Studies on the effect of carbon dioxide in insect treatment with phosphine

Y.L. Ren, *† I.G. O'Brien* and C.P. Whittle†

Abstract

Studies on the toxicity of mixtures of phosphine and carbon dioxide to adults of *Cryptolestes turcicus* (Grouvelle) showed that carbon dioxide enhanced the toxicity of phosphine. Studies on respiration of the tested insects showed an increase in phosphine consumption when carbon dioxide level increased. The optimum concentration of carbon dioxide for synergy with phosphine was in the range of 5–35% (v/v). The experiment also shows that the action of phosphine is stimulated through carbon dioxide stimulating respiration. However, once the concentration of carbon dioxide is above 35%, the toxicity of phosphine is gradually decreased because of the effect of narcosis.

Introduction

Phosphine has been widely used to control stored-grain insects. It is highly toxic to insects, relatively easy to apply, not appreciably phytotoxic, does not taint most commodities and leaves little residue. Phosphine plays an important role in integrated pest management, and it is still one of the most important methods, not only in eradication, but also, in China, in quarantine treatment. However, phosphine resistance of the lesser grain borer (*Rhyzopertha dominica* (F.)) and the flat grain beetle (*Cryptolestes turcicus* (Grouvelle)) has occurred in China (Table 1) and for some strains the resistance is extremely high. In fact it is not possible to determine LD50 values for these strains by fixed time (20-hour) exposure methods (e.g. Anon. 1975). Significant resistance to phosphine has been noted previously elsewhere (e.g. Tyler et al. 1983).

The present study was initiated to investigate possible methods for enhancing the efficacy of phosphine fumigations and, in particular, to study the effect of carbon dioxide on phosphine toxicity and the relationship between toxicity, respiration and fumigant uptake. *C. turcicus*, an important pest in China, was chosen as a representative insect.

The normal gases in the insect environment have some influence on the toxicity of fumigants to insects. Various techniques have been employed to alter the normal gases of the atmosphere to enhance the effectiveness of fumigants. In vacuum fumigation fumigants are found to penetrate commodities faster and to be more toxic to insects than in the normal atmosphere (Bond and Monro 1967). Carbon dioxide is often added with some fumigants to reduce flammability and explosive hazard and may increase the toxic effect of certain fumigants (Cotton and Young 1929). Carbon dioxide has also been used as a carrier to enable phosphine to penetrate to the bottom of the silo (Leesch 1990). Friedlander and Navarro (1979, 1983, 1984) have reported that carbon dioxide appears to have an effect on the metabolism of stored grain insects when under higher concentration. Liang Quan (1980) found that co-application in carbon dioxide increases the susceptibility of five strains of grain beetles to phosphine. Qou Shi-Jie (1980) reported that carbon dioxide potentiates the toxicity of phosphine against adults of *Tribolium castaneum* and *Sitophilus oryzae* at low concentration, e.g. at 30°C with carbon dioxide above 11.8% (v/v), 0.009 mg (PH_3)/L and an exposure time of 72 hours, all the stored grain insects could be killed, but no quantitative comparison was made with the phosphine without CO_2. Cotton (1930) investigated the use of carbon dioxide with fumigants and concluded that it might increase the toxic effects of fumigants by satisfying the sorptive properties in commodities when it is used with the fumigants. Cotton and Young (1929) and Cotton (1932) studied the uses of carbon dioxide to stimulate respiration which resulted in the increased toxicity of some fumigants. Jones (1938) and Aliniazee and Lindgren (1969) were able to show that the concentration of carbon dioxide was a factor in increasing the toxic effect of methyl bromide and other fumigants to the red flour beetle, *Tribolium castaneum* (Herbst). Kashi and Bond (1975) showed that concentrations of carbon dioxide ranging from 1 to 50% potentiated the toxic effects of phosphine on *Sitophilus granarius* (L.) and *Tribolium confusum* du Val. Desmarchelier (1984) showed that 25% carbon dioxide increased the toxicity of phosphine to some stored-product insects but not to the most tolerant stages. Rajendran and Muthu (1989) showed that carbon dioxide increased the toxicity of phosphine to adults of *S. granarius* and *T. confusum*. All of these studies have concentrated on the effects of carbon dioxide on the toxicity of fumigants to insects, largely in a qualitative sense.

