Effect of Grain Fumigants on Lipids in Vivo and in Vitro

Ren Yonglin,† James M. Desmarchelier,*,‡ and Fiona Watson†

Faculty of Applied Science, University of Canberra, P.O. Box 1, Belconnen 2617, Australia, and CSIRO, Division of Entomology, P.O. Box 1700, ACT 2601, Australia

The effect of current and potential grain fumigants on food lipids was examined in two ways. First, wheat was fumigated in vivo and the lipid extracted and compared with that from unfumigated wheat. Second, wheatgerm and canola oil were fumigated in vitro, and the fate of both fumigant and lipid was examined. The fumigants tested were phosphine, methyl bromide, carbon disulfide, cyanogen, and carbonyl sulfide. Fumigation of wheat or oils had no effect on lipid composition as assessed by Fourier transform infrared or ultraviolet spectroscopy. In fumigation of lipids in sealed containers, some fumigant was sorbed by the lipid, but the fumigant was recovered intact after heating. The order of solubility of fumigant in lipid was carbon disulfide > methyl bromide > cyanogen > phosphine = carbonyl sulfide.

Keywords: *Fumigants; lipids; FTIR*

INTRODUCTION

Grain lipids are important for human and animal nutrition (Whitney, 1990) and in cereal chemistry (Pomeranz and Finney, 1969; Cooksson and Coppock, 1956). Fumigation of wheat with chemicals such as phosphine (PH_3) is used to prevent insect damage. Interactions between fumigants and grain chemicals have been studied in several ways. These include studies on the effects of fumigants on quality, such as baking properties (Matthews et al., 1970), studies on the effects of fumigants on certain chemicals in grains, such as tocopherols (Slover and Lehmann, 1972), and studies which determine the fate of the fumigant (Banks, 1986). However, no previous study has investigated the interaction between fumigants and lipids by measuring both the lipid and the fumigant and by studying the interactions on both whole grain (in vivo) and on extracted lipids such as canola oil (in vitro). This present publication addresses that lack, by studying both the effect of fumigants on lipids and the effect of lipids on the stability of fumigants. Techniques employed in this study include fumigant sorption (Banks, 1986), Fourier transform infrared spectroscopy (FTIR) (Lai et al., 1994; Freeman, 1968; Carnovale and Quaglia, 1973) and UV spectroscopy (Lai et al., 1994).

EXPERIMENTAL PROCEDURES

Materials. Wheat used was Australian standard white wheat, 11.4% moisture content, w/w, wet basis. Canola oil, wheatgerm oil, olive oil, and cod liver oil were purchased at local retail stores. Chloroform, carbon tetrachloride (Merck), light petroleum (bp 38−55 °C), hexane, and butanol (BDH) were of analytical grade. Carbonyl sulfide (COS), methyl bromide (CH_3Br) (Matheson), and PH_3 (BOC) were in cylinders, and carbon disulfide (CS_2) (Ajax) was a liquid. Cyanogen (C_2N_2) was prepared by the reaction of potassium cyanide with cupric ions (Brotherton and Lynn, 1959).

Fumigant Dosing and Analysis. Wheat (40 g) was placed in gastight Erlenmeyer flasks (750 mL) fitted with a septum injection system (Quickfit adaptor, cone screwthread, ST5, Bibby Sterilin, U.K.) and fumigated for 48 h at 25 °C with either CH_3Br, COS, CS_2, or C_2N_2 at 100 mg L^{-1} or with PH_3 at 50 mg L^{-1}. After 48 h, the wheat was transferred to a Petri dish and aired in a fume hood for 48 h prior to extraction of lipid. In addition, commercial wheat germ oil (1 g) in a 270 mL Erlenmeyer flask was dosed with 100 mg L^{-1} of either CH_3Br, COS, C_2N_2, CS_2, or PH_3 for 24 h at 30 °C. After airing for 4 h, the oil was dissolved in carbon tetrachloride (40 mL) for spectrometric analysis.

Fumigant concentration in the air (the headspace) above the lipid within the sealed Erlenmeyer flask was determined by gas−liquid chromatography (GC), using injection volumes of 20 μL. The sulfur gases were detected on a Tracor 220 M GC, equipped with a flame photometric detector (sulfur mode) after separation on a 2 m × 3 mm glass column packed with HayeSep Q (Alltech Associates) at 80 °C. Phosphine was analyzed using the same system (phosphorus mode). Methyl bromide was detected on a Shimadzu GC6AM GC, equipped with a flame ionization detector, after separation on a 2 m glass column packed with 4% SP2401, 6% SE30 on Chromosorb W, 60−80 mesh (Alltech Associates). Operation conditions were oven temperature of 140 °C and injection temperature of 200 °C. Cyanogen was analyzed on a Varian 3300 gas chromatograph, equipped with a nitrogen/phosphorus (TSD) detector, after separation on 50 m × 0.53 mm BP624 column (SGE). Operation conditions were column temperature of 120 °C, injection temperature of 160 °C, and detector temperature of 300 °C.

