will determine its future in the industry.

## Introduction

The increasing problems with current fumigants drive the need for research to develop new control methods, restore old fumigants and identify potential new fumigants. Carbonyl sulfide is one of the new fumigants, and ethyl formate and hydrogen cyanide are old fumigants under evaluation for use in stored-product insect control (Annis and Waterford 1996; Bell 2000). The toxicity exhibited by essential oils and their constituent monoterpenes marks them as potential alternative compounds to currently used fumigants (Shaaya et al. 1997; Huang et al. 2000; Isman 2000; Weaver and Subramanyam 2000; Lee et al. 2001, 2002). Lee et al. (2001) reported that 1,8-cineole, a primary component in eucalyptus and rosemary oil, displayed potent toxicity against *Sitophilus oryzae* (L.). However, in commodity fumigation, the understanding of sorption, desorption and residue on target grain, as well as worker safety and mammalian ingestion, are important issues for commercially available fumigants (Hilton and Banks 1997). The research reported here addresses sorption, desorption and residue analysis of 1,8-cineole as a potential fumigant of wheat. Also, the efficacy of 1,8-cineole at different filling ratios of wheat was examined with respect to the toxicity to the stored grain insects, *Sitophilus oryzae*, *Rhyzopertha dominica* (F.) and *Tribolium castaneum* (Herbst).

## Experimental procedures

### Materials and insects

1,8-cineole is a Fluka product (Cat. No. 470-82-6), and the other reagents used were analytical grade chemicals. All the wheat used was from a single source (variety Rosella, 11.5–12.5% moisture content). The flour culture medium for *T. castaneum* was made from the Rosella wheat with brewer's yeast added. Insects were cultured as follows: 1 g of *S. oryzae* on 800 g wheat, 0.86 g of *T. castaneum* on 734 g flour and 66 g yeast, and 0.56 g of *R. dominica* on 800 g wheat plus 250 mL flour. Parents were removed after 2 weeks and the culture media incubated for 8–9 weeks at 25°C. The adults used in the experiment were 2–3 weeks post-emergent.

### Sorption of 1,8-cineole on grains

Sorption studies were carried out using glass desiccators (2.5–2.7 L) containing stainless steel magnetic stirrers. The desiccator lids were sealed with glass stoppers containing a septum and a filter paper to capture the

injected oil and aid evaporation of the fumigant. The volume of each desiccator was measured by the amount of water it could hold. The wheat was placed in each desiccator at a 50% filling ratio (f.r.) and the desiccator sealed. Then 1,8-cineole was injected into each desiccator at a rate of 150 μL/L air of and the contents fumigated for 48 hr. Gas chromatographic analyses were performed with a Varian Star 3400 with ZB-Wax column (30 m × 0.53 mm i.d., film thickness: 1.00 μm) and flame ionisation detections (FID) made in conjunction with a Star chromatography workstation. The carrier gas was helium with flow rate 10 mL/min. The oven temperature was 150°C.

### Desorption of 1,8-cineole from grains

Samples for desorption analysis were prepared after a 48 hr fumigation in desiccators placed in a fume cupboard. The fumigated wheat was placed in empty 250 mL conical flasks (50% f.r.) as rapidly as possible, and the flasks sealed with glass stoppers containing a septum. Analysis of desorption of fumigant from treated wheat was carried out. The concentration of fumigant in the head space of the flasks was measured at 0, 1, 2, 4 and 24 hr after resealing. This procedure was repeated for additional fumigated wheat samples which were aerated 1, 2 and 4 days in open 1 L glass containers in a fume cupboard.

### Residue of 1,8-cineole in grains

Residues in wheat were determined after desorption studies. The wheat residues (200 g) were extracted with 100 mL hexane for 1 hour in the Nickens–Nickerson apparatus. Analysis of fumigant residue was performed under the same conditions as previously described. The GC oven was programmed as follows: start at 70°C and held for 2 min; from 70 to 80°C at 1°C per min; from 80 to 190°C at 10°C per min; from 190 to 250°C at 30°C per min; then constant at 250°C at 5 min. Injector temperature was 250°C. Detector temperature was 250°C. The carrier gas was helium at a flow rate of 10 mL/min.

