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FUMIGATION OF PADDY RICE AND RICE PRODUCTS WITH ETHYL FORMATE

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ABSTRACT

The phasing–out of methyl bromide (MB) has created a need for alternative fumigants to disinfest cereals such as rice. One possible alternative is ethyl formate (EtF). The interaction of EtF with barley, wheat and canola has been investigated previously, but more data on the fate of EtF during fumigation of rice commodities and its effects on their quality are needed. In this study, 5 kg samples of paddy rice, brown rice, white rice and rice flour were treated for 2 d with EtF applied at 60 $g \cdot m^{-3}$ at 10 and 25ºC. The loss of EtF from the headspace during fumigation was then measured at regular intervals. EtF loss from headspace was especially high during fumigation of paddy rice and rice flour. Remaining fumigant was removed from the commodity by progressive airing. Removal of EtF from all commodities was rapid at 25°C compared to 10°C. After remaining gas concentrations fell below the threshold limit value, EtF residues were determined in all commodities and untreated controls. At 25°C, residues were indistinguishable from background levels. At 10°C substantial residues were detected immediately after airing off. To investigate phytotoxicity of EtF towards paddy rice, the viability of paddy rice after fumigation was measured. It was found that EtF treatment at 60 $g \cdot m^{-3}$ had no effect on the viability of paddy rice.

INTRODUCTION

The phase-out of methyl bromide (MB) for all but pre-shipment and quarantine use has created a need for alternative fumigants for the disinfestation of durable food commodities. In Australia, one such alternative, ethyl formate (EtF), is already widely used for treatment of dried fruit (Simmons and Fisher, 1945; Vincent and Lindgren, 1972; Hilton and Banks, 1997). First stage field trials by the Stored Grain Research Laboratory (CSIRO Entomology), have taken place on wheat (Desmarchelier *et al.,* 1998) with no detrimental effects. EtF has not been systematically tested for fumigation of paddy rice and rice products. For any substance to be effective as a fumigant it is important that a lethal concentration is maintained for an adequate time without effecting product quality. Leakage, sorption and breakdown determine whether this is achieved in any given treatment. The rate of removal of EtF from the commodity after treatment is important for safe use of the fumigant and for the persistence of fumigant residues. This work is the first stage of assessing the applicability of EtF to rice commodities, opening a pathway for effective fumigation using a material other than MB. Here we report on the loss of EtF from the headspace during fumigation of rice commodities, removal of EtF after treatment, EtF residues and the effect of EtF treatment on viability of paddy rice.

MATERIALS AND METHODS

Fumigation treatments

Fumigations were carried out on duplicate 5 kg samples in sealed metal tins. In empty tins, target concentrations were reached easily and no significant EtF leakage occurred over 48 h (data not shown). Fumigations were carried out at 10ºC and 25ºC in controlled temperature rooms at 60 g m^{-3} for 2 d, based on laboratory data by Muthu *et al.,* 1984; Hilton and Banks, 1997 and Damcevski and Annis, 1998.

Fumigant application and measurement

Liquid ‘Eranol’ (manufactured by Orica, active ingredient EtF 901 g L^{-1}) was used for fumigation. Dosages were calculated based on the required vapour concentration, the volume of the empty container and the liquid density of the fumigant preparation.

A Varian 3600 CX gas chromatograph fitted with an AT-WAX column (60 m_0.53 mm ID_1.0 µm) was used for EtF analysis. Chromatographic conditions: Oven temperature: 100°C, Carrier gas: helium at 7 psi, Detector: Flame Ionisation Detector (FID) run at 275°C, Injector: 150°C, 40 µL injections. Concentrations were calculated on the basis of peak areas from the regression equations.

After 48 h of fumigation, the grain was stored at the fumigation temperature, and aired off by removing the lid and running the grain through a Börner divider inside a fume cupboard. The tins were resealed for 24 h before headspace concentrations were measured again. The airing-off procedure was repeated until headspace concentrations had stabilised below 0.3 g m^{-3}, i.e. below the Threshold Limit Value (TLV) of 100 ppm (ACGIH 1999).

