

Article

# Insecticidal Effects of Fumigants (EF, MB, and PH<sub>3</sub>) towards Phosphine-Susceptible and -Resistant *Sitophilus oryzae* (Coleoptera: Curculionidae)

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**Abstract:** This study was conducted to evaluate the insecticidal effects of ethyl formate (EF), methyl bromide (MB), and phosphine (PH<sub>3</sub>) fumigants against PH<sub>3</sub>-susceptible and -resistant strains of the rice weevil (*Sitophilus oryzae*), a major rice pest. The highest lethal concentration time 50 (LCt<sub>50</sub>) values of the PH<sub>3</sub>-susceptible strains were 255.797, 21.104, and 6.171 mg h L<sup>-1</sup> for EF, MB, and PH<sub>3</sub>, respectively, at pupal stage. The highest LCt<sub>50</sub> values of the PH<sub>3</sub>-resistant strains were 149.028 and 48.170 mg h L<sup>-1</sup> for EF and PH<sub>3</sub>, respectively, at late larval stage, and 43.520 mg h L<sup>-1</sup> for MB at pupal stage. In comparison to the susceptible strains, the PH<sub>3</sub>-resistant strain collected in South Korea had a resistance level 4 to 56 times higher. Use of the major quarantine fumigants EF, MB, and PH<sub>3</sub> indicated the existence of PH<sub>3</sub>-resistant rice weevils in South Korea for the first time.

Keywords: phosphine; resistance; Sitophilus oryzae; ethyl formate; methyl bromide

## 1. Introduction

Stored pests are known to cause damage to stored products, such as reduced grain weight, quality, commercial value, and seed survival [1]. More than 600 species of insects infesting stored grains belong to Coleoptera, among which the genus Sitophilus includes several important grain pest species [2–5]. *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae) reduces the amount and quality of stored grains and causes economic losses [1,6]. *S. oryzae* females make holes and lay an egg, and larval and pupal period is spent hidden in the stored grain [6]. *S. oryzae* larvae feed on proteins and vitamins by consuming the germ of cereals, and adults largely reduce the carbohydrate content by consuming the endosperm of the grains [1,7].

Methyl bromide (MB) and phosphine (PH<sub>3</sub>) fumigants are mainly used for fumigation of grain at storages and silos to control pests damaging stored grains [8]. MB is an old fumigant discovered in 1932 that has been used in virtually every field of disinfection and disinfestation, such as fruit, grains, wood, and necessities [9]. However, it has been designated as an environmentally destructive substance causing ozone depletion [10], and its use has been completely prohibited except for quarantine and pre-shipment purpose under the Montreal Protocol [11,12]. PH<sub>3</sub> is known to spread rapidly during fumigation, has low fumigant residue after treatment, and has no side effects on seed survival [13,14]. However, PH<sub>3</sub> resistance has been found in India, China, Vietnam, Morocco, Brazil, and Australia. In each country, *S. oryzae* has been reported to be phosphine-resistant [15–21].

As an alternative to these fumigants, the efficacy of ethyl formate (EF) against stored product insects have been investigated in a wide range of studies [22–24]. EF is a low-molecular-weight volatile



material characterized by little residue [25] and occurs naturally in a variety of products such as beer, rice, and beef [26]. It has been used for dry fruit insect control since 1927 and has also been used for the disinfection of cereals, stored dry beans, tomatoes, and preserved foods [27,28]. In the United States, pesticides have been registered [29] for the control of pests in storage products such as *Tribolium confusum* and *Ephestia figuliella* in raisins [30].

*S. oryzae* is an important pest in the management of postharvest agricultural products. Phosphine resistance of *S. oryzae* has risen sharply to 75% in developing countries by 2000 [20], requiring the management of resistance. Therefore, this study was conducted to investigate the resistance levels of the newly discovered PH<sub>3</sub>-resistant rice weevils of South Korea and compare these resistance levels to those of susceptible strains. EF, MB, and PH<sub>3</sub> fumigants were used to provide baseline data on rice weevil control through a comparison of treatments currently recommended by quarantine.

#### 2. Materials and Methods

#### 2.1. Insects

PH<sub>3</sub>-susceptible strain (control strains) of *S. oryzae* was reared at Murdoch University (WA, Australia), and the PH<sub>3</sub>-resistant strain of *S. oryzae* was reared at Chungbuk National University (Chungcheongbuk-do, South Korea). All tested insects were brought to the Plant Quarantine Technology Center before experiment (Gyeongsangbuk-do, South Korea). The breeding conditions were maintained at a temperature of  $27 \pm 1$  °C and relative humidity of 50–60% in a brown rice feed at the Plant Quarantine Technology Center. The presence or absence of *S. oryzae* eggs were confirmed by thinning the area covered with gelatinous fluid near the rice grain (Figure 1). *S. oryzae* eggs were used in the experiment within 2–3 days after oviposition of *S. oryzae* adults. The larvae within 1–2 and 22–25 days after hatching were used as early and late larvae, respectively. Pupae were collected within 3–4 days after pupation. Adult of *S. oryzae* were grown for 10–20 days after emergence. The inside of the rice was checked to determine whether larvae or pupae were present. It was difficult to observe the inside of the brown rice, so a stereomicroscope (MDG33, Leica, Wetzlar, Germany) was used. All insects were inoculated on an insect breeding dish ( $\Phi$  5.5 cm × 1.5 cm, SPL, Pocheon, Gyeonggi-do, Korea) and fed 2 g of brown rice.