Materials and Methods

Phosphine generation

Carbon dioxide and phosphine were generated and mixed in a fumehood in the device shown in Figure 1. The required mixture of gases was produced in the 500 mL fumigation chamber (B).

Phosphine was generated by mixing water and aluminium phosphide (E)

$$AlP + 3HOH \rightarrow Al(OH)_3 + PH_3$$

The phosphine–air mixture in generator (E) was transferred to the storage chamber (C) under pressure from (E), and saturated sodium chloride in (C) was forced into balance bottle (D2). The concentration of PH_3 for each batch was measured by gas chromatography.

* Faculty of Applied Science, University of Canberra, P.O. Box 1, Belconnen ACT 2616, Australia.
† Stored Grain Research Laboratory, Division of Entomology, CSIRO, GPO Box 1700, Canberra ACT 2601, Australia.

Preparation of apparatus

Test insects (20 mg, about 200 insects) were put into the fumigation chamber (B), stoppered and connected (J) to the rest of the apparatus. PH_3 was passed into (A), flushing tube 4, and discarded. In the same way, CO_2 (F) was used to flush tube 3, and discarded.

Mixing the gases

Because the required concentrations of CO_2 vary widely from 0 to 60% , and in order not to affect the test insects during the gas mixing operation, we employed a method of gas replacement under slight negative pressure by using the 500 mL hand pump (H). That is, with the tubes 2 and 5 connected, the cock (b) was opened and the pressure in the fumigation chamber (B) was reduced by not less than 76 mmHg, as measured by (I). CO_2 was then admitted to (B) by connecting tubes 3 and 5. This operation was repeated the required number of times (Table 2) to obtain approximately the necessary concentration of CO_2.

The data in Table 2 show the approximate relationship between the number of pump strokes and the final CO_2 concentration. The calculated volume of phosphine gas mixture for (B) is taken into (A) and CO_2 is added to achieve the required concentration. For example, when the required concentration of CO_2 is 30% (v/v), as indicated in Table 2, it is necessary to replace gases three times. The concentration of CO_2 was measured in the fumigation chamber (B) through (J), and according to the difference between target concentration and the determined value of CO_2 a calculated amount of CO_2 was added from the gas burette (A) together with the calculated required amount of PH_3. The mixed gases in the gas burette were admitted to the fumigation chamber (B) after one further pump stroke of equal volume. Cock (a) was then opened briefly to allow air to enter and restore atmospheric pressure in chamber (B). The final concentrations of CO_2 and PH_3 are determined by GC analysis and adjusted if necessary.

Determination of gas concentrations

Phosphine concentrations were determined using a Shimadzu instrument fitted with a flame photometric detector (FPD). Operating conditions were: 3 mm × 3 m glass column packed with 5% SE-30 (w/w) on 80–100 mesh Chromosorb 105, column temperature, 100°C; injection port and detector block temperatures, 110°C and 130°C. An Aerograph A-110-C model gas chromatograph was used for determination of carbon dioxide and oxygen. This instrument was equipped with a thermal conductivity detector (TCD) using a 4-filament hot wire with 3 mm × 1.5m stainless steel column packed with silica gel (100 mesh).

Treatment of insects

A phosphine-susceptible strain of flat grain beetle *C. turcicus* was collected in July 1984 from a grain storage in Sichuan province, China and cultured in the laboratory. Insects were reared at 30 ±1°C, 75 ± 2% r.h. Upon emergence, adults were transferred to 25°C, 70% r.h. and held at these conditions for at least 1 week before being used in experiments. All subsequent stages were conducted at 25°C and 70% r.h.

