台灣農業研究 (J. Taiwan Agric. Res.) 73(1):1–10 (2024)
https://doi.org/10.6156/JTAR.202403_73(1).0001

**Research Article**

# Synergistic Effect of Additional Gas on the Toxicity of Phosphine to *Sitophilus oryzae* and *Sitophilus zeamais* (Coleoptera: Dryophthoridae)

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## Abstract

Feng, W. B., C. Y. Lee, T. C. Wang, and M. C. Yao. 2024. Synergistic effect of additional gas on the toxicity of phosphine to *Sitophilus oryzae* and *Sitophilus zeamais* (Coleoptera: Dryophthoridae). J. Taiwan Agric. Res. 73(1):1–10.

*Sitophilus* spp. is the most serious pest in imported brown rice warehouses, and its phosphine resistance has been observed in Taiwan. To overcome the efficiency decline of phosphine fumigation to *Sitophilus* beetles, phosphine fumigation with additional gas is the most noticed practice internationally. In this study, we evaluated the toxicity increase of phosphine in the presence of additional 10–20% of $CO_2$, $O_2$, or $N_2$ to *Sitophilus* beetles for 20 h or 72 h of fumigation. We found that there was no synergism between phosphine and additional gas for phosphine-resistant *Sitophilus oryzae* (Linnaeus) strains with 20 h fumigation. However, when the fumigation period prolonged to 72 h, the $LC_{50}$ of phosphine with additional 10 or 20% of $CO_2$, or $N_2$ decreased from 13.8 to 2.9–11.0 µg $L^{-1}$ for Houbi strain of *S. oryzae*. We consider that additional $CO_2$, and $N_2$ can potentially enhance the toxicity of phosphine against phosphine-resistant stored-product pests in the imported rice warehouse.

**Key words:** *Sitophilus*, Phosphine, $CO_2$, $O_2$, $N_2$.

## INTRODUCTION

Based on the principle of international trade fairness requested by World Trade Organization (WTO), Taiwan must import a certain amount of brown rice or white rice from abroad. The 8% of people's livelihood demand of rice are imported per year, and 65% of which are purchased by the government and transported to designated warehouses. They will then be stored for half a year to two years as the emergency grain. Since Taiwan is in the subtropical region where the temperature and humidity are high and no clearly divided four seasons that make the warehouses an ideal environment for the growth of pests. Besides, most warehouses for imported rice were rebuilt from old fertilizer warehouses. Their temperature and humidity are always higher than outsides that tend to provide conditions for better reproduction of stored-product insects. As a result, the imported rice could be infested with the stored-product insects during three-month of storage. Among the stored-product pests, *Sitophilus* spp. are the most serious pests in Taiwan, such as the rice weevil (*S. oryzae*) and corn weevil (*S. zeamais* Motchulsky).

Without the rice husk protection during storage, the *Sitophilus* beetles can easily bore deep holes with their mouthparts to lay eggs

Received: October 13, 2023; Accepted: November 28, 2023.
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inside. After hatching, the larvae feed and grow inside the grains producing debris which further leads to the outbreak of secondary pest infestation such as the rusty grain beetle (*Cryptolestes ferrugineus* Stephens), saw-toothed grain beetle (*Oryzaephilus surinamensis* Linnaeus), and foreign grain beetle (*Ahasverus advena* (Waltl)). Moreover, since the imported rice is often stored in ton bags and the larva of *Sitophilus* beetle is internal feeder, the damage and loss of rice is barely observable from the surface of storage bags. When the weevil adults emerged from rice, huge loss of rice had occurred.

Currently, 57% aluminum phosphide or 66% magnesium phosphide tablets are used to produce phosphine to control the outbreaks of stored-product insects in warehouses during the storage periods. However, Yao *et al*. (2022) tested the phosphine susceptibility of 21 strains of rice weevil and 17 strains of corn weevil collected in imported brown rice warehouses, and found that rice weevils from 5 locations (Hukou, Ershuei, Huatan, Xihu, and Houbi) had developed phosphine resistance at higher than moderate level. The development of phosphine resistance in rice weevils may gradually lead to the control failure using phosphine fumigation. Consequently, the fumigation operator may have to put more aluminum phosphide tablets into the warehouses to avoid phosphine fumigation failure. This vicious circle will not only increase the selection pressure of phosphine resistance to stored-product pests in the wild, but also increase the frequency of phosphine fumigation application that in turn leads to the increase of financial burden on the government (Agriculture and Food Agency).