(a)

(b)

Figure 1. Cont.



(-)

**Figure 1.** Identification method of rice weevil infected grain. (**a**) Egg of *Sitophilus oryzae;* (**b**) an egg covered with rice; (**c**) inside egg with removed surface of rice.

## 2.2. Fumigants

 $\rm PH_3$  was purchased as ECO2Fume ( $\rm PH_3$  2% + CO\_2 98%) from Cytec (Sydney, NSW, Australia), and EF was purchased as liquid EF (EF 97%) from Aldrich Chemical Company Inc. (St. Louis, MO, USA). MB was used as a control, and Youngil MB (MB 98.5%) of Nonghyup Chemical (Seongnam, Gyeonggi-do, Korea) was used.

## 2.3. Fumigation System

Fumigant treatment was performed at the Plant Quarantine Technology Center. The fumigant treatment was applied in a fumigation chamber at a temperature of  $20 \pm 1$  °C and a relative humidity of  $60 \pm 10\%$ , and fumigation was carried out in a 12 L glass desiccator (DWK Life Sciences, Mainz, Germany). Tested insects were put in  $100 \times 40$  mm size insect breeding dish (SPL, Pocheon, Gyeonggi-do, Korea) before fumigation. More than 30 insects were carried in each dish and three dishes were used for each test as replicates.

PH<sub>3</sub>-susceptible *S. oryzae* was treated at a concentration of 0.01 to 4.00 mg/L for 20 h, but in the case of PH<sub>3</sub>-resistant *S. oryzae*, a high concentration exceeding 4 mg/L is required, so concentration was fixed to 0.50 mg/L and the treatment time was varied from 24 h to 504 h. EF was applied at 10 to 150 mg/L for 4 h, and MB was applied at 3 to 25 mg/L for 4 h. Test of PH<sub>3</sub> resistance was performed according to the Food and Agriculture Organization (FAO) criteria [31]. According to FAO test, a population was judged to have resistance when more than 2 individuals survived for 20 h at PH<sub>3</sub> 0.04 mg/L. EF and MB treatments were observed at 24 h after fumigation, and PH<sub>3</sub> treatment was observed at 72 h after fumigation due to the knock-down phenomenon. The insecticidal rate was determined by touching an insect body with a microscopic needle. It was judged as dead if there was no movement using a microscope (MDG33, Leica, Wetzlar, Germany).

## 2.4. Measurement of Fumigant Concentrations

To investigate the actual concentration in the treated samples, 60 mL of sample gas was extracted from all experimental groups using a 60 mL syringe and 1 L Tedlar bag (SKC, Dorset, United Kingdom). EF and MB samples were taken at 30 min and 1, 2, and 4 h, and PH<sub>3</sub> samples were taken at 30 min and 1, 4, and 20 h after fumigation. If fumigation time was longer than 20 h, samples were extracted at 24-h intervals until the completion of the experiment.

The concentrations of EF and MB were measured using an Agilent GC 7890A equipped with a flame ionization detector (FID) after separation on an Rtx-5 column (15 m × 250  $\mu$ m × 1  $\mu$ m, RESTEK, Bellefonte, PA, USA) operating in split mode (10:1). The PH<sub>3</sub> concentration was determined using an Agilent GC 7890A equipped with a flame photometric detector (FPD) and HP-PLOT/Q (30 m × 530  $\mu$ m × 40  $\mu$ m, Agilent, Santa Clara, CA, USA) operating in split mode (10:1). The injector and oven temperature were 200 °C. The detector temperature was 250 °C. The injection volumes and flow rates of EF, MB, and PH<sub>3</sub> were 60, 60, and 20  $\mu$ L and 1.5, 1.5, and 5 mL/min, respectively. The concentrations of EF, MB, and PH<sub>3</sub> were calculated based on peak areas against external standards.

#### 2.5. Determination of the Concentration $\times$ Time (Ct) of the Fumigants

Concentration is an important factor to determine efficacy of chemical insecticide, including fumigants. However, fumigation time also is an important factor particularly for the fumigant, although, importance can be varied by the kind of fumigant [32]. To examine the effect of both concentration and time, exposure of the fumigant was expressed as concentration × time (Ct) product. The Ct of fumigants was calculated using the equation of Monro [33].

