

Donahaye, E.J., Navarro, S. and Leesch J.G. [Eds.] (2001) Proc. Int. Conf. Controlled Atmosphere and Fumigation in Stored Products, Fresno, CA. 29 Oct. - 3 Nov. 2000, Executive Printing Services, Clovis, CA, U.S.A. pp. 639-649

## NATURAL OCCURRENCE OF CARBONYL SULFIDE AND ETHYL FORMATE IN GRAINS

Y.L. REN\* AND J.M. DESMARCHELIER

CSIRO Entomology, Stored Grain Research Laboratory, GPO Box 1700, ACT 2601  
Canberra, Australia

[\*e-mail: yonglin.ren@ento.csiro.au]

### ABSTRACT

Ethyl formate and carbonyl sulfide are potential stored-product fumigants. They are present naturally in the atmosphere, plants and soil. Natural levels of ethyl formate and carbonyl sulfide in newly harvested grain and stored wheat, barley, oats and canola are reported in this paper. Levels of ethyl formate and carbonyl sulfide in grains varied with the commodity, temperature, moisture content and the period of storage. The values ranged from 0.5 - 2 mg kg<sup>-1</sup> for ethyl formate and 0.02 - 1 mg kg<sup>-1</sup> for carbonyl sulfide. Ethyl formate and carbonyl sulfide were naturally present in grains at harvest, increased during the first 4-5 months of storage, and then began to decline, particularly at grain temperatures greater than 20°C and moisture contents greater than 9.5%. Regulatory authorities should consider these natural levels of ethyl formate and carbonyl sulfide when establishing Maximum Residue Limits (MRL) for these fumigants.

### INTRODUCTION

Ethyl formate (EtF) has a long history of use as a fumigant for dried fruit (Simmons and Fisher 1945; Vincent and Lindgren 1972; Banks and Hilton 1997) and other stored-products (Cotton and Roark 1928; Roark and Cotton 1929). Ethyl formate has been re-evaluated by Desmarchelier *et al.*, (1998) as an alternative to methyl bromide (MB) for stored grain. Carbonyl sulfide (COS) is regarded as one of the new potential alternative fumigants to replace MB (Banks *et al.* 1993; Catley 1993).

Natural levels of EtF in a range of products, such as vegetable, stored grain, and animal products, have been reviewed by Desmarchelier (1999). For example, EtF as a naturally occurring volatile is present in the following products:

0.15 mg kg<sup>-1</sup> in milk (Kami and Ohsaki 1986);

1.3 mg kg<sup>-1</sup> in cheese (Collin *et al.* 1993);

0.3 mg kg<sup>-1</sup> in wheat and 0.03-0.7 mg kg<sup>-1</sup> in wheat germ (Desmarchelier *et al.*, 1998);

0.2-1.0 mg kg<sup>-1</sup> in barley (Desmarchelier *et al.*, 1999); and

0.9-10 mg kg<sup>-1</sup> in barley products such as beer (Ginova 1984; Bohmann 1985).

Similarly, many natural sources of COS have been identified, including oceans, soils, volcanoes and marshes (Khalil and Rasmussen 1984; Brown *et al.*, 1986; Adams *et al.*, 1981). Therefore, COS is naturally present in the atmosphere, in water, soil, and plants, as well as many raw and processed foodstuffs, including cereals and oilseeds. The natural levels of COS in grains and oilseeds was found to be 0.02-0.07 mg kg<sup>-1</sup> (Ren 1997; Desmarchelier *et al.*, 1998; Ren *et al.*, in press). However, there is little information on the effect of temperature, moisture content, variety and the period of storage on natural levels of EtF and COS in stored grain. As part of a study to evaluate EtF and COS as grain fumigants, information about the natural occurrence of the fumigants in stored products is relevant to the establishment of Maximum Residue Limits (MRL) and to the acceptance of EtF and COS as fumigants of stored products.

In this report we have evaluated the effect of temperature, moisture content, commodity and the period of storage on the natural levels of COS and EtF in Australian wheat, barley, oat and canola. Selection of the grains was based on the fact that, wheat is the major cereal grain in Australia, and barley because it is the second major cereal grain, where quality is especially important to the brewing industry. Oat was selected because its composition is different from other cereals, with a lipid content of 5.5-6.5%, which is 2-3 times higher than that of wheat, barley or rye, and a fibre content of 11-12%, 2-4 times higher than in most cereals (Pomeranz 1987). Canola was selected due to it being the principal oilseed in Australia.