### Bioassay of 1,8-cineole against *Sitophilus oryzae* at different fill rates of wheat

Fumigation bioassays were carried out with 20 adult insects exposed in sealed conical flasks (250 mL) containing 20, 50, 80% f.r. wheat (based on bulk density). The flasks were fitted with glass stoppers containing filter paper and a septum. Different volumes of 1,8-cineole were injected into the conical flasks using a gastight syringe, with at least three of the concentrations being replicated three times. After evaporation of the 1,8-cineole, the flasks were shaken gently to thoroughly mix the 1,8-cineole, grain and insects. Control flasks with insects were run concurrently with the experiment, and after fumigation all cultures were held at 25 ± 0.5°C. Mortality was determined after 24 and 72 h of incubation.

### Bioassay of 1,8-cineole against other stored-grain insects

Fumigations were carried out in glass desiccators (2.5–2.7 L) containing stainless steel magnetic stirrers to aid gas mixing. The desiccator lids were sealed with glass stoppers containing a septum and a filter paper, as previously described. Five glass jars containing 30 insects were placed inside a desiccator, which was then sealed. Different volumes of 1,8-cineole per air volume were injected into the desiccator and none added to the control desiccator. After fumigation, insects were moved into clean vials and incubated (at 25 ± 0.5°C for 24 h) on 50 g wheat for 24 h before mortality determination. Any insect showing movement was considered to be alive. For comparison with other fumigants, the $LD_{50}$ and $LD_{95}$ values were calculated by probit analysis (Finney 1971).

## Results

### Sorption of 1,8-cineole on wheat

The loss of the 1,8-cineole in the headspace of the sealed fumigation system is shown in Figure 1 which plots the ratio of concentration to applied concentration (C/Co) against fumigation time. The results indicate that liquid 1,8-cineole was vaporised within two hours and was very stable, showing no chemical change in the headspace of the flask. Wheat strongly absorbed 1,8-cineole; after the 24 hr exposure, 40% of the 1,8-cineole had disappeared from the headspace of the fumigation container. There was initial rapid sorption over the first 24 h, followed by a more gradual sorption for the next 24 h.

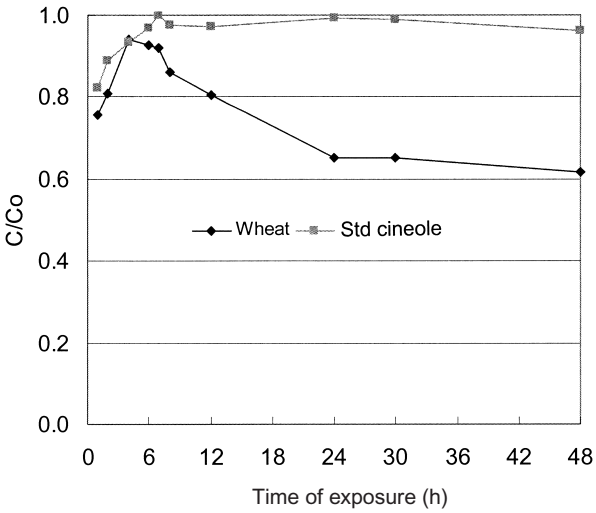


**Figure 1.** The loss of 1,8-cineole (150 μL/L air) during exposure to wheat in 2.4 L desiccators with 50% filling ratio and at room temperature (25 ± 3°C). C/Co is the ratio of concentration to applied concentration.

### Desorption of 1,8-cineole from wheat

The release of 1,8-cineole from fumigated wheat is shown in Figure 2. Desorption of 1,8-cineole was rapid

from wheat, with about 95% of the absorbed cineole being removed from wheat after 24 h aeration. After further aeration, less than 2% the absorbed 1,8-cineole remained. Desorption of 1,8-cineole from the unaired wheat was variable due to losses of 1,8-cineole during the transfer of the wheat from the desiccators to the desorption flask.

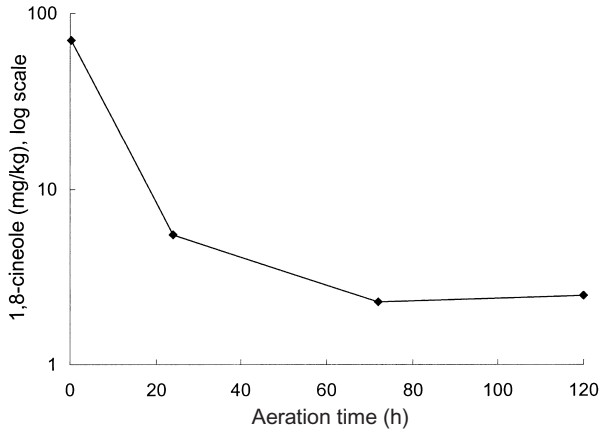


**Figure 2.** Amount of 1,8-cineole (150 µL/L air) released from fumigated wheat in a 2.4 L desiccator (temp. 25 ± 3°C, 50% filling ratio).

