0219

Fumigation Efficacy of Ethyl Formate on Wheat, Corn and Rice in Sealed Desiccators

He Yanping, Xu Li, Chen Sisi, Xu Guangwen, Xie Lingde

Abstract: Ethyl formate (EF) is a potential alternative fumigant to methyl bromide and phosphine for the control of insect pests in stored commodities. Effects of temperature, relative humidity and grain m. c. on toxicity of EF were studied using sealed desiccators containing maize, wheat or rice. Lower temperatures decreased efficacy of EF. A concentration of 80 mg/L EF killed >98% of *Sitophilus zeamais* Motschulsky placed on top of the grain exposed 72h at 16, 24 or 32℃. However, very low mortalities of *S. zeamais* placed under rice at all three temperatures and under wheat at 16℃. A concentration of 80 mg/L EF could not control *Rhyzopertha dominica* (F.) on top of wheat or rice and below all three grains at 16℃, 72h exposure. Increased r. h. increased efficacy of EF. At 35 and 65% r. h., very low mortalities of *S. zeamais* and *R. dominica* were found below maize after an applied concentration of 80 mg/L EF after 72h at 25℃. However, when r. h. was increased to 95%, it killed >90% of both pests. The efficacy of EF was reduced at higher grain moisture contents. At >12% m. c., mortality of *S. zeamais* and *R. dominica* under maize was below 20% even though higher r. h. of 60 and 90% were used.

Key words: ethyl formate, temperature, r. h., moisture content, mortality, fumigation

Introduction

Ethyl formate (EF) is an easily vaporised organic liquid. It has been investigated as a rapid disinfestant for many stored product pests and other pests[1,2,3]. EF has been used for disinfestation of pests in stored dried fruit since 1927[4]. However, it as a grain fumigant has waned following development of methyl bromide and phosphine[5]. Methyl bromide is now being phased out internationally under the Montreal Protocol because of its damaging effect on the ozone layer. Use of phosphine is under increasing pressure because of the frequency of resistance to it[6]. Ethyl formate has been considered as a candidate for development as a fumigant of stored grain.

The rapid toxic action of EF has been observed in many insects. Damcevski and Annis showed that EF could give >99% mortality in adult insects and >95% mortality in other stages with a 24 h exposure period[1,7,8]. EF also is an environment friendly fumigant. EF naturally occurs in many grains, vegetables, fruit, and animal products such as milk and cheese[5-9]. Its residues break down to the naturally occurring products, formic acid and ethanol[1]. However, EF is easily sorbed by all commodities and has poor penetration through bulk grain. Its fumigant toxicity is affected by many complex factors. Carbon dioxide (CO_2) can enhance fumigant toxicity of EF, and many other fumigants, to insects[10,11]. CO_2 mixed with EF can substantially reduce its ammability and has the effect of raising the lower ammability limit[12]. Furthermore, CO_2 has ability to improve the movement of fumigant through the grain[13]. Efficacy of EF against pests was markedly increased at increased r. h. in the absence of grain[14]. However, the effect was less in presence of grain, possibly due to the high solubility of EF in water (105 g/L at 20℃)[14,15]. Ren and Mahon[5] showed that using two dosages of 85 g/t, EF gave a high level of control of all stages of most of the test insects in the wheat, split faba beans (*Vicia faba*) and sorghum. Split faba beans and sorghum took up EF more strongly than wheat. EF residues in wheat were reduced to natural levels within 2 weeks from application, but in split faba beans and sorghum it took more than 4 weeks for residues to return to near background levels.

Maize weevil, *Sitophilus zeamais*, and lesser grain borer *Rhyzopertha dominica* are two serious pests of stored grain. High mortality of *R. dominica* by EF has been reported[8], but there is little reported data on EF against *S. zeamais*. Two pests were chosen for comparative studies.

In this paper, we report the influence of temperature, kind of grain, relative humidity and moisture content (m. c.) of grain on EF effects on these two species.