To overcome the decline of phosphine fumigation efficiency against the phosphine-resistant stored-product pests, several strategies had been recommended including increasing ambient temperature during fumigation, using alternate fumigants, and fumigation with additional gas (Nayak & Collins 2008; Boopathy *et al*. 2022). Among these recommendations, addition of gases such as $O_2$, $CO_2$, and $N_2$ to enhance the fumigation effect of phosphine is the most noticed practice internationally (Athié *et al*. 1998; Liu 2011; Manivannan *et al*. 2016; Constantin *et al*. 2020; Sakka *et al*. 2020). In the present study, several phosphine-resistant and susceptible strains of rice weevil and corn weevil from the collection of Yao *et al*. (2022) were used. The median lethal concentration ($LC_{50}$) of weevils to phosphine for 20 h and 72 h were measured with or without 10 or 20% of individual $CO_2$, $O_2$, or $N_2$, respectively. The potential improving strategies of phosphine fumigation against the phosphine-resistant stored-product pests in Taiwan will then be suggested based on the results of the three added gases in improving the phosphine insecticidal effect.

# MATERIALS AND METHODS

## Sampling site and insect rearing

The species of *Sitophilus* weevils used in this study were *S. oryzae* and *S. zeamais*. All strains of both species were collected from warehouses where the imported brown rice was kept. The adults of phosphine-susceptible strain of *S. oryzae* were collected from Yilan and the Lab strain (reared for more than 10 years without insecticide treatments), and the phosphine-resistant strains were collected from Hukou, Ershuei, Huatan, Xihu, and Houbi. The adults of phosphine-susceptible strains of *S. zeamais* were collected from Xinying, Longtian, and Tainan. Weevil adults were reared in a plastic container (radius: 12 cm, height: 5 cm) with oatmeal as food source and maintained in environmental chambers with temperature set at 27 ± 1℃ and relative humidity at 70 ± 5%, and 24-hour darkness.

## Synergism of fumigation between additional gas and phosphine

The 57% aluminum phosphide tablets were manufactured by Detia Freyberg GmbH (Laudenbach, Germany), and purchased from GIANT BEAR Company (Taipei, Taiwan).

The synergism experiments were slightly modified from the recommended FAO phosphine bioassay methods (Anonymous 1975). First, 3 g of 57% aluminum phosphide tablets were put into a glass jar with 5% sulfuric acid in a 20 L desiccator (called gas production site) to generate phosphine gas and waited for 1.5 h for the chemical reaction to complete. For each bioassay, 30 $F_2$ generation unsexed weevil adults (1-to-2-week-old) were placed in a ventilated plastic container (radius: 3.5 cm, height: 5.5 cm), then put into another 20 L desiccator (called tests site) for phosphine fumigation. For each bioassay, there were 3 replications with a control without phosphine fumigation. The 10 or 20% $CO_2$, $O_2$, and $N_2$ were injected through air flow meter respectively, then the required dose of phosphine gas was injected to the tests site with gas tight syringes. The phosphine specificity detector and tester (Uniphos Envirotronic Pvt Ltd, Valsad, India) were used to measure the phosphine concentration. All desiccators were placed in a controlled temperature space (27 ± 1℃, 75 ± 10% RH) and the fumigations were conducted for 20 h and 72 h, respectively. After completion of the fumigation, the phosphine gas and the other additional gases were removed with the fume hood. The weevil adults were fed with oatmeal for 24 h to allow time for them to recover, and the mortality (no response to touch) was observed after the recovery period. The Vaseline was used to insure the tightness of desiccators while the phosphine detector was used to detect the escaping of phosphine gas.