Residue analysis

Ethyl formate residues were determined by an improved method recently developed by Vu and Ren (personal communication). Briefly, samples were extracted in sealed flasks with ammonium nitrate solution for 24 h. The headspace of the flasks was then sampled and analysed as described above, except that oven temperature was 70ºC, injection volume was 50 µL and a DB FFAP column (30 m_0.53 mm ID) was used. Levels of EtF were calculated on the basis of peak areas compared to standard curves obtained from spiked standards. Analyses were carried out in duplicate.

Germination of paddy rice

Two replicates of four hundred seeds in lots of 100 were taken randomly from treated and control duplicate 0.5 kg paddy rice sub-samples. Averages reported are therefore based on the germination of 1,600 seeds (n = 16). Seeds were soaked in 80 mL of distilled water for 24 h (soaking method), or exposed to accelerated aging in an oven 50ºC for 24 h (oven method). Each lot of pre-treated seeds were spaced uniformly on sheet paper and placed in a germination cabinet for 7 d at 30ºC. Shoot length (mm), strong development (%), vigour (%), germination (%), abnormal development (%), non-viable seeds (%) and failure to germinate (%) were determined. Seeds were evaluated following the guidelines of the International Seed Testing Association (ISTA 1999).

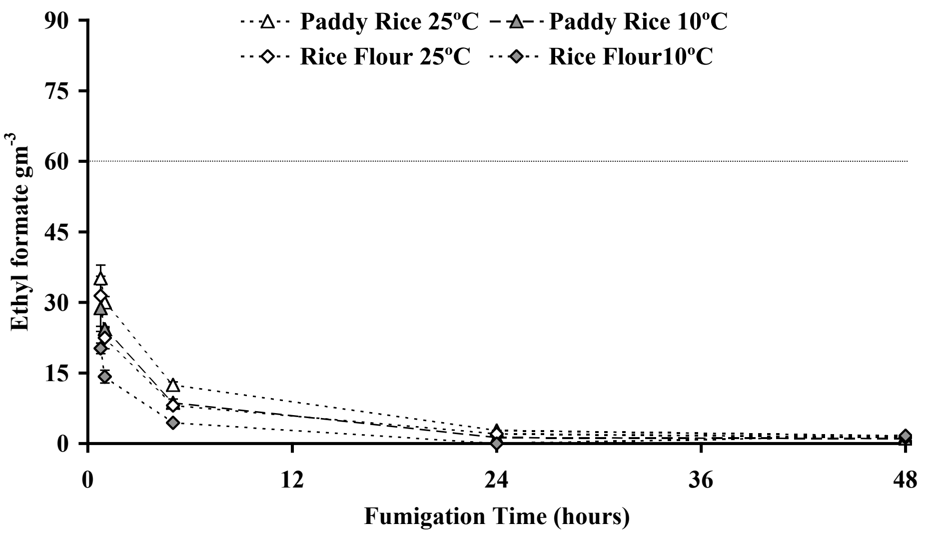

Fig. 1. Loss of ethyl formate from the headspace during fumigation of paddy rice and rice flour at 10°C and 25°C. Ethyl formate was applied at 60 g m^{-3} (dotted line). Error bars show standard error of the mean, n = 4.

RESULTS AND DISCUSSION

Loss of EtF from headspace during fumigation

Fumigation of paddy rice with EtF at 25ºC resulted in initial headspace concentrations approximately half to those expected in an empty container (Fig. 1). Average concentrations fell noticeably from 30 g m^{-3} after 1 h of fumigation to 13 g m^{-3} after 5 h. EtF concentration then continued to decline slowly, falling below 2 g m^{-3} by the end of the treatment period. For 10ºC, the average fumigant concentrations 1 h after application was lower at 24 g m^{-3} than at 25°C. At the end of the fumigation, 1 g m^{-3} of EtF remained in the headspace. Loss of EtF from the headspace in tins containing rice flour fumigated at 25 and 10°C was more rapid than in paddy rice (Fig. 1). At 25°C and 10°C, average EtF concentrations fell from 23 and 14 g m^{-3} measured 1 h after application to 8 and 4 g m^{-3} respectively after 5 h. At the end of the 2 d fumigation period 1 g m^{-3} of EtF was found in the headspace of the flour treated at 25°C and 2 g m^{-3} remained in the tins treated at 10°C.