#### 2.6. Statistical Analysis

All experiments were performed with 3 replicates. Percent mortality and standard error (SE) were determined via an Excel v. 2007. The lethal concentration time 50 (LCt<sub>50</sub>) and 99 (LCt<sub>99</sub>) values of *S. oryzae* were obtained using the Probit analysis [34] program (CSIRO 1998), with logarithmic transformation of data.

#### 3. Results

#### 3.1. Fumigation of Phosphine-Susceptible S. oryzae

The PH<sub>3</sub> susceptibility-related effects of PH<sub>3</sub>, EF, and MB fumigants on *S. oryzae* eggs, early larvae, late larvae, pupae, and adults were evaluated. PH<sub>3</sub> showed a 100% insecticidal rate against eggs, early larvae, and adults at 1.00 mg/L and an 85.56% insecticidal rate in late larvae at 1.00 mg/L (Figure 2). Pupae treated with 4.00 mg/L, the highest treatment concentration, showed an insecticidal rate of 86.04%. As a result of treatment with EF, eggs, early larvae, and adults showed insecticidal rates of 90% or more at an EF concentration of 70 mg/L (Figure 3). However, in the case of late larvae treated with 120 mg/L, the insecticidal rate was 94.43%. In the pupae, the highest treatment concentration of 150 mg/L was applied, but the insecticidal rate was as low as 73.30%. MB showed a 100% insecticidal rate at 10 mg/L for eggs, 8 mg/L for adults, and 7 mg/L for early larvae but 25 mg/L for pupae and 20 mg/L for late larvae (Figure 4). These results indicate that PH<sub>3</sub>-susceptible *S. oryzae* is most tolerant in the pupal and late larval stages. Among PH<sub>3</sub>, EF, and MB fumigants, LCt<sub>50</sub> was the lowest (0.295 mg h/L) when treated with PH<sub>3</sub>, and the highest LCt<sub>50</sub> (255.797 mg h/L) was obtained by treating the pupae with EF. PH<sub>3</sub> showed a high insecticidal rate at a low concentration but had to be treated for a long time, and EF had to be treated at a high concentration, though the treatment time was short.

#### 3.2. Evaluation of Phosphine-RESISTANT S. oryzae by Fumigation Time of Phosphine

Assessment of PH<sub>3</sub> resistance was conducted according to the FAO criteria. *S. oryzae* collected from South Korea was treated with 0.04 mg/L PH<sub>3</sub> for 20 h and compared with PH<sub>3</sub>-susceptible *S. oryzae* distributed from Australia. The PH<sub>3</sub>-susceptible *S. oryzae* showed a 98.3% insecticidal rate, and *S. oryzae* in South Korea was 21.0%. According to the FAO resistance evaluation criteria, *S. oryzae* in South Korea is considered to have resistance. The effect of PH<sub>3</sub> on PH<sub>3</sub>-resistant *S. oryzae* was evaluated by setting the concentration to 0.5 mg/L and then by treatment time. As a result, the eggs, early larvae, late larvae, pupae, and adults showed 100% insecticidal rates when treated for 72, 120, 168, 336, and 504 h, respectively (Figure 5).



**Figure 2.** Mortality of phosphine susceptible *S. oryzae* exposed to phosphine (PH<sub>3</sub>) fumigant for 20 h at 20 °C in 12 L desiccator.



**Figure 3.** Mortality of phosphine susceptible *S. oryzae* exposed to ethyl formate (EF) fumigant for 4 h at 20 °C in 12 L desiccator.



**Figure 4.** Mortality of phosphine susceptible *S. oryzae* exposed to methyl bromide (MB) fumigant for 4 h at 20 °C in 12 L desiccator.



**Figure 5.** Mortality of phosphine resistant *S. oryzae* exposed to PH<sub>3</sub> fumigant for 0.5 mg/L at 20 °C in 12 L desiccator.

#### 3.3. Alternative Fumigants for PH<sub>3</sub>-Resistant S. oryzae

The effect of EF and MB fumigants on  $PH_3$ -resistant *S. oryzae* eggs, early larvae, late larvae, pupae, and adults was evaluated. EF showed insecticidal rates of 100% at 70 mg/L for egg, early larvae, and pupae, 100% at 90 mg/L for adults, and 88.90% at 90 mg/L for late larvae (Figure 6).

MB treatment showed a 100% insecticidal rate at 8 mg/L for eggs, 10 mg/L for early larvae and adults, whereas late larvae and pupae showed a 98.9% insecticidal rate at 20 mg/L (Figure 7). The LCt values were analyzed based on the effect of the fumigants on PH<sub>3</sub>-resistant *S. oryzae*. The LCt<sub>50</sub> value of EF was the highest (149.028 mg h/L), and the LCt<sub>50</sub> of MB was the lowest (25.840 mg h/L). The LCt50 of EF was 5.76 times higher than that of MB.