### Statistical analysis

All analyses were performed using SPSS program (Version 22). The Probit analysis was used to analyze the concentration-mortality data, and obtain the $LC_{50}$ and 95% fiducial limits (FL) of $LC_{50}$ of each treatment. The relative ratios of phosphine fumigation toxicity were then calculated by the $LC_{50}$ with the extra gas added divided by the $LC_{50}$ without the extra gas added for each strain. A relative ratio > 1.00 indicated decreased phosphine toxicity and a relative ratio < 1.00 indicated increased phosphine toxicity.

## RESULTS

### Synergism of 20 h co-fumigation

As compared to fumigation with phosphine alone for 20 h in *S. oryzae*, results showed that co-fumigation with 10–20% $CO_2$ resulted in the $LC_{50}$ decreasing to 0.49- to 0.93-fold for Yilan and lab strains (Table 1). However, the $LC_{50}$ increased to 1.00- to 1.83-fold for all phosphine-resistant strains except the Huatan strain. As phosphine co-fumigation with 10% $N_2$, the $LC_{50}$ decreased to 0.72- to 0.81-fold for Huatan and Hukou strains (Table 1). However, the $LC_{50}$ of phosphine with 20% $N_2$ was similar to that with only phosphine for Huatan strain. The $LC_{50}$ of phosphine with 10–20% $O_2$ increased to 1.27- to 2.99-fold for all strains except for the lab strain at 10% $O_2$ (Table 1).

In *S. zeamais*, results showed that co-fumigation with 10–20% $CO_2$ resulted in decreases of $LC_{50}$ to 0.22- to 0.60-fold for Longtian and Tainan strains, while the $LC_{50}$ of phosphine with 10–20% $CO_2$ did not change for the Xinying strain (Table 2). The $LC_{50}$ of phosphine with 10–20% $N_2$ increased to 1.38- to 1.64-fold for all strains except for the all Longtian treatments and Tainan + 10% $N_2$ (Table 2). For the Longtian and Tainan strain of corn weevils, the $LC_{50}$ of phosphine with 10–20% $O_2$ decreased to 0.40- to 0.91-fold (Table 2). Conversely, the $LC_{50}$ of phosphine with 10–20% $O_2$ increased to 1.44- to 1.76-fold for Xinying strain.

### Synergism of 72 h co-fumigation

Results of 72 h fumigation for the Houbi strain of *S. oryzae* (Table 3) indicated that the $LC_{50}$ of phosphine with 10–20% $CO_2$ or $N_2$ decreased to 0.21- to 0.80-fold as compared to that with only phosphine. However, the $LC_{50}$ of phosphine with 10–20% $O_2$ increased to 1.10- to 1.70-fold.

**Table 1.** The $LC_{50}$ of *Sitophilus oryzae* to phosphine co-fumigation with 10 or 20% $CO_2$, $N_2$ or $O_2$ for 20 h.