Extraction and Analysis of Lipids. After fumigation, wheat was ground to a fine flour and portions (5 g) extracted with vigorous shaking with petroleum ether (15 mL) for 30 min followed by two extractions with butanol (each 15 mL), for 1 h each. The filtrates were pooled, the solvents were removed under nitrogen, and the residue was dissolved in carbon tetrachloride (5 mL) for spectrometric analysis. A similar procedure was applied to untreated wheat (control). In addition, control or fumigated wheat was ground into flour and portions (5 g) were extracted in a Soxhlet apparatus with hexane by refluxing for 8 h. The solvent was removed under nitrogen and the residue dissolved in 5 mL of carbon tetrachloride for spectrometric analysis. All lipid extractions were performed in triplicate.

Lipid composition was determined by both UV spectroscopy on a Pharmacia LKB Novaspec II spectrometer and by FTIR spectroscopy on a Digilab FTS-7 spectrometer (Bio-Rad Laboratories) with an IR controller. UV absorbance was measured in 1 cm cells over the range 280−400 nm and IR absorbance in 1 mm sodium chloride cells over the range 1000−3600 cm^{-1}. The effect of fumigation on lipid composition was assessed by comparison with control samples. This comparison involved

† University of Canberra.
‡ CSIRO.
* To whom correspondence should be addressed [FAX (06) 2464206; e-mail jimd@ento.csiro.au].

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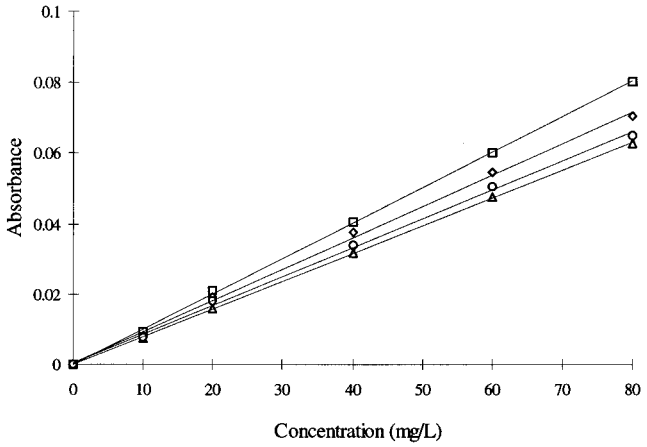

Figure 1. Absorbance at 2920 cm^{-1} plotted against concentration of lipid: □ olive oil; ◇, cod liver oil; ○, wheatgerm oil; △, canola oil.

visual inspection of the whole spectrum and quantitation at 1165, 1375, 1460, 1745, 2855, 2930, and 3010 cm^{-1}. Dose response curves were measured at each wavenumber for solutions of each of four commercial oils (cod liver oil, olive oil, canola oil, and wheatgerm oil), containing 10, 20, 40, 60, and 80 μg of oil per milliliter of solvent.

RESULTS AND DISCUSSION

As expected, absorbance in the IR was linear with the amount of oil at each tested wavenumber. This is illustrated in Figure 1 for four oils (wheatgerm oil, canola oil, olive oil, and cod liver oil), where oil concentration is plotted against absorbance at 2920 cm^{-1}. Plots of absorbance versus concentration at the other wavenumbers were also linear.

The IR spectra of lipids extracted from fumigated wheat are compared with that of unfumigated (control) wheat in Figure 2, which shows the percent transmittance at the seven wavenumbers where absorption was most intense. Visual inspection of the spectra revealed no new peaks as a result of fumigation, and there was no effect from any tested fumigant on the transmittance of any of the major peaks. The standard error (SE) in transmittance between the six samples (five fumigated and one control) was low (Figure 2). It averaged less than 0.72%, the standard error between replicates. Carnovale and Quaglia (1973) related lipid damage during storage of grain to the triglyceride peak at 1160 cm^{-1} and the COOH peak at 1710 cm^{-1}. The triglyceride peak (1165 cm^{-1}, Figure 2) and the small COOH peak (not shown) were not effected by fumigation.