### 3.4. LCt Analysis of the PH<sub>3</sub>-Susceptible and PH<sub>3</sub>-Resistant Rice Weevils

The  $LCt_{50}$  and  $LCt_{99}$  values of the PH<sub>3</sub>-susceptible and PH<sub>3</sub>-resistant strains of rice weevils were analyzed based on the results of the effects of the three fumigants.

The LCt analysis results for EF showed that the LCt<sub>50</sub> values of EF were confirmed to be the highest at pupal stage (255.797 mg h/L) and the lowest at early larval stage (60.110 mg h/L). The LCt<sub>50</sub> values of EF on the PH<sub>3</sub>-resistant strains showed a different aspect with the previous one, that egg stage was the lowest (60.034 mg h/L) and late larval stage was the highest (149.028 mg h/L). PH<sub>3</sub>-resistant strain showed higher LCt<sub>50</sub> than PH<sub>3</sub>-susceptible strain at early larval stages, but lower LCt<sub>50</sub> at late larval and pupal stages, which are tolerant stages (Table 1).

The LCt<sub>50</sub> values of MB for the PH<sub>3</sub>-susceptible strain were the highest at pupal stage (21.104 mg h/L) and the lowest at egg stage (9.997 mg h/L), and the LCt<sub>50</sub> value of MB for the PH<sub>3</sub>-resistant strain were the highest at pupal stage (43.520 mg h/L) and the lowest at early larval stage (14.900 mg h/L). PH<sub>3</sub>-resistant strain showed higher LCt<sub>99</sub> than PH<sub>3</sub>-susceptible strain at all stages except adult, but did not exceed 2.06-fold (Table 2).

As a result of LCt analysis for PH<sub>3</sub>, the LCt<sub>50</sub> values of the PH<sub>3</sub>-susceptible strain were the highest at pupal stage (6.171 mg h/L) and the lowest at adult stage (0.295 mg h/L). The LCt<sub>50</sub> values of the PH<sub>3</sub>-resistant strain were the highest at late larval stage (48.170 mg h/L) and the lowest at egg stage (6.595 mg h/L). The highest concentration needed to control the late larvae was different from that needed to control the susceptible strain. The concentration needed to control the eggs was 57.206 mg h/L, the concentration needed to control the early larvae was 107.914 mg h/L, the concentration need to control the late larvae was 241.311 mg h/L, the concentration need to control the pupae was 91.760 mg h/L, and the concentration needed to control the adults was 25.938 mg h/L. Control of rice weevil late larvae and pupae using PH<sub>3</sub> should be considered (Table 3).



Figure 6. Mortality of phosphine resistant S. oryzae exposed to EF fumigant for 4 h at 20 °C in 12 L desiccator.



**Figure 7.** Mortality of phosphine resistant *S. oryzae* exposed to MB fumigant for 4 h at 20 °C in 12 L desiccator. **Table 1.** Lethal concentration time (LCT) of *S. oryzae* exposed to EF fumigant at 20 °C in 12 L desiccator.

Strain <sup>a</sup>	Stages	n	LCT <sub>50</sub> (mg h/L) (95% CL <sup>b</sup> )	RR <sub>50</sub> c	LCT <sub>99</sub> (mg h/L) (95% CL)	<b>RR</b> 99	$Slope \pm SE$	df	x <sup>2</sup>
S	Egg	542	75.795 (35.294–99.852)	1.00	186.623 (162.962–233.473)	1.00	$5.946 \pm 0.921$	5	0.00
	Early larva	450	60.110 (40.398–78.077)	1.00	213.792 (160.004–346.215)	1.00	$4.222\pm0.332$	4	0.04
	Late larva	600	160.491 (97.945–265.422)	1.00	775.478 (389.069–14622.164)	1.00	$3.401 \pm 0.789$	6	8.13
	Pupa	600	255.797 (209.902–340.130)	1.00	2373.015 (1289.504–6737.892)	1.00	$2.405\pm0.233$	6	0.80
	Adult	540	77.711 (58.090–100.392)	1.00	316.190 (204.109–837.332)	1.00	$3.818 \pm 0.494$	5	1.97
R	Egg	760	60.034 (2.415–98.658)	0.79	217.692 (127.285–2673.091)	1.17	$4.159 \pm 1.378$	7	40.65
	Early larva	630	64.450 (45.514–81.298)	1.07	311.913 (224.910–557.809)	1.46	$3.398 \pm 0.396$	6	5.58
	Late larva	540	149.028 (85.417–191.420)	0.93	449.200 (289.551–5031.511)	0.58	$4.856 \pm 1.089$	5	17.00
	Pupa	540	140.408 (53.470–187.556)	0.55	312.447 (217.735–10214.042)	0.13	$6.698 \pm 1.307$	5	7.38
	Adult	540	66.043 (43.299–87.325)	0.85	394.584 (266.764–807.344)	1.25	$2.997 \pm 0.32$	5	3.27

<sup>a</sup> Strain = S; Susceptible, R; Resistance. <sup>b</sup> CL = confidence limits. <sup>c</sup> RR = LCT of the R strain/LCT of the S strain.