## MATERIALS AND METHODS

### Preparation of the commodities

Four Australian representative grains (wheat, oats, barley and canola) were used. They were collected by hand, prior to harvesting to exclude any possible contamination. All commodities (1.5 kg) were placed into sealed jars (2 L), and a varying amount of distilled water was added to the jars to adjust the moisture content (m.c.). They were allowed to equilibrate for 2 weeks at three different temperatures, 15±1°C, 25±2°C and 35±2°C, and the m.c.'s (wet basis) were measured following the procedures of the International Standard (ISO R 712-1968). The following m.c.'s were obtained;

11.9%, 12.9% and 13.9% for wheat

12.1%, 13.0% and 14.9% for barley

10.8%, 12.5% and 13.4% for oats

5.1%, 6.7% and 7.9% for canola.

### Reagents and apparatus

A cylinder of compressed COS gas was purchased from Matheson Gas Company, USA, via BOC, Australia. All the reagents used were analytical grade. Acetone was supplied by Biolab Scientific, Clayton, Victoria, Australia. Ethyl

formate was supplied by Tech Ajax, Sydney, Australia. All other chemicals were obtained from BDH AnalaR, England.

Extractions were carried out in 250 mL Erlenmeyer flasks with a ground-glass joint (Crown Scientific, N.S.W, Australia, Cat. No. FE250/3) fitted with a Quik Fit stopper containing a half hole septa (Alltech Associates, P/N 6526).

#### **Determination of natural levels of COS and EtF**

Carbonyl sulfide was determined on a Shimadzu GC6AM GC (Shimadzu Seisakusho, Kyoto, Japan), equipped with a flame photometric detector (FPD). Separation was achieved on a 1 m x 3 mm (i.d.) glass column packed with HayeSep Q (Alltech Associates, Cat. No. 2801) at 140°C and carrier flow (N<sub>2</sub>) of 40 mL min<sup>-1</sup> at 0.8 psi. Concentrations of ETF were determined using a Varian Star 3600 CX series (Varian Associates, Inc., USA), equipped with a flame ionisation detector (FID), after separation on a 30 m x 0.53 mm (i.d.) megabore capillary column DB FFAP (J & W 125-3212) at an oven temperature of 70°C.

The natural levels of COS and EtF were determined by analysis of the headspace over the commodity plus the extraction solvent, following the procedure described by Desmarchelier *et al.*, (1998). For analysis of COS, the commodities (50 g) were extracted in sealed flasks (250 mL) with 50 mL of acidified aqueous acetone (H<sub>3</sub>PO<sub>4</sub>+water+acetone, 1:2.2:12.5, v/v/v) solution for 10 h. For analysis of EtF, the commodities (50 g) were extracted in sealed flasks (250 mL) with 70 mL of ammonium nitrate (70%, w/v) solution for 24 h. An aliquot of the headspace (50 µL) was injected directly into the GC, and the levels of COS or EtF were calculated on the basis of peak areas. Periodically, the peak areas were calibrated using a spiked standard. The data recorded in the figures are the mean of duplicate samples.

#### **Preparation of the gas standards, and the fortified samples**

A dilute gas was prepared by injecting a measured volume of the concentrated gaseous COS or the liquid ETF into a flask (1 L) containing five glass beads (2-3 mm o.d.). After mixing, the diluted gas was used to prepare both the fortified samples and the gas standards. The spiked samples, carried out in duplicate, were prepared by injecting a measured volume of the diluted gas into a sealed flask containing the commodity plus the extraction solution. For the Gas Chromatograph analysis of COS, complete elution of solvent or solvent vapour was required before further injection, so a minimum interval of 10 min was kept between injections.

## **RESULTS**

#### **Natural levels of carbonyl sulfide in the commodities**

Carbonyl sulfide was found to be naturally present in Australian wheat, barley, oats and canola during the 9-10 months of storage, but levels varied with commodity, moisture content, temperature and the period of storage (Fig. 1). The levels of COS were greater at higher m.c.'s and high grain temperatures. The values ranged from 0.005 mg kg<sup>-1</sup> to 0.500 mg kg<sup>-1</sup> of COS in all the tested cereal grains and canola (Fig. 1). Carbonyl sulfide occurred naturally in all the newly harvested grains, eg. 0.005 - 0.040 mg kg<sup>-1</sup> in wheat, barley and oats; 0.010 - 0.500 mg kg<sup>-1</sup> in canola. In wheat and canola concentrations increased 3-10 times during the 5-7 months of storage, and then began to decline, particularly,

when the grain temperature and m.c. were rising. Carbonyl sulfide in oats increased during the first 3 months of storage, and then declined slightly. However, in canola COS levels increased during 9 months of experimental storage, except in the samples with the highest m.c. (7.9%) or the highest grain temperature of 35°C. In both cases COS declined after 6 months of storage (Fig. 1).