### 1,8-cineole residues on wheat

The levels of 1,8-cineole residues in fumigated wheat after 1, 2, 4 and 6 days of aeration are shown in Table 1. The 1,8-cineole residues were rapidly removed within the first 2 days. Once the level of 1,8-cineole had fallen to 60 mg/kg, it was difficult to remove more of the fumigant. The results are consistent with those of wheat fumigated with carbon disulfide (Ren 1996). The residues correlated well with the rate of desorption.

**Table 1.** Residues of 1,8-cineole (mg/kg) in fumigated wheat following different aeration times.

| Length of aeration (hr) | Residue in wheat (mg/kg) | Loss per day (mg/kg) |
|---|---|---|
| 24 | 84.6 | – |
| 48 | 72.2 | 12.4 |
| 96 | 67.1 | 2.55 |
| 114 | 61.7 | 2.70 |

### Toxicity of 1,8-cineole to *S. oryzae* at different fill rates of wheat

For 24 h fumigation, mortalities at concentrations of 30, 50, 100 and 150 µL/L air of 1,8-cineole with 20, 50 and 80% f.r. for wheat are shown in Figure 3. Concentrations of 1,8-cineole of 30 and 50 µL/L air gave low mortalities with all filling ratios of wheat tested. Concentrations of 100 and 150 µL/L gave 88 and 98% mortality with a 20% f.r., 30 and 68% with a 50% f.r. and 17 and 57% with an 80% f.r.

For a 72 h fumigation, toxicities of 1,8-cineole at concentrations of 50, 100 and 150 µL/L air with 20, 50 and 80% f.r. wheat are shown in Figure 4. Concentrations of 50, 100 and 150 µl/L air showed 49, 97 and 100% kill with a 20% f.r., 35, 65 and 90% kill with a 50% f.r. and 14, 51 and 77% kill with an 80% f.r.

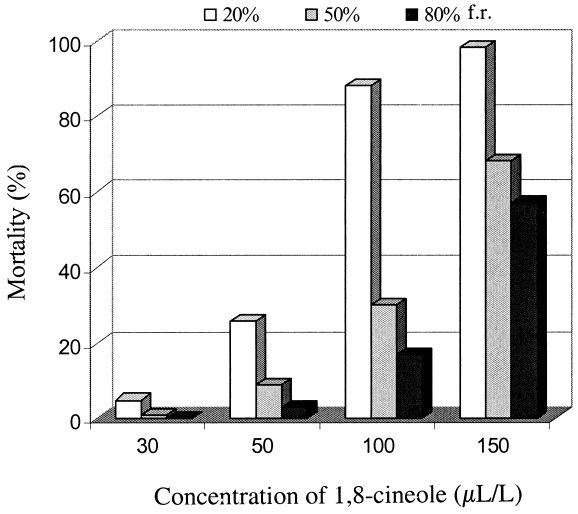


**Figure 3.** Mortality of *Sitophilus oryzae* resulting from 24 h fumigation of wheat with different dosages of 1,8-cineole at various filling ratios.

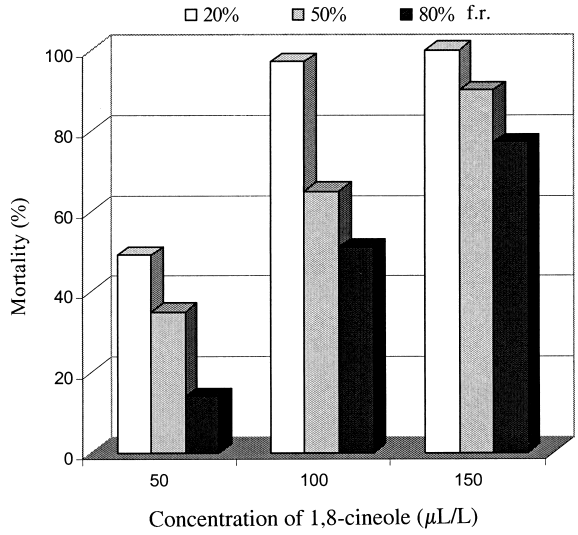


**Figure 4.** Mortality of *Sitophilus oryzae* resulting from 72 h fumigation of wheat with different dosages of 1,8-cineole at various filling ratios (f.r.).