Strain <sup>a</sup>	Stages	n	LCT <sub>50</sub> (mg h/L) (95% CL <sup>b</sup> )	RR <sub>50</sub> <sup>c</sup>	LCT <sub>99</sub> (mg h/L) (95% CL)	RR99	Slope $\pm$ SE	df	x <sup>2</sup>
S	Egg	576	9.997 (7.125–12.255)	1.00	24.111 (21.131–28.229)	1.00	$6.086 \pm 0.554$	5	0.041
	Early larva	540	12.113 (7.018–14.187)	1.00	19.929 (17.331–30.090)	1.00	$10.762\pm2.137$	5	10.61
	Late larva	540	18.952 (15.578–21.901)	1.00	60.351 (49.342–82.503)	1.00	$4.626 \pm 0.373$	5	0.53
	Pupa	540	21.104 (11.907–29.393)	1.00	67.795 (44.055–255.928)	1.00	$4.591 \pm 0.826$	5	30.65
	Adult	540	17.824 (16.772–18.433)	1.00	22.297 (20.853–26.931)	1.00	$23.929\pm3.761$	5	0.04
R	Egg	545	17.842 (6.726–22.183)	1.78	24.683 (16.463–27.220)	1.02	$16.509 \pm 3.323$	5	0.00
	Early larva	540	14.900 (13.461–17.159)	1.23	34.098 (26.534–53.390)	1.71	$6.471 \pm 0.601$	5	0.96
	Late larva	630	25.840 (21.031–32.636)	1.36	68.905 (48.205–161.691)	1.14	$5.462 \pm 0.863$	6	17.13
	Pupa	450	43.520 (35.203–73.578)	2.06	73.072 (50.790–186.850)	1.08	$10.339 \pm 1.080$	4	0.43
	Adult	540	16.397 (14.842–17.388)	0.92	23.792 (21.992–27.719)	1.07	$14.392\pm1.655$	5	0.00

Table 2. Lethal concentration time (LCT) of S. oryzae exposed to MB fumigant at 20 °C in 12 L desiccator.

<sup>a</sup> Strain = S; Susceptible, R; Resistance. <sup>b</sup> CL = confidence limits. <sup>c</sup> RR = LCT of the R strain/LCT of the S strain.

Table 3. Lethal concentration time (LCT) of *S. oryzae* exposed to PH<sub>3</sub> fumigant at 20 °C in 12 L desiccator.

			I CT <sub>ro</sub> (mg h/I)		I CToo (mg h/I)				
Strain <sup>a</sup>	Stages	n	(95% CL <sup>b</sup> )	RR <sub>50</sub> <sup>c</sup>	(95% CL)	RR99	Slope ± SE	df	<i>x</i> <sup>2</sup>
S	Egg	540	0.440 (0.042–0.861)	1.00	4.625 (2.911–17.448)	1.00	$2.277 \pm 0.425$	5	7.73
	Early larva	540	0.602 (0.307–0.907)	1.00	12.243 (7.436–29.192)	1.00	$1.779\pm0.173$	5	0.55
	Late larva	540	3.901 (2.799–5.862)	1.00	119.032 (49.421–529.324)	1.00	$1.567 \pm 0.133$	5	2.33
	Pupa	632	6.171 (3.462–11.297)	1.00	2450.358 (536.688–9983.186)	1.00	$0.895\pm0.099$	6	6.90
	Adult	540	0.295 (0.225–0.342)	1.00	0.634 (0.555–0.797)	1.00	$6.996 \pm 0.776$	5	1.37
R	Egg	717	6.595 (0.773–13.142)	14.99	57.206 (42.018–99.369)	12.37	$2.480 \pm 0.512$	7	1.03
	Early larva	725	28.456 (0.943–45.574)	47.27	107.914 (60.927–276614.079)	8.81	$4.019 \pm 1.390$	7	67.32
	Late larva	633	48.170 (26.561–105.326)	12.35	241.311 (108.523–5887.895)	2.03	$3.325\pm0.845$	6	25.57
	Pupa	630	29.106 (24.576–33.114)	4.72	91.760 (77.518–116.869)	0.04	$4.666 \pm 0.364$	6	2.71
	Adult	540	16.550 (12.113–19.290)	56.10	25.938 (22.889–30.448)	40.91	$11.924 \pm 1.581$	5	0.26

<sup>a</sup> Strain = S; Susceptible, R; Resistance. <sup>b</sup> CL = confidence limits. <sup>c</sup> RR = LCT of the R strain/LCT of the S strain.