#### **Natural levels of ethyl formate in the commodities**

Ethyl formate was found to be naturally present in Australian wheat, barley, oats and canola during the 10 months of storage. Levels varied with the commodity, temperature of storage, m.c. and the period of storage (Fig. 2). The values ranged from 0.1 - 0.6 mg kg<sup>-1</sup> of EtF in all the tested grain samples. As with COS, EtF also occurred naturally in the newly harvested grain, e.g. 0.1-0.4 mg kg<sup>-1</sup> in wheat, barley and oats; and 0.2-0.4 mg kg<sup>-1</sup> in canola. Ethyl formate in canola and oats increased 2-5 times during the first 7 months of storage, and then began to decline, particularly, when grain temperature and m.c. were rising. At 15°C grain temperature and low m.c., such as 5.1% m.c. in canola or 10.8% m.c. in oats, the background levels of EtF in canola and oats had no significant change during the 10 months storage. (Fig. 2). However, at the same temperature in wheat and barley, the EtF background levels did increase during the 3-9 months of storage. At a m.c. of 11.9-12.9% for wheat and 10.8% for barley, and at 25°C grain temperature, the EtF background levels also increased during the first 3 months of storage, but then declined (Fig. 2). However, in both wheat and barley, when the grain temperature was higher, such as at 25°C and 35°C, the EtF background levels decreased from the first month of storage, even with a low m.c. (Fig. 2).

## **DISCUSSION**

#### **Natural levels of COS in the commodities**

Since COS was found to be present in newly harvested grains, it has been demonstrated that it occurs naturally during plant growth. It could be formed by two ways:

(i) Adsorption during growth: It has been observed that there are large amount of COS in emissions from vegetation and soil (Bremner and Banwart 1976; Crutzen 1983; Brown *et al.* 1986; Kluczewski *et al.* 1985) and it is much greater from areas of high biological productivity (Adams *et al.* 1981; Khalil and Rasmussen 1984). Emission of COS increased with the application of organic manure and was positively correlated with the total sulfur content in the soil (Yang *et al.*, 1998).

(ii) Enzymatic synthesis or biological hydrolysis (Brown *et al.*, 1986; Taylor *et al.* 1983; Schildknecht and Rauch 1962; Sze and Ko 1979; Ren 1999).

Carbonyl sulfide is continually present in all stored commodities, increasing during the first 3-9 months of storage. This probably results from metabolism and the activity of microorganisms in the grain, since the rate of increase of COS was dependent on the grain temperature and m.c. These results are consistent with those reported for naturally occurring COS present during the malting and the brewing processes (Anness and Bamforth 1982). The levels of COS formed