In agreement with Lai et al. (1994), the maximum UV absorbance of wheat lipid occurred over a broad band centered at 320 nm, an area of absorption of highly conjugated systems and which typically results in some visible color (Dyer, 1965). There was no effect from fumigation in UV absorbance at any wavelength, including 320 nm, the absorbance maximum, and 340 nm, where the effect of wavelength on absorbance is much greater than at the absorbance maximum (Figure 3). Lack of observed effect applied to fumigated wheat, whether extracted by petroleum ether and butanol or by Soxhlet extraction, and to fumigated wheat germ oil. The standard error (SE) in absorbance between the six samples, five fumigated and one control, from the mean of replicate determinations, was low (Figure 3) and less than 0.0027, the SE between replicates.

Partitioning of fumigants into wheatgerm oil from air is shown in Figure 4. After injection of the fumigant into sealed flasks containing oil, the headspace concentration of CS_2, C_2N_2 and CH_3Br declined rapidly, but then remained constant. When the flasks containing the lipids were heated to 100 °C, the concentrations in the headspace increased. These processes of initial loss of fumigant from the headspace followed by recovery after heating is explained by an initial sorption of fumigant by oil (i.e., partitioning of fumigant into oil) but no subsequent breakdown of fumigant on the lipid. In contrast to initial sorption of CH_3Br and CS_2, there was little partitioning of PH_3 and COS into wheatgerm oil, and concentrations in the headspace remained

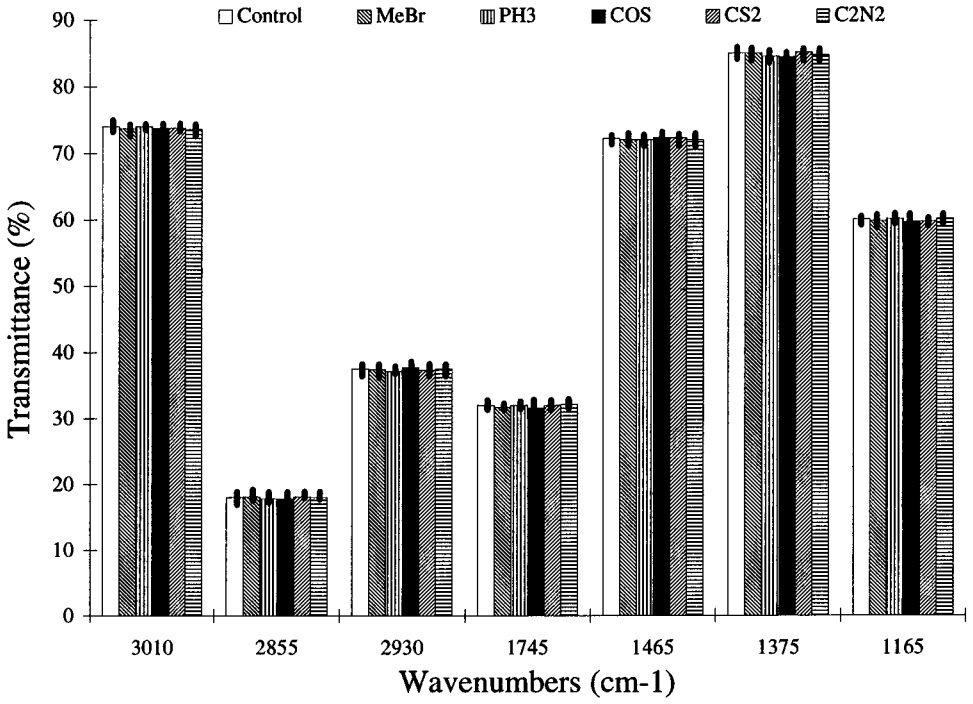

Figure 2. IR transmittance of lipids, at seven wavenumbers, in lipid extracted from control wheat (bar 1) and wheat fumigated with CH_3Br, PH_3, COS, CS_2, and C_2N_2 (bars 2−6, respectively).

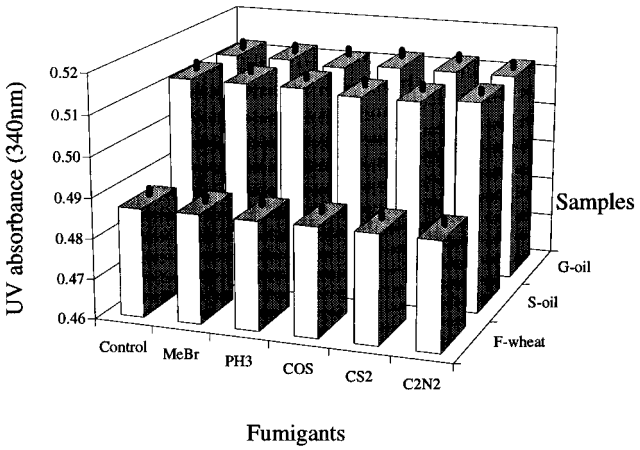

Figure 3. Absorbance of wheat lipids at 340 nm, in control wheat and in wheat after fumigation with CH_3Br, PH_3, COS, CS_2, and C_2N_2: F-wheat, lipid extracted from wheat with petroleum ether plus butanol; S-oil, lipid extracted from wheat by Soxhlet extraction; G-oil, wheatgerm oil.