Brown and white rice lost EtF less rapidly than paddy rice and rice flour (Fig. 2). At 25°C, 1 h after application, average EtF concentrations found in the headspace of tins containing brown and white rice were 66 and 39 g m^{-3} respectively. After 5 h concentrations had fallen to 26 and 25 g m^{-3}, respectively. At 10°C the concentration of fumigant over brown rice was 26 g m^{-3} 1 h after application. Concentration dropped by slightly less than half over 5 h and 4.2 g m^{-3} remained at the end of the fumigation period. In white rice treated at 10°C, 1 h after application 24 g m^{-3} of EtF were present; levels fell to 18 g m^{-3} after 5 h and 10 g m^{-3} remained after 2 d of fumigation.

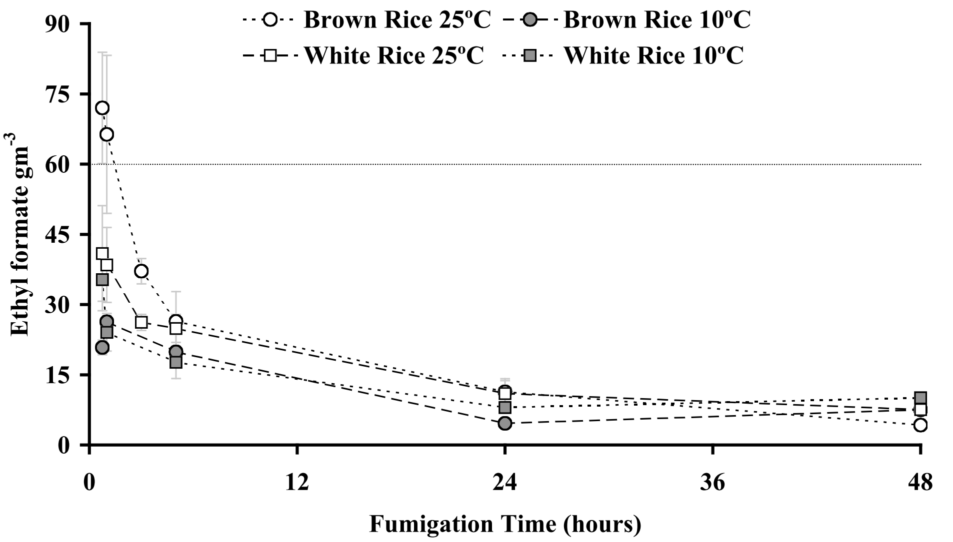

Fig. 2. Loss of ethyl formate from the headspace during fumigation of paddy, brown and white rice at 10°C and 25°C. Ethyl formate was applied at 60 g m^{-3} (dotted line). Error bars show standard error of the mean, n = 4.

The observed EtF concentrations for brown and white rice remaining after 12 h of fumigation at 25°C (Table 1) were adequate for the control of adult *Sitophilus oryzae* (L.) (Annis, 1999; Damcevski and Annis, 1998), *Rhyzopertha dominica* (F.) and *Tribolium castaneum* (Herbst) (Desmarchelier *et al.,* 1998). Treatment rates were marginal in paddy and to low in rice flour and doses would have to be increased to achieve efficacious fumigation. Data on ethyl formate toxicity at 10°C is limited, but based on available data similar conclusions can be drawn to those for 25°C observations. In a recent trial container fumigation of a load of dried sultanas at low temperatures (average 11.6°C) *Oryzaephilus surinamensis* (L.) and *Tribolium confusum* (Jacquelin du Val) adults (both field strains ex Merbein, Victoria, Australia) were successfully controlled at similar doses (Reuss and Tarr, unpublished data). Damcevski and Annis (in press) report complete control of all life stages of *R. dominica*, *T. castaneum* and *S. oryzae* adults at 16°C at 60 g m^{-3} for 24 h.