1. *College of Food Science and Engineering*, *Wuhan Polytechnic University*, *Wuhan* 430023, *P. R. China.*

Materials and Methods

Insects

All test insects were reared at Wuhan Polytechnic University in electronically controlled incubators 27 ± 1℃ and 75% 5% r. h.. *Sitophilus zeamais* was reared on whole wheat, *Rhyzopertha dominica* on broken wheat, *Tribolium castaneum* on whole wheat flour with 5% yeast. The wheat to be used was sterilized at 80℃ for 2h. The moisture content was then adjusted to 13% ± 1% w. b. Adults (7 – 14 days old) were used in this study.

Chemicals

The EF used was analytical grade (≥97% purity), purchased from Tianjin Basifu Chemical Ltd.

EF Treatment at Different Temperatures

The insects were treated with EF in gastight 15 L glass desiccators, sealed with glass stoppers fitted with a septum for gas injection. Four fifths of the volume of the desiccators were filled by 12% m. c. grain (maize, wheat or rice). Each test replicate used 30 adult maize weevils and 30 adult lesser grain borers. Three replicates of each kind of insect were put below the grain and also on the top of the grain. 1mL liquid EF was added onto a filter paper in a dish on the grain surface. The final calculated concentration of EF in the desiccators was 80 mg/L. EF treatments were carried out at 16, 24 and 32℃, each at 70% r. h. in electronically controlled incubators. After the exposure of 72h, the desiccators were opened and mortalities were assessed for each replicate.

EF Treatment at Different Humidities

Test insects were treated in desiccators, as above. The desiccators were filled to two-thirds capacity with 6 kg maize. Prior to being sealed, the desiccators, with grain were held at 25℃ at 35%, 65% or 95% r. h for 30 minutes. The containers were then sealed and 1mL EF was applied introduced through the septum using a gas-tight syringe. After 72h, the desiccators were opened and mortalities were assessed for every replicate. Maize at 10% m. c. was used in r. h. 35% r. h. tests, and 12% m. c. maize was used in 65% and 95% r. h. tests.

EF Fumigation at Different r. h. and m. c. of Grain

Fumigations were carried out as above at 25℃ using maize of different moisture contents. The moisture contents and resulting r. h. is given in Table 1.

Table 1. Fumigation condition of EF for corn at 25℃

Weight of grain (g)	Grain m. c. (%)	r. h. (%)	Concentration of EF (mg/L)
6	9.2	30	80
6	12.7	60	80
6	16.25	80	80

Results

EF Treatment at Different Temperatures

Table 2 shows that a concentration of 80 mg/L EF killed >98% of *S. zeamais* placed on the top of the maize, wheat or rice after exposure of 72h at 16, 24 and 32℃ (Table 2). Very low mortalities were observed for insects placed below under rice at all three test temperatures and under the wheat at 16℃. A initial concentration of 80 mg/L EF could not control *R. dominica* after 72h at 16℃ (Table 3) did not kill all of the test insects either on top of or below the grain. It killed more than 90% lesser grain borers both on the top of the maize and wheat and underneath the two grains at 24℃ and 32℃. Lower mortalities of lesser grain borer were found in the rice at the three temperatures. These results showed that higher temperature increased the efficacy of EF. EF fumigant efficacy varied with the kind of grain used (Tables 2, 3).

Table 2. Mortality (%) of maize weevils with EF applied at 80 mg/L after 72h at different temperatures [a]

Grain		16℃	24℃	32℃
Maize	top	100.0 ±0.0a	100.0 ±0.0a	100.0 ±0.0a
	bottom	97.0 ±2.6a	100.0 ±0.0a	98.8 ±2.1a
Wheat	top	92.4 ±10.3a	100.0 ±0.0a	100.0 ±0.0a
	bottom	29.7 ±13.6a*	98.9 ±1.9b	100.0 ±0.0b
Rice	top	98.9 ±1.9a	100.0 ±0.0a	98.9 ±1.9a
	bottom	0.0 ±0.0a*	5.7 ±2.1b*	6.9 ±1.7b*

[a] Results are the means SD ($n = 3$). Means within columns followed by the same letter are not significantly different ($p > 0.05$,

LSD Fisher's multiple range test).