#### 4. Discussion

In most species, egg and pupal stages are reported to be the most tolerant developmental stages to the fumigants, whereas *S. oryzae* pupae were the most tolerant [35]. In *Phthorimaea operculella*, pupal resistance was the strongest under EF treatment [36]. *Rhyzopertha dominica* and *Tribolium castaneum* showed 100% mortality under 24 h EF treatment, while *S. oryzae* survived in the immature stages [37]. In another study of *S. oryzae* and *T. castaneum*, the tolerance of eggs was the lowest and that of the pupae was the highest [22]. In this study, the PH<sub>3</sub>-sensitive *S. oryzae* and PH<sub>3</sub>-resistant *S. oryzae* were treated with PH<sub>3</sub>, EF, and MB fumigants, and the PH<sub>3</sub>-resistant *S. oryzae* was treated with EF. The lowest tolerance was confirmed in eggs.

There is a growing worldwide problem of PH<sub>3</sub> resistant grain pests, and PH<sub>3</sub> resistant *S. oryzae* has also been reported in various countries. As a result of the FAO test (0.04 g/m<sup>3</sup>) on *S. oryzae*, *R. dominica*, and *T. castaneum* for the detection of PH<sub>3</sub> resistance in Morocco, 18 of 19 groups of *S. oryzae* showed PH<sub>3</sub>

resistance [18]. As PH<sub>3</sub>-resistant *S. oryzae* is increasing, FAO test was performed to confirm the PH<sub>3</sub> resistant *S. oryzae* of South Korea. Here, we report the first confirmed the existence of PH<sub>3</sub>-resistant *S. oryzae* in South Korea. In general, exposure time of pests to PH<sub>3</sub> takes three to five days to have an insecticidal effect, while exposure time of *Sitophilus* sp. to PH<sub>3</sub> requires 12 days or more [13,38]. Chaudhry [13] found that resistant pests did not meet sufficient resistance levels at past disinfection sites, even with the long-term use of phosphine to control *S. oryzae*, but under insufficient disinfection time, some surviving insects have rebuilt their population. Insecticides must be applied repeatedly at overdose levels to cause resistance. Therefore, the airtightness of fumigation chamber should be maintained and fumigation time should be considered when applying PH<sub>3</sub> in the field.

Efficacy of PH<sub>3</sub> was found to be affected by the exposure time more than the concentration [39]. Therefore, in this study, the concentration was fixed and the treatment time was varied to control PH<sub>3</sub>-resistant *S. oryzae*. PH<sub>3</sub>-resistant *S. oryzae* treated with 0.5 mg/L PH<sub>3</sub> for 502 h was able to achieve 100% mortality. Therefore, PH<sub>3</sub> should be exposed for approximately 21 days to control PH<sub>3</sub>-resistant *S. oryzae*. PH<sub>3</sub> could be used to treat the PH<sub>3</sub> resistant *S. oryzae* for 100% mortality rate, but a long exposure time is needed. It cannot be used in the field because of long exposure time. Therefore EF, which shows effect in short time, can used to control PH<sub>3</sub> resistant *S. oryzae* instead of PH<sub>3</sub>. EF was first reported to be effective in box-car grain fumigation in Australia, and the use of EF in grains has been proposed for the last 40 years, but it has been difficult to apply in the field because of the disadvantages of low insecticidal activity and high flammability compared to other fumigants [40]. In this study, EF was treated at 20 °C, and 90 mg/L or more had to be added to control all of the PH<sub>3</sub>-susceptible and -resistant *S. oryzae*. Since EF has a low explosion threshold (85 g/t), more studies should be conducted to increase the efficacy of EF at lower concentration to avoid explosion.

## 5. Conclusions

EF was used as a substitute to treat PH<sub>3</sub>-susceptible and -resistant *S. oryzae*, but EF required a higher concentration of fumigant than that of other fumigants. To use EF to control *S. oryzae*, it is necessary to carry out studies to increase the insecticidal effect at a low concentration.

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## References

- 1. Dal Bello, G.; Padin, S.; Lastra, C.L.; Fabrizio, M. Laboratory evaluation of chemical-biological control of the rice weevil (*Sitophilus oryzae* L.) in stored grains. *J. Stored Prod. Res.* **2000**, *37*, 77–84. [CrossRef]
- 2. Khan, A.R.; Selman, B.J. On the mortality of *Tribolium castaneum* adults treated sublethally as larvae with pirimiphos methyl, *Nosema whitei* and pirimiphos methyl—*N. whitei* doses. *Entomophaga* **1988**, *33*, 377–380. [CrossRef]
- 3. Marsans, G.J. *Manejo y Conservacion de Granos;* Hemisferio Sur Editorial: Buenos Aires, Argentina, 1987; p. 266.
- 4. Pinto, A.R., Jr.; Furiatti, R.S.; Pereira, P.R.V.S.; Lazzari, F.A. Evaluation of insecticides for control of *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae), and *Rhyzopertha dominica* (Fab.) (Coleoptera: Bostrichidae) in stored rice. *An. Soc. Entomol. Bras.* **1997**, *26*, 285–290. [CrossRef]
- 5. Rajendran, S. Postharvest pest losses. In *Encyclopedia of Pest Management*; Pimentel, D., Ed.; Marcel Dekker, Inc.: New York, NY, USA, 2002; pp. 654–656.