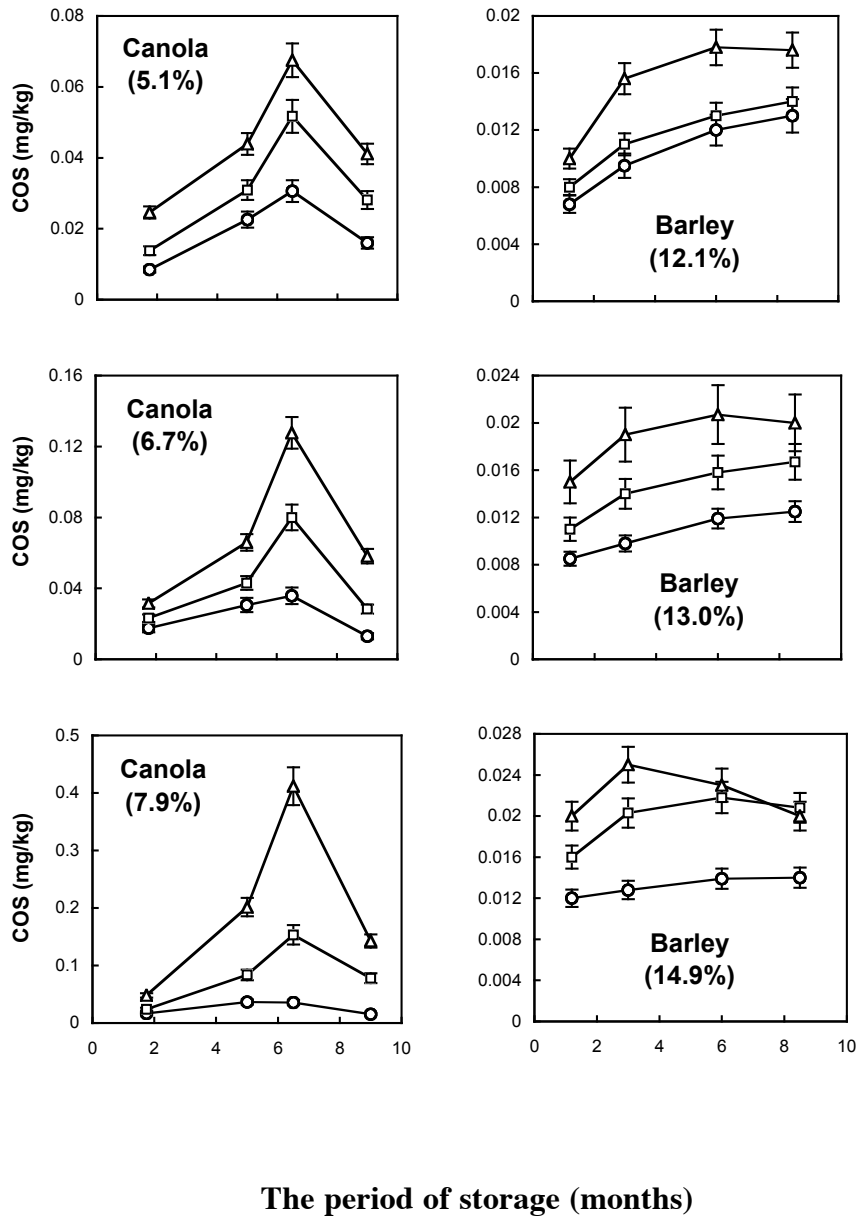


Fig. 1. Natural occurrence of carbonyl sulfide in barley, canola, oats and wheat, at different temperature, moisture content and period of storage (○ ○ ○ , at 15°C; □ □ □ , at 25°C; △ △ △ , at 35°C).