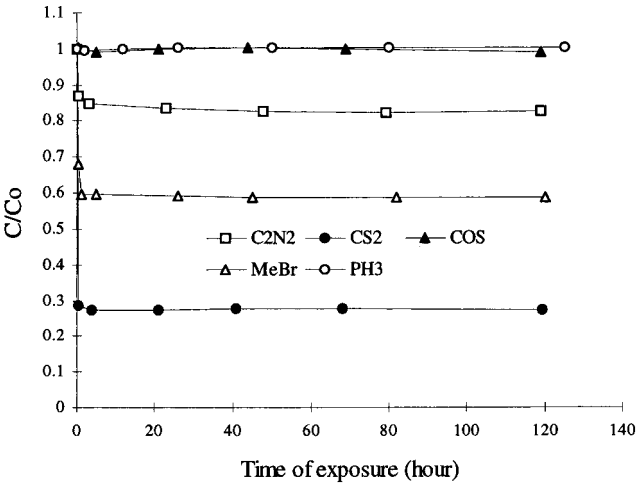

Figure 4. Headspace concentration (C) of fumigants in air over wheatgerm oil, as a ratio to concentration in the absence of oil (C_o).

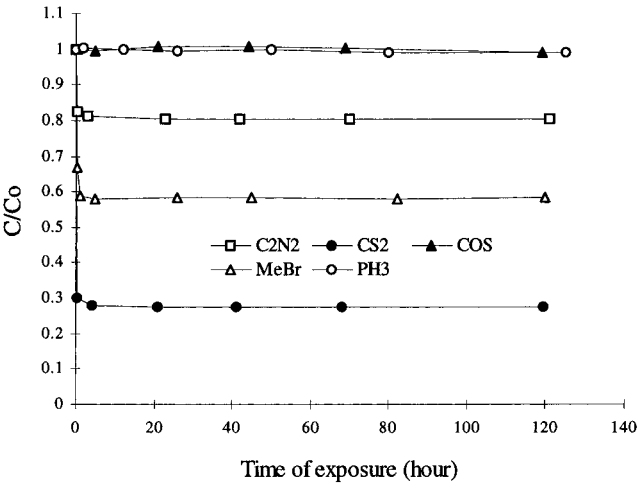

Figure 5. Headspace concentration (C) of fumigants in air over canola oil, as a ratio to concentration in the absence of oil (C_o).

constant. The behavior of fumigants over canola oil (Figure 5) was almost identical with that over wheatgerm oil (Figure 4).

Although each fumigant was applied to wheat at concentrations well in excess of those used in fumigations (Monro, 1969; Desmarchelier, 1994), there was no effect on lipids, as shown by identity of IR and UV spectra. In addition, the concentration of each fumigant remained constant, after initial sorption, indicating no reaction between lipid and fumigant. These results are consistent with the finding of Slover and Lehmann (1972) that periodic fumigation of wheat with either CH_3Br or PH_3 had no significant effect on tocopherols, although storage itself had a minor effect. In our study, the lack of reaction between lipids and fumigants was shown by studies on both the lipid and the fumigant, and by studies in vivo and in vitro.

The reversible sorption of fumigants by lipids may be relevant to residues in foodstuffs, rather than to changes in lipid composition. Grain lipids (Figures 4 and 5) were essentially stable solvent systems for the tested fumigants but were much better solvents for CH_3Br and CS_2 than for PH_3 or COS. It is postulated that the relative degree of partitioning of fumigants into lipids from air is an important factor in explaining the relative persistence of fumigants on commodities and on attributes such as ease of penetration and ease of desorption. Consistent with this hypothesis, PH_3 and COS were more easily blown through wheat than were CS_2 and CH_3Br (Desmarchelier, 1994).

CONCLUSION

No irreversible reactions between fumigants and lipids were detected using procedures, including UV and FTIR spectra and changes in fumigant concentration, which were capable of detecting such reactions. This is a pleasing result from the point of view of human and animal nutrition. The effect of lipids on the retention of fumigants in commodities appears to be more relevant to nutrition and consumer safety than does the possible effect of fumigants on lipid composition.

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