| Treatments | $N^z$ | $LC_{50}$ (95% $FL^y$) (μg $L^{-1}$) | Slope | Chi-square | Relative Ratio$^x$ |
|---|---|---|---|---|---|
| Lab (RR = 2.22$^w$) | 450 | 3.3 (2.9–3.9) | 2.30 | 30.22 | 1.00 |
| Lab + 10% $CO_2$ | 450 | 2.6 (2.2–3.0) | 2.43 | 8.54 | 0.79 |
| Lab + 20% $CO_2$ | 450 | 1.6 (1.3–1.8) | 1.80 | 19.59 | 0.49 |
| Lab + 10% $N_2$ | 450 | 4.0 (3.6–4.5) | 3.21 | 15.08 | 1.21 |
| Lab + 20% $N_2$ | 450 | 3.2 (2.6–3.9) | 3.23 | 26.66 | 0.97 |
| Lab + 10% $O_2$ | 450 | 3.1 (2.8–3.5) | 3.14 | 14.59 | 0.94 |
| Lab + 20% $O_2$ | 450 | 4.2 (3.8–4.7) | 3.87 | 2.72 | 1.27 |
| Yilan (RR = 1.00) | 450 | 1.5 (0.9–2.2) | 2.46 | 970.54 | 1.00 |
| Yilan + 10% $CO_2$ | 450 | 1.4 (0.9–1.8) | 1.87 | 33.59 | 0.93 |
| Yilan + 20% $CO_2$ | 450 | 0.9 (0.6–1.2) | 1.62 | 24.32 | 0.60 |
| Yilan + 10% $N_2$ | 450 | 2.3 (1.6–3.2) | 2.77 | 64.64 | 1.53 |
| Yilan + 20% $N_2$ | 450 | 3.3 (2.3–4.3) | 2.37 | 53.09 | 2.20 |
| Yilan + 10% $O_2$ | 450 | 3.3 (3.0–3.6) | 4.81 | 6.44 | 2.20 |
| Yilan + 20% $O_2$ | 450 | 3.4 (3.1–3.7) | 4.30 | 12.53 | 2.27 |
| Ershuei (RR = 15.13) | 540 | 22.7 (14.8–35.7) | 3.00 | 65.21 | 1.00 |
| Ershuei + 10% $CO_2$ | 450 | 41.6 (38.7–44.5) | 4.52 | 3.43 | 1.83 |
| Ershuei + 20% $CO_2$ | 450 | 29.7 (24.5–34.7) | 2.60 | 27.17 | 1.31 |
| Ershuei + 10% $N_2$ | 450 | 44.1 (39.1–50.0) | 2.37 | 6.18 | 1.94 |
| Ershuei + 20% $N_2$ | 540 | 37.4 (30.9–43.9) | 1.60 | 17.33 | 1.65 |
| Ershuei + 10% $O_2$ | 540 | 46.4 (40.9–51.8) | 2.08 | 10.17 | 2.04 |
| Ershuei + 20% $O_2$ | 450 | 30.6 (26.3–34.8) | 2.29 | 1.15 | 1.35 |
| Huatan (RR = 17.47) | 450 | 26.2 (21.2–35.2) | 1.35 | 10.96 | 1.00 |
| Huatan + 10% $CO_2$ | 540 | 16.7 (12.8–20.5) | 1.36 | 1.93 | 0.64 |
| Huatan + 20% $CO_2$ | 540 | 26.3 (21.1–32.2) | 1.26 | 7.81 | 1.00 |
| Huatan + 10% $N_2$ | 540 | 18.8 (15.1–22.4) | 1.59 | 6.23 | 0.72 |
| Huatan + 20% $N_2$ | 450 | 27.9 (19.2–35.5) | 1.16 | 3.47 | 1.06 |
| Huatan + 10% $O_2$ | 540 | 76.1 (60.4–104.0) | 0.92 | 12.29 | 2.90 |
| Huatan + 20% $O_2$ | 540 | 78.3 (69.5–88.1) | 2.25 | 6.11 | 2.99 |
| Hukou (RR = 25.87) | 540 | 38.8 (35.3–42.4) | 3.25 | 20.45 | 1.00 |
| Hukou + 10% $CO_2$ | 450 | 51.7 (40.9–73.6) | 1.15 | 3.94 | 1.33 |
| Hukou + 20% $CO_2$ | 450 | 59.4 (47.5–85.4) | 1.27 | 4.43 | 1.53 |
| Hukou + 10% $N_2$ | 450 | 31.5 (26.7–36.3) | 2.03 | 6.25 | 0.81 |
| Hukou + 20% $N_2$ | 450 | 55.9 (46.4–72.7) | 1.52 | 9.38 | 1.44 |
| Hukou + 10% $O_2$ | 540 | 98.5 (76.2–149.0) | 0.91 | 18.40 | 2.54 |
| Hukou + 20% $O_2$ | 540 | 93.9 (85.1–104.0) | 2.72 | 6.14 | 2.42 |
| Xihu (RR = 36.40) | 540 | 54.6 (46.0–66.8) | 1.57 | 14.50 | 1.00 |
| Xihu + 10% $CO_2$ | 450 | 61.0 (51.4–78.1) | 1.73 | 7.67 | 1.12 |
| Xihu + 20% $CO_2$ | 540 | 85.1 (65.9–134.0) | 1.14 | 17.22 | 1.56 |
| Xihu + 10% $N_2$ | 540 | 91.3 (77.7–116.0) | 2.15 | 13.25 | 1.67 |
| Xihu + 20% $N_2$ | 540 | 68.2 (55.1–94.6) | 1.94 | 29.77 | 1.25 |
| Xihu + 10% $O_2$ | 720 | 122.0 (94.5–185.0) | 1.10 | 6.59 | 2.23 |