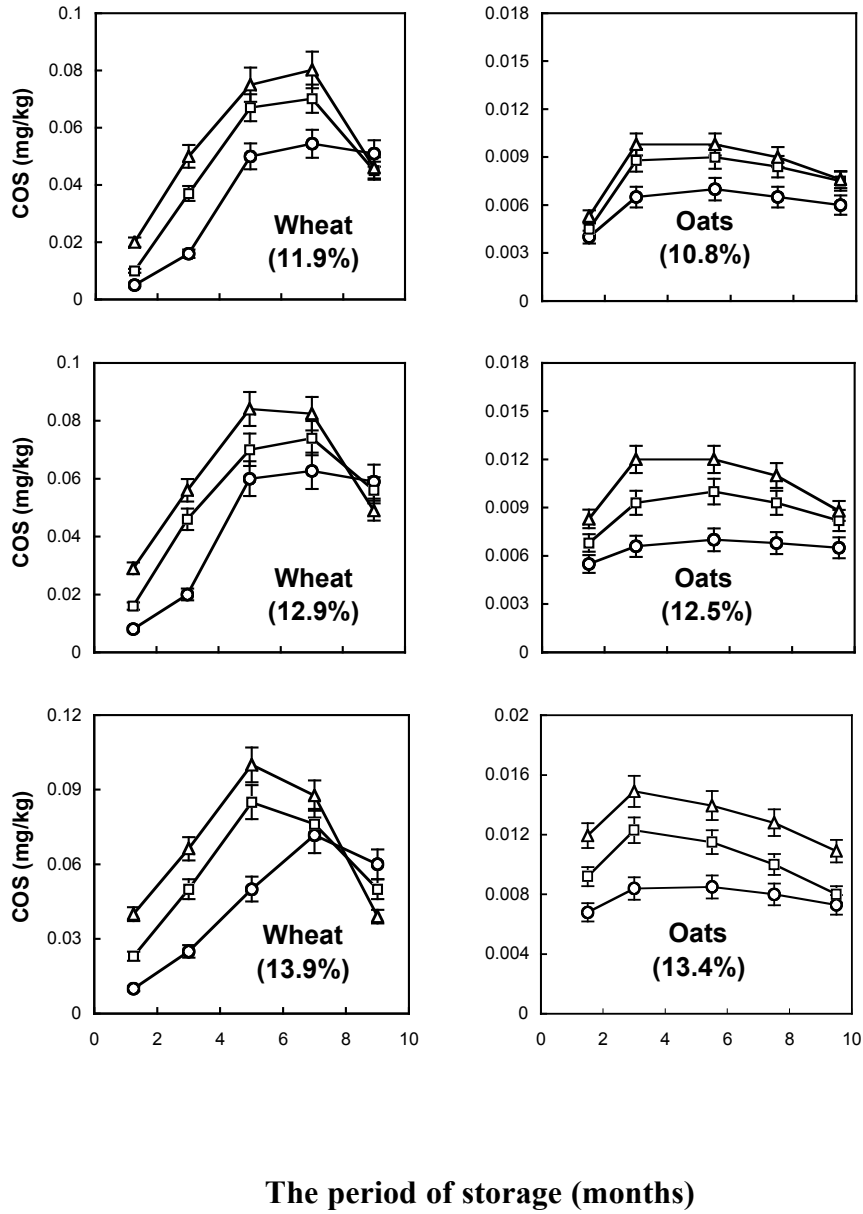


Fig. 1. (continued) Natural occurrence of carbonyl sulfide in barley, canola, oats and wheat, at different temperature, moisture content and period of storage (○ ○ ○ , at 15°C; □ □ □ , at 25°C; △ △ △ , at 35°C).

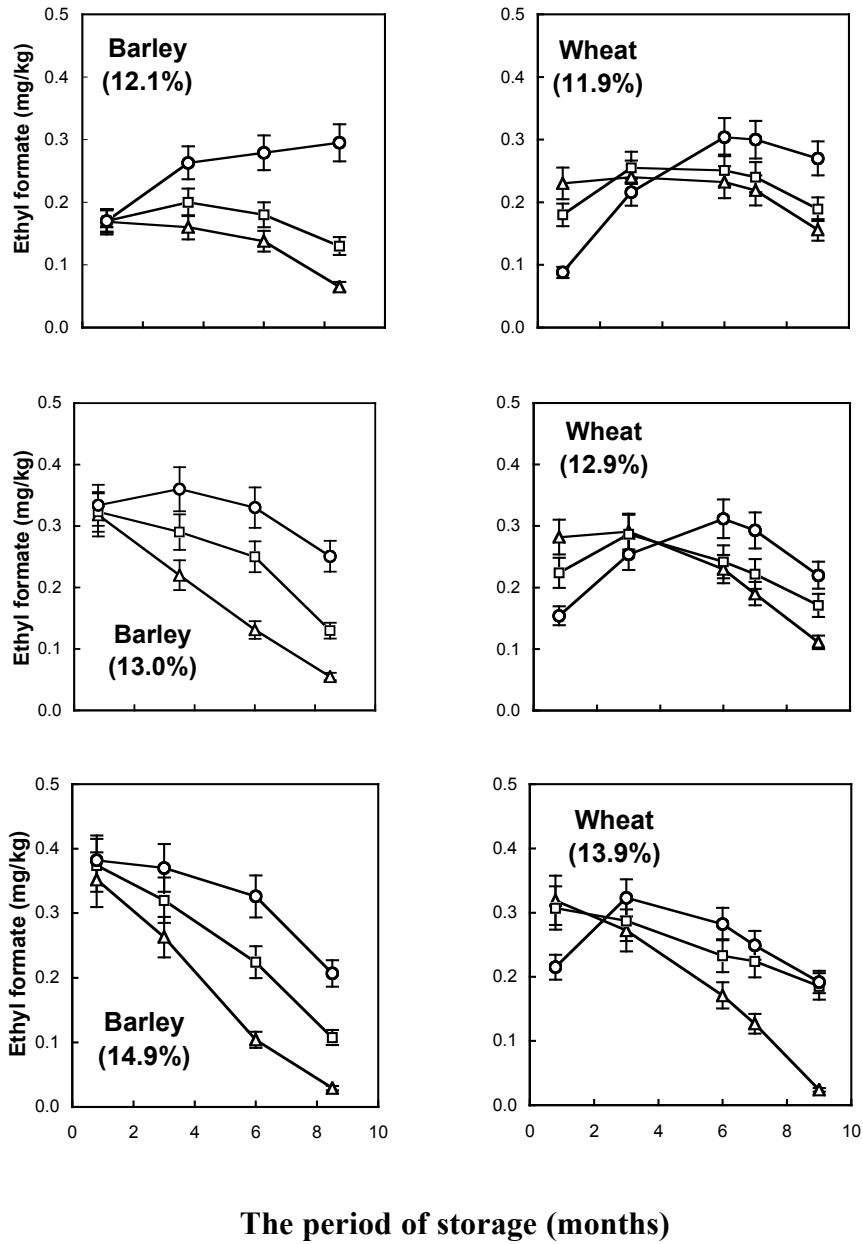


Fig. 2. Natural occurrence of ethyl formate in wheat, barley, oats and canola, at different temperature, moisture content and period of storage (□ O□ , at 15°C; □ □ , at 25°C; □ □□ , at 35°C).