TABLE 1
Approximate average Ethyl formate headspace concentrations (g m^{-3}) at fixed times in rice commodities treated at 10° and 25°C. All commodities were dosed at 60 gm^{-3}

	Paddy Rice	Brown Rice	White Rice	Rice Flour
Initial[1] 25°C	30	66	39	23
12 h 25°C	9	22	22	9
Initial[1] 10°C	24	26	24	14
12 h 10°C	6	15	15	4

[1] measurement after 1 h.

There was considerable variation in measurements taken during the first hour of fumigation. In some replicates, levels of EtF exceeding the flammability limit in air (85 g m^{-3}) were found in the first 30 min following application. When a sealed system is first dosed with EtF fumigation the system undergoes a mixing phase in which fumigant partly exists in liquid form and the generating vapour is actively distributing throughout the available gas spaces and loss of fumigant is minimal. The high boiling point of EtF (55°C) results in slower volatilisation at low temperatures. Consequently, longer mixing times and lower maximum headspace concentrations are likely to occur at lower fumigation temperatures (Damcevski and Annis, in press). Mixing problems can be overcome by using application techniques that are currently under development at the CSIRO Stored Grain Research Laboratory. One such technique was recently described by Allen *et al.* (in press).

Removal of fumigant from commodity following fumigation

EtF could be removed from rice products through unforced ventilation. Removal of residual fumigant from brown and white rice at 10°C was the slowest (Table 2). EtF was lost faster from rice flour and paddy rice fumigated and then stored at 10°C, with levels dropping below the TLV of 0.3 g m^{-3} after 3 airings. In rice commodities fumigated and subsequently stored at 25°C, removal of EtF from the headspace was quickly and easily achieved. Removal of EtF from rice commodities fumigated at temperatures close to 25°C should be an easy task achievable by natural ventilation alone. Forced aeration may be necessary to rapidly reduce fumigant levels in grain bulks or when fumigating at low temperatures.

TABLE 2
Concentration of ethyl formate (EtF) in headspace after repeated airing by unforced ventilation after fumigation and subsequent storage at 10° and 25°C. Standard error of the mean is shown (n = 4)

Commodity	Repeated airing	EtF at 10ºC (g m^{-3})	EtF at 25ºC (g m^{-3})
Paddy rice	1	0.9±0.07	0.7±0.04
	2	0.3±0.02	0.3±0.04
	3	0.2±0.01	0.1±0.02
	4	0.2±0.02	0.0±0.00
Brown rice	1	2.9±0.09	1.1±0.11
	2	0.7±0.05	0.2±0.01
	3	0.4±0.03	0.0±0.01
	4	0.3±0.01	0.0±0.00
White rice	1	2.9±0.07	0.9±0.23
	2	0.6±0.08	0.2±0.01
	3	0.3±0.02	0.0±0.02
	4	0.2±0.01	0.0±0.00
Rice flour	1	0.8±0.02	0.4±0.04
	2	0.2±0.03	0.1±0.01
	3	0.2±0.02	0.0±0.00
	4	0.2±0.02	0.0±0.00

Residues

Residues found in fumigated and untreated samples are shown in Table 3. Treatment temperature did substantially affect level of residues. At 10°C residues of all commodities were much greater than background level, especially in paddy rice (11.4 mg·kg^{-1}) and rice flour (5.9 mg·kg^{-1}). Desmarchelier *et al.* (1998) stated that EtF residues decayed much faster on warm grain than on cool grain. They suggested a withholding period of 4 weeks after EtF treatment of grain to allow for residues to fall below 1 mg·kg^{-1}.

In Australia, the experimental Maximum Residue Limit (MRL) for EtF for use on dried fruits is set at 1.0 mg·kg^{-1} (National Registration Authority (NRA) 2000). All untreated rice samples had EtF levels below the MRL for dried fruits (Table 3). Currently, the NRA specifies no EtF MRL for grains, because it is considered that the residues are the same as those in natural food components and are of no toxicological significance (NRA 2000). The occurrence of EtF residues in foodstuffs has recently been reviewed by Desmarchelier (1999). Desmarchelier *et al.,* (1999) reported EtF concentrations exceeding the MRL for dried fruits in untreated barley and sultanas. Ramarathnam and Kulkarni (1984) reported EtF in extracted volatiles of scented rice not treated with EtF. Therefore, residues found after the fumigation in rice should be compared to naturally occurring EtF levels. Residues of all samples treated at 25°C were below those found in the untreated product (Table 3).