[*] Mortalities of pests is significantly different between top and down of the same grain($p<0.05$, Student's t test).

Table 3. Mortalities of lesser grain borers with EF applied at 80 mg L^{-1} for 72h at different temperatures(%)[a]

Grain		16℃	24℃	32℃
Maize	top	94.6 ±5.0a	97.7 ±4.0a	98.9 ±2.0a
	bottom	74.1 ±0.8a *	92.0 ±4.9b	93.4 ±5.8b
Wheat	top	51.9 ±10.2a	98.9 ±1.9b	100.0 ±0.0b
	bottom	5.6 ±3.8a*	94.4 ±6.9b	92.2 ±10.7b
Rice	top	68.6 ±6.5a	63.3 ±3.3a	61.2 ±6.2a
	bottom	3.4 ±0.1a*	4.4 ±1.9a*	11.5 ±3.2b*

[a] Results are the means SD($n=3$). Means within columns followed by the same letter are not significantly different($p>0.05$, LSD Fisher's multiple range test).

[*] Mortalities of pests is significantly different between top and down of the same grain($p<0.05$, Student's t test).

Efficacy of EF Fumigation at Different r. h.

Increased r. h. increased the efficacy of EF. At 35 and 65% r. h., very low mortalities of *S. zeamais* and *R. dominica* were found under the maize with an initial concentration of 80 mg/L EF after 72h at 25℃. However, when r. h. was increased 95%, it killed more than 85% of both pests (Table 4).

Table 4. Mortalities of the two pests with EF applied at 80 mg/L after 72h at 25℃ for maize at different r. h. (%)[a]

Pest		16℃	24℃	32℃
S. zeamais	top	92.3 ±1.7a	100.0 ±0.0b	97.8 ±1.9b
	bottom	22.9 ±5.0a*	71.9 ±0.8b *	96.8 ±5.6c
R. dominica	top	96.3 ±6.4a	89.4 ±9.8a	93.0 ±3.2a
	bottom	40.9 ±3.2a*	64.7 ±3.3b*	86.3 ±13.9c

[a] Results are the means SD($n=3$). Means within columns followed by the same letter are not significantly different($P>0.05$, LSD Fisher's multiple range test).

[*] Mortalities of pests is significantly different between top and bottom of the same grain($P<0.05$, Student's t test).

Effect of r. h. and m. c. of Maize to EF Fumigation

Both r. h. and m. c. of maize affect the efficacy of EF. An initial concentration of 80 mg/L, EF gave lower mortalities of both *S. zeamais* and *R. dominica* with the lowest r. h. (30%) (Table 5). At >12% m. c., mortalities of *S. zeamais* and *R. dominica* below the maize were <20%, even with higher r. h. (60% and 80%) (Table 5). These results were far below the mortalities observed for *S. zeamais* (71.9%) and *R. dominica* (64.7%) below maize at 65% r. h., 12% m. c. and 25℃ (Table 4). This shows that m. c. of grain affected efficacy of EF much more than r. h. (Tables 4, 5).

Table 5. Mortalities of two pests with EF applied at 80 mg/L after 72h at 25℃ for the different m. c. of maize(%)[a]

Pest		r. h. 30% m. c. 9.2%	r. h. 60% m. c. 12.7%	r. h. 80% m. c. 16.3%
S. zeamais	top	53.5 ±3.2a	98.5 ±2.6b	97.5 ±4.3b
	bottom	5.1 ±0.8a*	16.9 ±3.6b*	13.3 ±6.7b*
R. dominica	top	29.8 ±3.1a	60.4 ±17.0b	54.9 ±11.7b
	bottom	5.3 ±1.8a*	16.7 ±5.8b*	7.5 ±2.9a*

[a] Results are the means SD($n=3$). Means within columns followed by the same letter are not significantly different($P>0.05$, LSD Fisher's multiple range test).