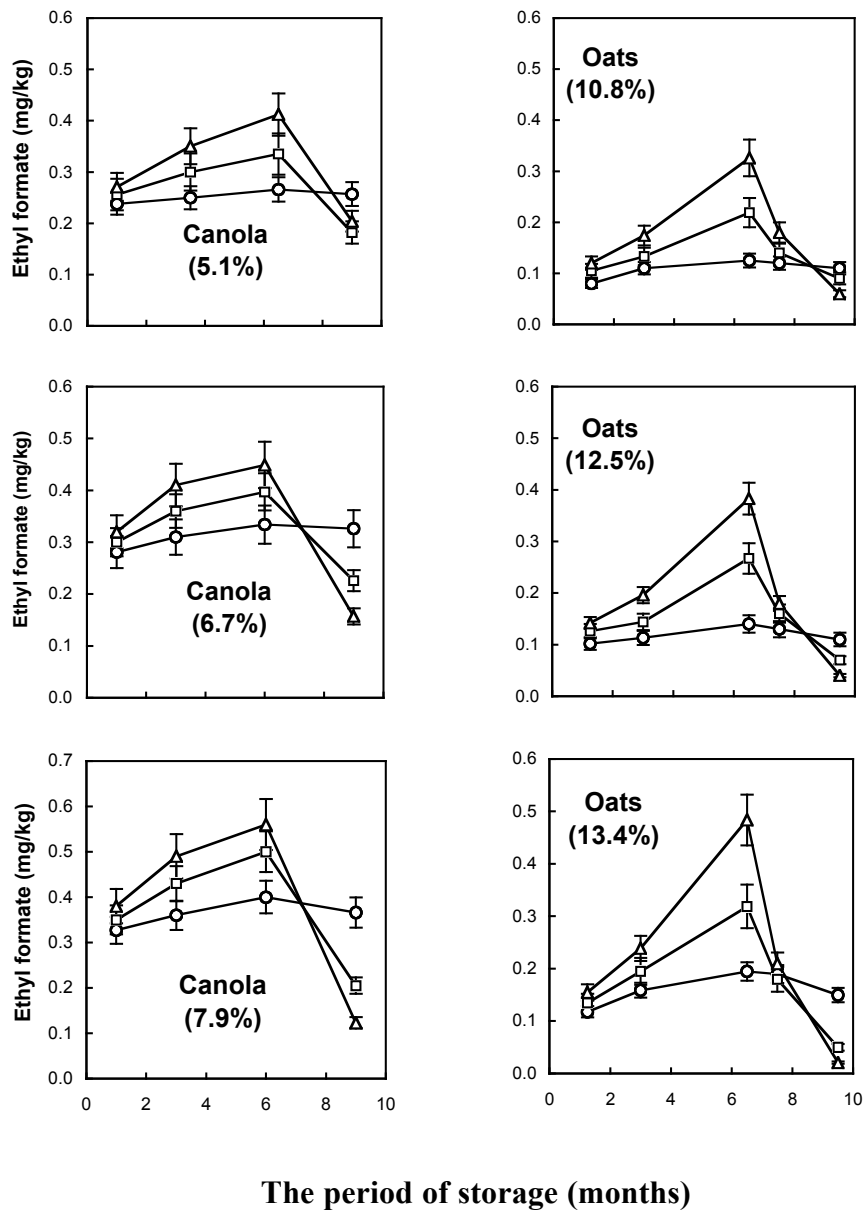


Fig. 2. (continued) Natural occurrence of ethyl formate in wheat, barley, oats and canola, at different temperature, moisture content and period of storage (□ ○ □ , at 15°C; □ □ □ , at 25°C; □ □ □ , at 35°C).

in all the tested grains are in agreement with previous laboratory and field trial studies. They were 0.005 - 0.100 mg kg<sup>-1</sup> in wheat, 0.005 - 0.050 mg kg<sup>-1</sup> in



barley, 0.005 - 0.030 mg kg<sup>-1</sup> in oats, and 0.02 - 0.60 mg kg<sup>-1</sup> in canola (Ren *et al.* in press; Desmarchelier 1999; Desmarchelier *et al.* 1998; Ren 1997).