**Table 1.** The $LC_{50}$ of *Sitophilus oryzae* to phosphine co-fumigation with 10 or 20% $CO_2$, $N_2$ or $O_2$ for 20 h. (continued)

| Treatments | N[z] | $LC_{50}$ (95% FL[y]) (µg $L^{-1}$) | Slope | Chi-square | Relative Ratio[x] |
|---|---|---|---|---|---|
| Xihu + 20% $O_2$ | 630 | 73.6 (66.8–81.4) | 2.51 | 9.51 | 1.35 |
| Houbi (RR = 49.20) | 690 | 73.8 (54.5–114.0) | 1.14 | 19.30 | 1.00 |
| Houbi + 10% $CO_2$ | 450 | 103.0 (59.6–134.7) | 1.03 | 37.33 | 1.40 |
| Houbi + 20% $CO_2$ | 450 | 106.0 (102.0–112.0) | 6.50 | 11.06 | 1.44 |
| Houbi + 10% $N_2$ | 720 | 148.0 (134.0–170.0) | 3.32 | 9.84 | 2.01 |
| Houbi + 20% $N_2$ | 720 | 147.0 (133.0–168.0) | 3.31 | 11.57 | 1.99 |
| Houbi + 10% $O_2$ | 450 | 120.0 (110.0–137.0) | 4.33 | 8.90 | 1.63 |
| Houbi + 20% $O_2$ | 540 | 122.0 (112.0–137.0) | 3.58 | 4.49 | 1.65 |

[z] Number of insects tested.
[y] Estimated lethal concentration values with fiducial limits (FL).
[x] For each strain, the relative ratio = $LC_{50}$ of additional gas treatment divided by the $LC_{50}$ of no additional gas treatment. For example, the relative ratio of Lab + 10% $CO_2$ = 2.6 ($LC_{50}$ of Lab + 10% $CO_2$)/3.3 ($LC_{50}$ of Lab) = 0.79.
[w] RR = Resistance ratio.

**Table 2.** The $LC_{50}$ of *Sitophilus zeamais* to phosphine co-fumigation with 10 or 20% $CO_2$, $N_2$ or $O_2$ for 20 h.