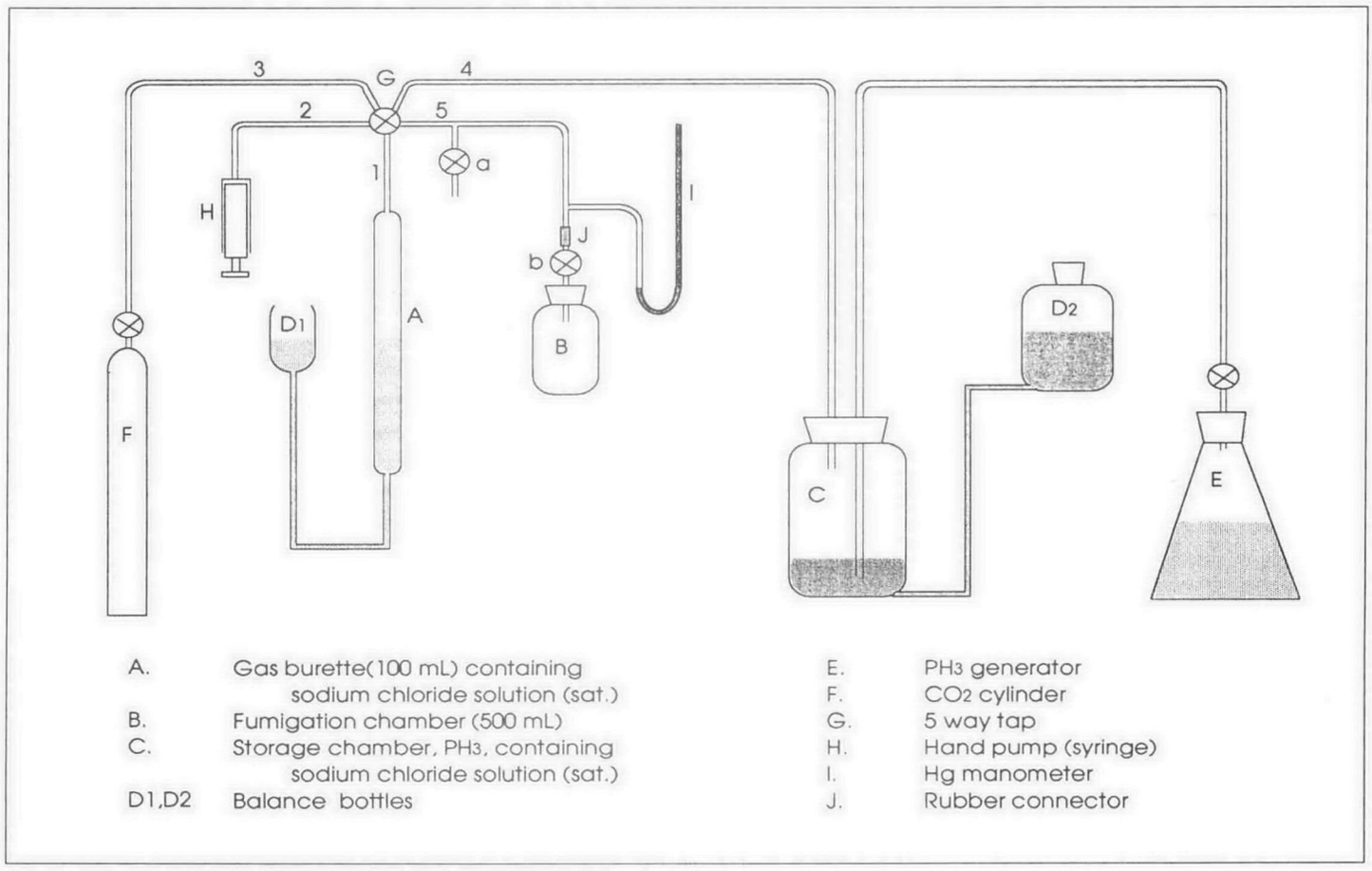

Fig. 1. Apparatus for generating and mixing phosphine with carbon dioxide.

Batches of adults were exposed to each dosage (concentration × treatment time, mg.hour/L) and then transferred to petri dishes containing a thin layer of wholemeal wheat flour and stored under normal atmosphere. The end-point mortalities for individual treatment concentrations were determined over 14 days following termination of the exposure (Anon. 1989).

Measuring toxicity of phosphine under different carbon dioxide levels

Four batches of 50 adults were treated at each concentration of phosphine in 500 mL bottles under different concentrations of carbon dioxide (normal air, 5, 10, 15, 30, 35, 45 and 60%) for a 24-hour exposure period, following which they were transferred to petri dishes for the determination of mortalities. The phosphine concentrations used were 0.02, 0.05, 0.1, 0.2 and 0.5 mg/L. Data were analysed by probit analysis (Finney 1952). Standard errors of the slopes were in the range 0.16–0.74.

Measuring toxicity of CO_2 and of PH_3 in CO_2

Four batches of 50 adults were treated at each concentration of carbon dioxide in 500 mL bottles under different concentrations of phosphine for a 24-hour exposure period, following which they were transferred to petri dishes for the determination of mortalities. The carbon dioxide concentrations were: normal air, 3, 5, 10, 15, 19, 25, 28, 38, 50 and 58% (v/v); phosphine concentrations were 0, 0.071 and 0.104 mg/L for each carbon dioxide level.