[*] Mortalities of pests is significantly different between top and down of the same grain($P<0.05$, Student's t test).

Discussion

In general, fumigants show high toxicities at higher temperatures[16], but there is little data published on effects of temperature to EF. Damcevski et al. proposed that this should be studied[14]. Our results showed that EF gave higher mortality of pests at high temperatures, especially below a layer of grain (Tables 2,3). However, higher mortalities of *S. zeamais*, *R. dominica* and *Tribolium castaneum* were found at lower temperatures in the absence of grain (these Proceedings). It is suggested that higher temperatures increase uptake of EF, through increased respiration, and also increase fumigant movement thus increasing its effectiveness in presence of grain.

The presence or absence of grain and its amount directly affects the efficacy of EF. There is little loss of EF without wheat[14]. The loss of EF from the headspace above wheat has been studied observed. The loss is attributed to sorption and subsequent breakdown by the wheat[1,14,17,18]. Also, the influence of a commodity on the ef cacy of fumigation is due both to its sorption behaviour and also its effect on the metabolism of the insects[5]. Damcevski et al. reported the effect of wheat on the metabolism of adult stored-product Coleoptera[19]. Changes in the respiration and metabolism of *S. oryzae* were observed when in the presence of wheat[14]. Results in this paper showed effectiveness of a set dosage of EF was influenced by the type of grain treated (Tables 2, 3). This implies that the kind of grain influences the sorption abilities to EF. Also, the different grains may affect metabolism of pests differently. This idea merits further study.

Results in this paper are consistent with previous observations that EF showed higher toxicity to pests in higher r. h. (Table 4). EF is sorbed by most commodities[15,20,21], especially where they have high moisture content or are warm[14]. Tables 4 and 5 show that higher m. c. of grain inhibited efficacy of EF more strongly than higher r. h. However, higher r. h. can increase the m. c. of grain, so the effect of r. h. on EF action is complex.

The m. c. of grain should be considered when EF is to be used to fumigate stored grain. In order to get best effectiveness of EF, it is necessary to choose the moost favorable conditions of temperature, kind of grain, m. c. of grain and r. h..

This research will maximize the ability of the "green" fumigant, ethyl formate, as a fumigant for stored grain.

Acknowledgements

This work was supported by the National Project of Scientific and Technical Supporting Programs of China During the 11th Five-year Plan(NO. 2006BADO2A18 -01)

References

[1] Damcevski K A, Annis P C. Two old fumigants for the new millennium. In: Zalucki, M P, Drew R A I, White, G. G. (eds.), Pest management-future challenges. Proceedings of the Sixth Australasian Applied Entomological Research Conference, Brisbane, Australia, 1998, 2: 307

[2] Stewart J K, Aharoni Y. Vacuum fumigation with ethyl formate to control the green peach aphid in packaged head lettuce. Journal of the American Society of Horticultural Science, 1983, 108: 295 -298

[3] Aharoni Y, Nitzan Y, Copel A. Natural volatiles to control the California red scale on harvested grapefruit. Tropical Science, 1987, 27: 155 -157

[4] Simmons P, Gertler S I. Ethyl formate and isopropyl formate as fumigants for packages of dried fruits. Journal of Economic Entomology, 1945, 38: 715 -716

[5] Ren Y L, Mahon D. Fumigation trials on the application of ethyl formate to wheat, split faba beans and sorghum in small metal bins. Journal of Stored Products Research, 2006, 42: 277 -289.