| Treatments | N[z] | $LC_{50}$ (95% FL[y]) (µg $L^{-1}$) | Slope | Chi-square | Relative ratio[x] |
|---|---|---|---|---|---|
| Xinying (RR = 1.32[w]) | 450 | 2.5 (1.5–3.7) | 2.61 | 95.48 | 1.00 |
| Xinying + 10% $CO_2$ | 450 | 2.5 (2.2–3.0) | 2.67 | 19.72 | 1.00 |
| Xinying + 20% $CO_2$ | 450 | 2.5 (2.0–3.1) | 2.43 | 28.36 | 1.00 |
| Xinying + 10% $N_2$ | 450 | 4.1 (3.8–4.5) | 0.57 | 1.81 | 1.64 |
| Xinying + 20% $N_2$ | 450 | 4.0 (3.5–4.5) | 2.92 | 13.45 | 1.60 |
| Xinying + 10% $O_2$ | 450 | 3.6 (2.7–4.6) | 2.85 | 39.93 | 1.44 |
| Xinying + 20% $O_2$ | 450 | 4.4 (3.8–5.1) | 2.42 | 15.45 | 1.76 |
| Longtian (RR = 5.32) | 450 | 10.1 (8.8–11.8) | 1.92 | 16.24 | 1.00 |
| Longtian + 10% $CO_2$ | 450 | 2.9 (2.5–3.3) | 2.64 | 13.58 | 0.29 |
| Longtian + 20% $CO_2$ | 450 | 2.2 (1.9–2.5) | 2.54 | 10.36 | 0.22 |
| Longtian + 10% $N_2$ | 450 | 4.9 (3.9–6.0) | 2.52 | 22.77 | 0.49 |
| Longtian + 20% $N_2$ | 450 | 6.4 (5.7–7.1) | 3.32 | 14.23 | 0.63 |
| Longtian + 10% $O_2$ | 450 | 4.0 (3.2–4.9) | 2.92 | 31.18 | 0.40 |
| Longtian + 20% $O_2$ | 450 | 7.1 (5.3–10.4) | 1.92 | 42.90 | 0.70 |
| Tainan (RR = 6.16) | 540 | 11.7 (9.6–15.1) | 1.79 | 6.18 | 1.00 |
| Tainan + 10% $CO_2$ | 450 | 5.3 (4.3–6.5) | 2.50 | 24.38 | 0.45 |
| Tainan + 20% $CO_2$ | 450 | 7.0 (4.9–11.5) | 1.24 | 23.74 | 0.60 |
| Tainan + 10% $N_2$ | 450 | 8.3 (6.1–12.7) | 1.26 | 30.90 | 0.71 |
| Tainan + 20% $N_2$ | 450 | 16.1 (11.0–32.2) | 0.99 | 50.34 | 1.38 |
| Tainan + 10% $O_2$ | 450 | 7.9 (6.1–11.0) | 2.10 | 24.52 | 0.68 |
| Tainan + 20% $O_2$ | 450 | 10.7 (8.9–13.7) | 1.41 | 12.52 | 0.91 |

[z] Number of insects tested.
[y] Estimated lethal concentration values with fiducial limits (FL).
[x] For each strain, the relative ratio = $LC_{50}$ of additional gas treatment divided by the $LC_{50}$ of no additional gas treatment.
[w] RR = Resistance ratio.

**Table 3.** The $LC_{50}$ of *Sitophilus oryzae* to phosphine co-fumigation with 10 or 20% $CO_2$, $N_2$ or $O_2$ for 72 h.

| Treatments | N[z] | $LC_{50}$ (95% FL[y]) ($\mu g\ L^{-1}$) | Slope | Chi-square | Relative ratio[x] |
|---|---|---|---|---|---|
| Houbi (RR = 49.20[w]) | 450 | 13.8 (11.4–16.7) | 1.56 | 8.52 | 1.00 |
| Houbi + 10% $CO_2$ | 450 | 6.8 (5.2–8.6) | 1.47 | 10.71 | 0.49 |
| Houbi + 20% $CO_2$ | 450 | 2.9 (1.6–4.3) | 0.92 | 4.52 | 0.21 |
| Houbi + 10% $N_2$ | 450 | 11.0 (9.2–13.0) | 1.77 | 7.14 | 0.80 |
| Houbi + 20% $N_2$ | 450 | 10.5 (6.7–15.3) | 2.08 | 24.08 | 0.76 |
| Houbi + 10% $O_2$ | 450 | 15.2 (9.6–25.0) | 2.24 | 27.37 | 1.10 |
| Houbi + 20% $O_2$ | 450 | 23.5 (19.2–29.8) | 1.80 | 12.13 | 1.70 |

[z] Number of insects tested.
[y] Estimated lethal concentration values with fiducial limits (FL).
[x] For each strain, the relative ratio = $LC_{50}$ of additional gas treatment divided by the $LC_{50}$ of no additional gas treatment.
[w] RR = Resistance ratio.