- Follett, P.A.; Snook, K.; Janson, A.; Antonio, B.; Haruki, A.; Okamura, M.; Bisel, J. Irradiation quarantine treatment for control of *Sitophilus oryzae* (Coleoptera: Curculionidae) in rice. *J. Stored Prod. Res.* 2013, 52, 63–67. [CrossRef]
- 7. Plarre, R. An attempt to reconstruct the natural and cultural history of the granary weevil, *Sitophilus granarius* (Coleoptera: Curculionidae). *Eur. J. Entomol.* **2010**, *107*, 1–11. [CrossRef]
- 8. Zettler, J.L.; Arthur, F.H. Chemical control of stored product insects with fumigants and residual treatments. *Crop Prot.* **2000**, *19*, 577–582. [CrossRef]
- 9. Ducom, P. Methyl bromide alternatives. In Proceedings of the 9th International Conference on Controlled Atmosphere and Fumigation in Stored Product, Antalya, Turkey, 15–19 October 2012; Volume 15, p. 19.
- 10. Yagi, K.; Williams, J.; Wang, N.; Cicerone, R.J. Agricultural soil fumigation as a source of atmospheric methyl bromide. *Proc. Natl. Acad. Sci. USA* **1993**, *90*, 8420–8423. [CrossRef]
- 11. Spurgeon, D. End agreed for Ozone-destroying pesticide. Nature 1997, 389, 319. [CrossRef]
- 12. UNEP. Handbook for the Montreal Protocol on Substances that Deplete the Ozone Layer; UNEP/Earthprint: Nairobi, Kenya, 2006; pp. 10–11.
- 13. Chaudhry, M.Q. A review of the mechanisms involved in the action of phosphine as an insecticide and phosphine resistance in stored-product insects. *Pestic. Sci.* **1997**, *49*, 213–228. [CrossRef]
- 14. Chaudhry, M.Q. Phosphine resistance. Pestic. Outlook 2000, 11, 88–91. [CrossRef]
- 15. Athié, I.; Gomes, R.A.; Bolonhezi, S.; Valentini, S.R.; De Castro, M.F.P.M. Effects of carbon dioxide and phosphine mixtures on resistant populations of stored-grain insects. *J. Stored Prod. Res.* **1998**, *34*, 27–32. [CrossRef]
- Rajendran, S. Phosphine resistance in stored insect pests in India. In *Stored Products Protection, Proceedings* of the 7th International Working Conference on Stored-product Protection, Beijing, China, 14–19 October 1998; Jin, Z., Liang, Q., Liang, Y., Tan, X., Guan, L., Eds.; Sichuan Publishing House of Science and Technology: Chengdu, China, 1999; pp. 635–641.
- Zeng, L. Development and countermeasures of phosphine resistance in stored grain insects in Guangdong of China. In *Stored Products Protection, Proceedings of the 7th International Working Conference on Stored-product Protection, Beijing, China, 14–19 October 1998;* Jin, Z., Liang, Q., Liang, Y., Tan, X., Guan, L., Eds.; Sichuan Publishing House of Science and Technology: Chengdu, China, 1999; pp. 642–647.
- 18. Benhalima, H.; Chaudhry, M.Q.; Mills, K.A.; Price, N.R. Phosphine resistance in stored-product insects collected from various grain storage facilities in Morocco. *J. Stored Prod. Res.* **2004**, *40*, 241–249. [CrossRef]
- Pimentel, M.A.G.; Faroni, L.R.D.A.; da Silva, F.H.; Batista, M.D.; Guedes, R.N. Spread of phosphine resistance among Brazilian populations of three species of stored product insects. *Neotrop. Entomol.* 2010, 39, 101–107. [CrossRef] [PubMed]
- 20. Nguyen, T.T.; Collins, P.J.; Ebert, P.R. Inheritance and characterization of strong resistance to phosphine in *Sitophilus oryzae* (L.). *PLoS ONE* **2015**, *10*, e0124335. [CrossRef] [PubMed]
- 21. Nguyen, T.T.; Collins, P.J.; Duong, T.M.; Schlipalius, D.I.; Ebert, P.R. Genetic conservation of phosphine resistance in the rice weevil *Sitophilus oryzae* (L.). *J. Hered.* **2016**, *107*, 228–237. [CrossRef] [PubMed]
- 22. Muthu, M.; Rajendran, S.; Krishnamurthy, T.S.; Narasimhan, K.S.; Rangaswarmy, J.R.; Jayaram, M.; Majumder, S.K. Ethyl formate as a safe general fumigant. In Proceedings of the International Conference on Practical Aspects of CAF in Stored Products, Perth, Australia, 11–22 April 1983; Ripp, B.E., Ed.; Elsevier: New York, NY, USA, 1984; pp. 369–393.
- 23. Hilton, S.J.; Banks, H.J. Ethyl formate as a fumigant of sultanas; sorption and efficacy against six insect pests species. In Proceedings of the International Conference on Controlled Atmosphere and Fumigation in Stored Products, Nicosia, Cyprus, 21–26 April 1996; Donahaya, E.J., Navarro, S., Varnava, A., Eds.; Printco: Nicosia, Cyprus, 1997; pp. 409–422.
- 24. Damcevski, K.; Dojchinov, G.; Haritos, V.S. VAPORMATETM, a formulation of ethyl formate with CO2, for disinfestation of grain. In *Stored Grain in Australia 2003, Proceedings of the Australian Postharvest Technical Conference, Canberra, Australia, 25–27 June 2003;* Wright, E.J., Webb, M.C., Highley, E., Eds.; CSIRO: Canberra, Australia, 2003; pp. 199–204.
- 25. Desmarchelier, J.M.; Ren, Y.L. Analysis of fumigant residues-a critical review. J. AOAC Int. 1999, 82, 1261–1280.
- 26. Desmarchelier, J.M. Ethyl formate and formic acid: Occurrence and environmental fate. *Postharvest News Inf.* **1999**, *10*, 7–12.