[6] Benhalima H, Chaudry M Q, Mills K A, Price N R, Phosphine resistance in stored - product insects collected from various grain storage facilities in Morocco. Journal of Stored Products Research, 2004, 40: 241 -249

[7] Damcevski K A, Annis P C. Does ethyl formate have a role as a rapid grain fumigant Preliminary ndings. In: Donahaye, E J, Navarro S, Leesch J. (eds.), Proceedings of the International Conference on Controlled Atmosphere and Fumigation in Stored Products, Fresno, California, USA, 2001, 91 99

[8] Damcevski K A, Annis P C. The response of three stored product insect species to ethyl formate vapour at different temperatures. In: Wright E J, Banks H J, Highley E. (eds.), Stored Grain in Australia 2000. Proceedings of the Australian Postharvest Technical Conference, Adelaide, Australia. Stored Grain Research Laboratory, CSIRO Entomology, Australia, 2002, 78 81

[9] Vu T L, Ren Y L, Natural levels of ethyl formate in stored grains determined using an improved method of analysis. Journal of Stored Products Research, 2004, 40: 77 -85

[10] Nicolas G, Sillans D. Immediate and latent effects of carbon dioxide on insects. Annual Re-

view of Entomology, 1989, 34: 97 – 116
[11] Simpson T, Bikoba V, Mitcham E J. Effects of ethyl formate on fruit quality and target pest mortality for harvested strawberries. Postharvest Biology and Technology, 2004, 34: 313 – 319
[12] Haritos V S, Damcevski K A, Dojchinov G. Improved efficacy of ethyl formate against stored grain insects by combination with carbon dioxide in adynamic' application. Pest Management Science, 2006, 62: 325 – 333
[13] Leesch J G. Carbon dioxide on the penetration and distribution of phosphine through wheat. Journal of Economic Entomology, 1992, 85: 157 – 161
[14] Damcevski K A, Annis P C. Influence of grain and relative humidity on the mortality of *Sitophilus oryzae* (L.) adults exposed to ethyl formate vapour. Journal of Stored Products Research, 2006, 42: 61 – 74
[15] Hilton S J, Banks H J. Ethyl formate as a fumigant of sultanas: sorption and ef cacy against six pest species. In: Donahaye E J, Navarro S, Varnava A. (eds.), Proceedings of the International Conference on Controlled Atmosphere and Fumigation in Stored Products, Nicosia, Cyprus. Nicosia: Printco Ltd. 1997, 409 – 422
[16] Bai X G. Stored products pest and management. Science Press. 2002, 451 – 480
[17] Damcevski K A, Annis P C. Does ethyl formate have a role as a rapid grain fumigant Preliminary ndings. In: Donahaye, E J, Navarro S, Leesch J. (eds.), Proceedings of the International Conference on Controlled Atmosphere and Fumigation in Stored Products, Fresno, California, USA, Executive Printing Services, Clovis, CA, USA, 2001, 9199Bell D M, Cogan C H, Highley P M(eds.). Advances in stored product protection. Proceedings of the Eighth International Working Conference on Stored Product Protection, York, England, 22 26 July 2002. , Wallingford CAB International. 2002, 533 – 538
[18] Desmarchelier J M, Allen S E, Ren Y, Moss R, Vu L T. Commercial scale trials on the application of ethyl formate, carbonyl sulphide and carbon disulphide to wheat. CSIRO Entomology Technical Report No. 75, 1998, 1 – 63
[19] Annis P C, van S Graver J E. Fumigation and controlled atmospheres in the90s and beyond. In: Naewbanij J O, Manalay A A (eds.), Proceedings of the 14th ASEAN Seminar on Grain Postharvest Technology, Manila, Philippines, 5 8 November 1991. ASEAN Grains Postharvest Programme, Bangkok, Thailand, 1993, 283 – 296
[20] Banks H J, Behaviour of gases in grain storages. In: Champ, B R, Highley E., Banks H J (eds.). Fumigation and controlled atmosphere storage of grain. Proceedings of an International Conference, Singapore, 14 18 February 1989. ACIAR Proceedings 25, 1990, 96 – 107