## DISCUSSION

Since 1975, reports indicated that additional gases such as $CO_2$, $N_2$, and $O_2$ could potentially enhance the toxicity of phosphine against the phosphine-resistant stored-product pests. Athié *et al*. (1998) indicated that phosphine fumigation for 20 h with additional 10–20% $CO_2$ decreased the $LC_{50}$ of phosphine to 0.11- to 0.90-fold for phosphine-resistant *S. oryzae* and *Rhyzopertha dominica* (L.). Constantin *et al*. (2020) found that phosphine fumigation for 48 h with additional 30.99% $CO_2$ decreased the $LC_{50}$ of phosphine to 0.12-fold for phosphine-resistant *C. ferrugineus* in Australia. The increased toxicity of phosphine with $CO_2$ was also found in the red flour beetle (*Tribolium castaneum* (Herbst)), the wheat weevil (*Sitophilus granarius* (L.)), and the flour mill beetle (*Cryptolestes turcicus* (Grouvelle)) (Kashi & Bond 1975; Rajendran & Muthu 1989; Ren *et al*. 1994). In addition, the decreased reproductive rates of the survival beetles after co-fumigation of phosphine and $CO_2$ were observed by Constantin *et al*. (2020) when compared to the control group. However, the fumigation of phosphine with 10–20% $CO_2$ did not enhance the toxicity of phosphine to all phosphine-resistant strains of the rice weevil and the $LC_{50}$ values increased to 1.12- to 1.83-fold as compared with that of phosphine alone for 20 h fumigation in this study. But phosphine fumigation with 10–20% $CO_2$ decreased the $LC_{50}$ value of Houbi strain to 0.21- to 0.49-fold as the fumigation time was extended to 72 h. Lambkin (2001) indicated that there was no synergistic effect between phosphine and 10% $CO_2$ to phosphine-resistant *R. dominica* unless the fumigation time was prolonged to 72 h. Wong-Corral *et al*. (2013) reported that 5 d of co-fumigation between phosphine and 50–90% $CO_2$ were needed to reach 100% mortality for all developmental stages of the cowpea weevil (*Callosobruchus maculatus* Fabricius). Other studies reported that fumigation toxicity of phosphine cannot be boosted with addition of 5–40% $CO_2$ in short fumigation periods (Rajendran 1990; Ren *et al*. 1994). By contrast, Valizadegan *et al*. (2012) indicated that the fumigation periods required for killing the immature stages of *O. surinamensis*, *Lasioderma serricorne* (Fabricius), and *Plodia interpunctella* (Hubner) could be reduced to 1 d as the phosphine fumigation was applied with an addition of 24% $CO_2$. Obviously, the effect of fumigation time on the toxicity of phosphine differs among the developmental stages of insects and species. However, the reasons about the differences of the co-fumigation time in enhancing the phosphine toxicity have not been mentioned yet.

There are 2 possible mechanisms for enhancing the phosphine toxicity with additional $CO_2$. One is to increase the metabolic rate of aerobic respiration, thereby increasing the toxicity of phosphine (Kashi & Bond 1975). The other one is to promote the opening of the insect spiracle to increase gas exchange rates, thus enhancing the amount of phosphine flowing into the insect body (McGovran 1932; Mitcham *et al*. 2006). However, it does not mean that the higher the concentration of $CO_2$, the better the synergistic effect. Some studies have found antagonism between high concentration of $CO_2$ and phosphine. Kashi & Bond (1975) indicated that adding 60–80% $CO_2$ did not inhibit the insect's aerobic metabolism which might be related to narcosis (Winks 1985) that finally led to increased resistance in insects to phosphine. The key point for synergism between $CO_2$ and phosphine might be the proportions of $CO_2$ and $O_2$ in the fumigation space. Constantin *et al*. (2020) speculated that the proportions of $CO_2$ and $O_2$ in the space should be 25–35% and 8–15%, respectively that led to the synergism between $CO_2$ and phosphine. However, differences of phosphine toxicity caused by the ratio of $CO_2$ and $O_2$ were rarely investigated.