- 27. Simmons, P.; Fisher, C.K. Ethyl formate and isopropyl formate as fumigants for packages of dried fruits. *J. Econ. Entomol.* **1946**, *38*, 715–716. [CrossRef]
- 28. Budavari, S.; O'Neill, M.; Smith, A.; Heckleman, P. *The Merck Index. An Encyclopedia of Chemicals, Drugs, and Biologicals,* 11th ed.; Merck & Co. Rahway: Rahway, NJ, USA, 1989.
- 29. Simpson, T.; Bikoba, V.; Tipping, C.; Mitcham, E.J. Ethyl formate as a postharvest fumigant for selected pests of table grapes. *J. Econ. Entomol.* 2007, *100*, 1084–1090. [CrossRef]
- 30. Ren, Y.; Lee, B.; Mahon, D.; Xin, N.; Head, M.; Reid, R. Fumigation of wheat using liquid ethyl formate plus methyl isothiocyanate in 50-tonne farm bins. *J. Econ. Entomol.* **2008**, 101, 623–630. [CrossRef]
- 31. Bond, E.J.; Monro, H.A.U. *Manual of Fumigation for Insect Control*; FAO Plant production and Protection Paper No 54; Food and Agriculture Organization of the United Nations: Rome, Italy, 1984; p. 432.
- 32. Bell, C.H. Fumigation in the 21st century. Crop Prot. 2000, 19, 563–569. [CrossRef]
- 33. Monro, H.A.U. Manual of fumigation for insect control. FAO Agric. Stud. 1969, 79, 381. [CrossRef]
- 34. Finney, D.J. Probit Analysis, 3rd ed.; Cambridge University Press: London, UK, 1971.
- 35. Hole, B.D.; Bell, C.H.; Mills, K.A.; Goodship, G. The toxicity of phosphine to all developmental stages of thirteen species of stored product beetles. *J. Stored Prod. Res.* **1976**, *12*, 235–244. [CrossRef]
- Kim, H.K.; Lee, S.W.; Kim, J.I.; Yang, J.O.; Koo, H.N.; Kim, G.H. Synergistic effects of oxygen on phosphine and ethyl formate for the control of *Phthorimaea operculella* (Lepidoptera: Gelechiidae). *J. Econ. Entomol.* 2015, 108, 2572–2580. [CrossRef] [PubMed]
- Damcevski, K.A.; Annis, P.C. The response of three stored product insect species to ethyl formate vapour at different temperatures. In *Stored Grain in Australia 2000, Proceedings of the Australian Postharvest Technical Conference, Adelaide, Australia, 1–4 August 2000;* Wright, E.J., Banks, H.J., Highley, E., Eds.; CSIRO: Canberra, Australia, 2002; pp. 78–81.
- 38. Anon. Fumigation Standard No. 12. Bull. OEPP EPPO Bull. 1984, 23, 212.
- 39. Price, L.A.; Mills, K.A. The toxicity of phosphine to the immature stages of resistant and susceptible strains of some common stored product beetles, and implications for their control. *J. Stored Prod. Res.* **1988**, 24, 51–59. [CrossRef]
- 40. Lee, B.H.; Huh, W.; Ren, Y.L.; Mahon, D.; Choi, W.S. New Formulations of Ethyl Formate to Control Internal Stages of *Sitophilus oryzae*. J. Asia Pac. Entomol. 2007, 10, 369–374. [CrossRef]



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