#### **Natural levels of EtF in the commodities**

The presence of EtF in newly harvested grains demonstrated that EtF occurs naturally during plant growth. The effect of moisture content on the natural levels of EtF is associated with the grain temperature, which indicates that naturally occurring EtF is a product of metabolism in commodities and microorganisms. The results are consistent with the natural levels of EtF in barley reported by Desmarchelier *et al.* (1999), who found that levels decreased from 1.0 mg kg<sup>-1</sup> to 0.2 mg kg<sup>-1</sup> under high temperature storage conditions. The results are also consistent with naturally occurring EtF found in micro-organisms, such as *Penicillium italicum* and *P. digitatum*, which are reported to have increased with increasing m.c. and temperature (Yuen *et al.*, 1995). In fact, if no EtF is found in the commodities at high m.c. and high temperature, this is due to its break down to ethanol and formic acid. Yuen *et al.* (1995) have pointed out that EtF may have antimicrobial properties when concentrations of natural levels of EtF are high and exposure time is long. That is, the production of further EtF in microorganisms can be inhibited by the presence of EtF. This may provide an explanation for the decline of EtF levels after a period of storage, particularly, when grain temperature and/or m.c. are high.

### **CONCLUSIONS**

Carbonyl sulfide and EtF occur naturally in stored grains at harvest and during periods of storage. They are formed during growth in the field and also might be formed during storage from the metabolism of the commodity and its microorganisms. This indicates that levels of COS or EtF should not be of concern with respect to the quality of the grain. This is a pleasing result from the point of view of the effect of any residual levels of COS or EtF, left after use as a fumigant on the quality of food-products and the environment. Therefore, these natural levels of COS or EtF should be considered by regulatory authorities when establishing Maximum Residue Limits (MRL) for these fumigants.

### **ACKNOWLEDGEMENTS**

The authors thank Drs. E. J. Wright, R. Reuss, and Mrs. D. A. Mahon for helpful advice and the Grain Research Development Corporation (GRDC), the Stored Grain Research Laboratory (SGRL) agreement for financial assistance. We thank Le Trang Vu for technical assistance and G. Russell for organising all the samples for this study.

### **REFERENCES**

- Adams, D.F., Farwell, S.O., Pack, M.R. and Robinson, E. (1981) Biogenic sulphur emissions from soils in eastern and southeastern United States. *Journal of Air Pollution Control Assessment*, **31**, 1083-1089.
- Anness, B.J. and Bamforth, C.W. (1982) Dimethyl sulphide - A review. *Journal of Institute of Brewing* **88**, 244-252.