The present study indicated that the fumigation periods could affect the synergistic effects between $CO_2$ and phosphine. This phenomenon may be related to phosphine resistance. The mechanisms of phosphine resistance were known to include structural changes in dihydrolipoamide dehydrogenase (DLD) (Zuryn *et al*. 2008; Schlipalius *et al*. 2012; Chen *et al*. 2015), decreased respiratory rate (Pimentel *et al*. 2007, 2009), active excretion of phosphine (Price 1984; Chaudhry & Price 1992; Pratt 2003), and upregulation of detoxification enzyme genes (Oppert *et al*. 2015; Yang *et al*. 2018; Wang *et al*. 2020). Among the mechanisms, decreased respiratory rate and active excretion of phosphine may have negative effects on the additional $CO_2$ and $N_2$. When the phosphine-resistant insects are in a space with high concentrations of $CO_2$ or $N_2$, the phosphine-resistance mechanisms in these insects might block the flow of the gas into their body that resulted in no synergism in short fumigation periods, or promote the over-expression of phosphine-resistant mechanisms that eliminated phosphine toxicity. Other studies indicated that insects may try to adapt to the high $CO_2$ concentration by minimizing the metabolic activity in the short fumigation period (Blomberg & Siegbahn 2014; Levy-de la Torre *et al*. 2018). However, since insects could not maintain the low respiration rate with slow metabolic activity and excludability of gas when the fumigation periods prolonged to 72 h, that finally led to the synergism between $CO_2$ and phosphine. Nevertheless, further evidence is needed.

In summary, we reported that co-fumigation of phosphine with 10–20% $CO_2$ or $N_2$ for 72 h could enhance the toxicity. The fumigation periods in imported rice warehouses are mostly 7–14 d, which is much longer than the fumigation periods conducted in the present study. In addition, $CO_2$ and $N_2$ are being considered as safe gases because of their low toxicity to human and environment. On the other hand, there was no synergistic effect between phosphine and 10–20% $O_2$ for 72 h fumigation, nevertheless high levels of oxygen can suppress the fire risk of phosphine during fumigation (Bond & Miller 1988; Ohtani *et al*. 1989). Therefore, we consider that co-fumigation of $CO_2$ or $N_2$ can be one of the potential methods for controlling the phosphine-resistant *Sitophilus* weevils in the imported rice warehouses. Besides, even if the weevils survive under co-fumigation condition, their reproductive rate may decrease after fumigation that in turn reduces the required fumigation frequency and the fumigation costs (Manivannan *et al*. 2016). The application potential of co-fumigation of insecticides with gases for pest control in the imported rice warehouses, and their control efficacy deserve further study.

## ACKNOWLEDGMENTS

We are grateful to Jin-Xia Li, Gui-Xiang

Hong, and Shu-Xia Hu for assisting the experiment. This study was supported by a grant from Agriculture and Food Agency, Ministry of Agriculture, Taiwan.

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# 磷化氫添加氣體對於米象及玉米象 (鞘翅目：椰象鼻蟲科) 之毒性協力效應

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## 摘要

馮文斌、李啟陽、王泰權、姚美吉。2024。磷化氫添加氣體對於米象及玉米象 (鞘翅目：椰象鼻蟲科) 之毒性協力效應。台灣農業研究 73(1):1–10。

米象屬昆蟲 (*Sitophilus* spp.) 為臺灣進口米倉庫中最嚴重的積穀害蟲，在臺灣已發現對磷化氫產生抗藥性，為克服磷化氫燻蒸對米象屬害蟲防治效果的下降，額外添加氣體與磷化氫共同燻蒸為當前國際最關注的方法。在本研究中，我們評估額外添加 10–20% 二氧化碳、氧氣或氮氣與磷化氫共同燻蒸對米象屬甲蟲之致死效果的提升，我們發現燻蒸 20 h，磷化氫與三種氣體對於抗藥性米象不會產生任何協力作用，然而將燻蒸時間延長至 72 h，添加 10–20% 二氧化碳與氮氣即有助於提升磷化氫對於後壁品系米象之致死效果，其 $LC_{50}$ 從 13.8 μg $L^{-1}$ 降至 2.9–11.0 μg $L^{-1}$，因此我們認為在磷化氫燻蒸時添加二氧化碳或氮氣，具有提升磷化氫對於進口米倉庫內磷化氫抗藥性積穀害蟲的致死效果。

**關鍵詞**：米象類、磷化氫、二氧化碳、氧氣、氮氣。

投稿日期：2023 年 10 月 13 日；接受日期：2023 年 11 月 28 日。

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