TABLE 3
Ethyl formate (EtF) residues (mg·kg^{-1}) after airing of fumigant from rice commodities treated at 25°C and 10°C

		EtF fumigation at 10ºC		EtF fumigation at 25ºC		Untreated	
Replicate:		1	2	1	2	1	2
commodity	Paddy rice	11.4	13.0	0.1	0.1	0.2	0.1
	Brown rice	1.7	2.1	0.2	0.2	0.3	0.3
	White rice	1.1	1.1	0.1	0.1	0.3	0.2
	Rice flour	5.9	6.3	0.1	0.1	0.2	0.2

Viability of paddy rice

In this study EtF was shown to have no or very low phyto-toxicity towards rice seed under the treatment schedule applied. Treatment had no effect on shoot length, strong development, low vigour or germination (Table 4). Similarly, fumigation did not increase the number of seeds showing abnormal development, being non-viable or failing to germinate (Table 5).

For the conservation of seed grain and maintenance of grain quality it is critical that the seed is tolerant to fumigation treatment. Desmarchelier *et al.* (1998) reported no negative effect of EtF treatment on wheat quality.

TABLE 4
Shoot length, strong development and low vigour in prepared (soaking or oven method) germinated paddy rice after fumigation with ethyl formate at 10°C and 25°C. Standard deviation is given (n = 16)

Parameter	Method	Fumigation 10ºC	Fumigation 25ºC	Untreated
Shoot length (mm)	*Soaking*	55 ± 3.1	56 ± 2.8	61 ± 5.0
	Oven	55 ± 2.1	57 + 2.0	54 ± 2.5
Strong development (%)	*Soaking*	87 ± 3.2	86 ± 4.2	87 ± 3.1
	Oven	82 ± 2.1	84 ± 3.3	84 ± 4.5
Low vigour (%)	*Soaking*	5 ± 2.1	5 ± 2.3	6 ± 2.2
	Oven	6 ± 2.2	5 ± 2.1	5 ± 2.3
Germination (%)	*Soaking*	92 ± 2.5	91 ± 3.6	93 ± 1.9
	Oven	88 ± 2.5	89 ± 3.3	89 ± 4.0

CONCLUSION

Fumigation of milled rice products with EtF at 25°C promises to be an efficacious pest control measure. The high rate of loss of EtF from the headspace of fumigated paddy rice and rice flour would require the application of higher dosages of fumigant to achieve efficacious insect control in these commodities. A dosage of 60g m^{-3} applied at 25°C resulted in EtF residues in fumigated commodities that were indistinguishable from background levels. At lower temperatures residues might be a problem immediately after treatment, especially in paddy rice and rice flour. Quality of paddy rice measured by viability parameters, such as shoot length, vigour and abnormal development, was not affected by EtF treatment.

TABLE 5
Abnormal development, non-viable seed and root development in the absence of a shoot in prepared (soaking or oven method) non-germinated paddy rice after fumigation with ethyl formate at 10° and 25°C. Standard deviation is given (n = 16)

Parameter	Method	Fumigation 10ºC	Fumigation 25ºC	Untreated
Abnormal development	*Soaking*	1 ± 1.1	2 ± 1.8	1 ± 1.2
	Oven	3 ± 1.2	2 + 0.9	2 ± 1.7
Non-viable seed (%)	*Soaking*	4 ± 2.0	5 ± 2.3	3 ± 1.6
	Oven	6 ± 1.6	7 ± 2.5	6 ± 3.0
Root developed only (%)	*Soaking*	3 ± 2.0	3 ± 1.8	3 ± 1.5
	Oven	3 ± 1.4	3 ± 1.7	3 ± 2.2
Failure to germinate (%)	*Soaking*	8 ± 2.5	9 ± 3.6	7 ± 1.9
	Oven	12 ± 2.5	11 ± 3.3	11 ± 4.0

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