### Toxicity of 1,8-cineole against different stored-grain insects

Toxicity of 1,8-cineole to various species of stored-grain insects is shown in Figure 5. The $LD_{50}$ and $LD_{95}$ dosages for *S. oryzae* are 31.0 and 47.0 µL/L of air, for *T. castaneum* 15.7 and 18.8, and for *R. dominica* 8.1 and 21.6. On the basis of the $LD_{95}$ dosages, the *S. oryzae* strain

tested is twice as tolerant as the strains of *T. castaneum* and *R. dominica* tested.

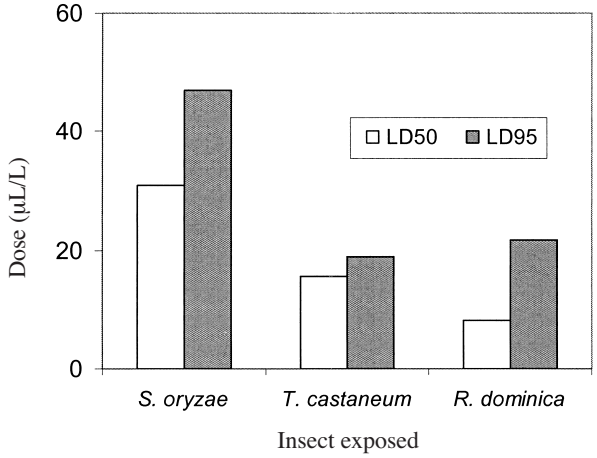


**Figure 5.** Toxicity of 1,8-cineole against three major stored grain insects, without wheat.

## Discussion

When developing a new fumigant to meet regulatory requirements, it is necessary to have an understanding of the sorption, desorption properties and residues on the target grain, as well as the toxicity of the fumigant to the target insect pests (Hilton and Banks 1997). Fumigants are absorbed onto grain either by physical or a combination of physical and chemical forces and sometimes the reaction is irreversible (Banks 1985). The research presented here examined the sorption and desorption properties of 1,8-cineole used as a fumigant, and measured the residues following fumigation. A literature search revealed no sorption studies on 1,8-cineole or any other candidate monoterpenes proposed as fumigants.

Barton et al. (1995) reported 1,8-cineole to be chemically stable during storage, and relatively safe, with minimal environmental and occupational health implications. The results of this research not only supplement the findings of Barton et al. (1995), but also show that physical forces were more important than chemical forces in the sorption of 1,8-cineole on grain. Additionally, the accepted safe levels of 1,8-cineole (European Commission 2002) should more rapidly and easily allow the determination of critical operational values such as the threshold limit value (TLV). An acceptable daily intake (ADI) of 1,8-cineole from flavoured foodstuffs has already been set in France, at 4.5 mg per person (Council of Europe 2001). This study measured 1,8-cineole residues on wheat of 84 mg/kg after 1 day and 61 mg/kg after 6 days aeration. These levels might lead to an intake over the ADI limit for 1,8-cineole set in France. However, levels are likely to be much lower in commercial-scale fumigation entailing forced aeration, longer aeration times and commodity processing time. In addition, 1,8-cineole is not considered as either a hazardous air pollutant or an ozone destroyer in the Clean Air Act. Neither do Worksafe Australia criteria indicate that it should be classified as hazardous.

While 1,8-cineole shows promise as a material with potential for use as a fumigant, in terms of toxicity it is far from being a usable fumigant in the short term. Commercial reality is that unless the market is large enough and the potential for profit is significant, a commercial product will never be developed. The market size will be dependent on the number and types of treatments possible. These will be determined by issues such as taint of commodities, overcoming sorption and penetration and a range of other details associated with its potential use. Potential for profit to suppliers of a 1,8-cineole product is likely to be a major problem, as there are few intellectual property advantages to offset the inevitable capital costs associated with manufacture, registration, and marketing. While there is no possibility of patenting the insecticidal use of 1,8-cineole, development of a patentable device, formulation or application method may offset this disadvantage to some extent.

## Acknowledgments

We thank Drs Bruce Halliday and YongLin Ren, and Mrs Daphne Mahon, for their help in proofreading this manuscript. We specially thank Mrs Daphne Mahon for providing the experimental protocol for sorption and desorption work.

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