- Anon. (1968) ISO R 712-1968 (E). Cereals and cereal products - Determination of moisture content (Routine method). ISO Recommendation, reported April 1968.
- Banks, H.J., Desmarchelier, J.M. and Ren Y.L. (1993) Carbonyl sulphide as a fumigant. International Patent Application PCT/AU 93/00018.
- Banks, H.J. and Hilton, S.J. (1997) Ethyl formate as a fumigant of sultanas: sorption and efficacy against six pest species. In: *Proc. Int. Conf. on Controlled Atmosphere and Fumigation in Stored Products*, (Edited by Donahaye, E.J., Navarro, S. and Varnava, A.), 21-26 April 1996, Printco Ltd., Nicosia, Cyprus, 409-422.
- Bohmann, J.J. (1985) Aging behaviours of beer. Part 4. Aging trials combined with gassing by carbon dioxide, nitrogen, air and oxygen. *Monats fuer Brauwissen*, **38**, 79-85.
- Bremner, J.M. and Banwart, W.L. (1976) Sorption of sulphur gases by soils. *Soil Biological Biochemistry*, **8**, 79-83.
- Brown, K.A., Kluczewski, S.M. and Bell, N.B. (1986) Metabolism of [35S]carbonyl sulfide in perennial ryegrass (*Lolium perenne* L.) and radish (*Raphanus sativus* L.). *Environment and Experimental Botany*, **26**, 355-365.
- Catley, A.C. (1993) Carbonyl sulphide, a potential methyl bromide substitute. NAPPO Newsletter **13**, 2.
- Collin, S., Osman, M., Delcambre, S., El-Zayat, A. and Dufour, J.P. (1993) Investigation of volatile flavour components in fresh and ripened Domiati cheese. *Journal of Agricultural and Food Chemistry*, **41**, 1659-1663.
- Cotton, R.T. and Roark, R.C. (1928) Fumigation of stored product insects with certain alkyl and alkylene formates. *Industry and Engineering Chemistry*, **20**, 380.
- Crutzen, P.J. (1983) Atmospheric interactions - homogeneous gas reactions of C, N and S containing compounds. In: *The major biogeochemical cycles and their interactions*. SCOPE 21 (Edited by Boiln B. and Cook R.B.), Wiley, New York. 67-114.
- Desmarchelier, J.M. (1999) Ethyl formate and formic acid: occurrence and environmental fate. *Postharvest News and Information*, **10**, 7N-12N.
- Desmarchelier, J.M., Allen, S.E., Ren, Y.L., Moss, R. and Vu, L.T. (1998) Commercial-scale trials on the application of ethyl formate, carbonyl sulphide and carbon sulphide to wheat. CSIRO Entomology, *Technical report*, No. 75.
- Desmarchelier, J.M., Johnston, F.M. and Vu, L.T. (1999) Ethyl formate, formic acid and ethanol in air, wheat, barley and sultanas: analysis of natural levels and fumigant residues. *Pesticide Science*, **55**, 815-824.
- Ginova, T. (1984) Effect of the yeast strains on the content of some aroma components of beer. *Khraniteina proomishlenost* **33**, 24-26; *Chemical Abstracts* 101, 209020e.
- Kami, T. and Ohsaki, K. (1986) Volatile constituents of the milk of cow fed with soiling dent corn. *Japanese Journal of Zootechnical Science*, **57**, 902-906.
- Khalil, M.A.K. and Rasmussen, R.A. (1984) Global sources, lifetime and mass balance of carbonyl sulfide (COS) and carbon disulfide (CS<sub>2</sub>) in the earth's atmosphere. *Atmospheric Environment*, **18**, 1805-1813.
- Kluczewski, S.M., Brown, K.A. and Bell, N.B. (1985) Deposition of [35S]-carbonyl sulfide to vegetable crops. *Radiation Protection Dosimetry*, **11**, 173-177.
- Pomeranz, Y. (1987) *Modern Cereal Science and Technology*. VCH Publishers, New York, pp 40.

- Ren, Y.L. (1997) Carbonyl sulphide as a fumigant for grain and timber – efficacy towards organisms and formation of residues. Ph. D. thesis, Faculty of Applied Science, University of Canberra, Australia.
- Ren, Y.L. (1999) Is carbonyl sulfide a precursor for carbon disulfide in vegetation and soil? Interconversion of carbonyl sulfide and carbon disulfide in fresh grain tissues in vitro. *J. Agric. and Food Chem.*, **47**, 2141-2144.
- Ren, Y.L., Desmarchelier, J., Allen, S. and Weller, G. (2001) Carbonyl sulfide (COS) trials on barley and canola in 40 t farm bin. In: *Proc. Australian Postharvest Technical Conf.*, Adelaide, Australia, 1-4 Aug. 2000. Stored Grain Research Laboratory, Canberra, (in press).
- Roark, R.C. and Cotton, R.T. (1929) Tests of various aliphatic compounds as fumigants. U.S. Department of Agriculture, *Technical Bulletin*, No.162, 52.
- Schildknecht, H. Von and Rauch, G. (1962) On plant defence chemicals III. The gas from horseradish. *Z. Naturforschung*, **17B**, 800-803.
- Simmons, P. and Fisher, C.K. (1945) Ethyl formate and Isopropyl formate as fumigants for packages of dried fruits. *J. Econ. Entomol.*, **38**, 715.
- Sze, N.D. and Ko, K.W. (1979) CS<sub>2</sub> and COS in the stratospheric sulphur budget. *Nature*, **280**, 308-310.
- Taylor, G.E., Mclaughlin, S.B., Shriner, D.S and Selvidge, W.J. (1983) The flux of sulphur-containing gases to vegetation. *Atmospheric Environment*, **17**, 789-796.
- Vincent, L.E. and Lindgren, D.L. (1972) Hydrogen phosphide and ethyl formate: Fumigation of insects infesting dates and other dried fruits. *J. Econ. Entomol.* **65**, 1667.
- Yang, Z., Kong, L., Zhang, J., Wang, L. and Xi, S. (1998) Emission of biogenic sulfur gases from Chinese rice paddies. *Science of the Total Environment*, **224** (1-3), 1-8.
- Yuen, CMC, Paton, J.E., Hanawati, R. and Shen, L.Q. (1995) Effect of ethanol, acetaldehyde and ethyl formate vapour on the growth of *Penicillium italicum* and *P. digitatum* on oranges. *J. Horticultural Sci.*, **70**, 81-84.