Measuring oxygen consumption and intake of phosphine

The method used to determine oxygen consumption by *C. turcicus* exposed to carbon dioxide has been described by Aliniazee (1971). Three batches (20 mg each) of adults were treated in a 250 mL bottle at each concentration of carbon dioxide (normal atmosphere and 10, 20, 30, 40, 50, and 60%) and the same concentration of phosphine (0.104 mg/L) was added to each bottle. Gas samples were taken after 48 hours and the oxygen and phosphine levels were determined by gas chromatography. The consumptions were determined as O_2 mg/g(insect) and PH_3 µg/g(insect) per 24-hour period.

Results

The effect of carbon dioxide on intake of phosphine and oxygen by insects

Oxygen consumption in an atmosphere of 0.1 mg/L phosphine was found to increase with CO_2 concentration, and when insects were exposed to greater than 20% (v/v) carbon dioxide, oxygen consumption was more than double the normal consumption. The intake of phosphine by the insects was also more than double at that concentration of CO_2 (Fig. 2). However, with further increases in concentration of carbon dioxide, phosphine taken up by insects was increased slightly, but oxygen consumption was decreased in concentrations of CO_2 above 30% (see Fig. 2).

The effect of carbon dioxide on phosphine toxicity

We confirmed that phosphine toxicity to *C. turcicus* was influenced by the concentration of carbon dioxide used in the experiments. Phosphine toxicity increased with increasing concentration of carbon dioxide but decreased beyond 35% CO_2. The LC_{90} was achieved with lower concentrations of phosphine as the CO_2 concentration increased. It was found that the concentration range of carbon dioxide for maximum enhancement of phosphine toxicity is 15–35% (see Fig. 3).

The mortality curve of *C. turcicus* is shown in Figure 4. Two concentrations of phosphine were used in the treatment of adult insects over a wide range of CO_2 concentrations (0–60%). We obtained a typical dosage–mortality curve under constant concentration of phosphine and increasing concentrations of carbon dioxide, and though carbon dioxide is

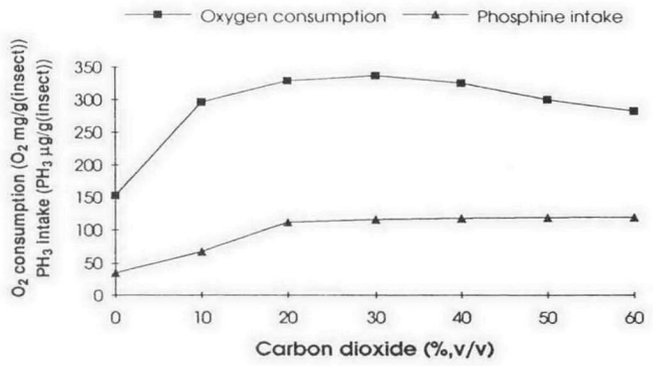

Fig. 2. Oxygen consumption and intake of phosphine (dosed at 0.1040.002 mg/L) by *C. turcicus* under carbon dioxide over 24 hours.

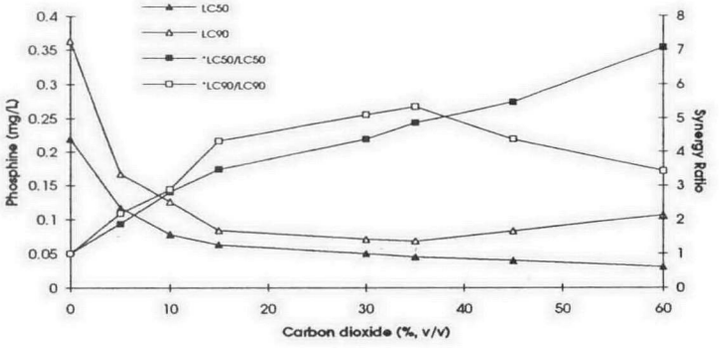

Fig. 3. The effect of carbon dioxide on the toxicity of phosphine (24 hours exposure). Toxicities (LC_{50}▲ and LC_{90}△) and Synergy ratios (*LC_{50}/LC_{50} ■ and *LC_{90}/LC_{90} □) vs CO_2 concentration. *LC_{50} and *LC_{90} values were determined in air, LC_{50} and LC_{90} with CO_2.

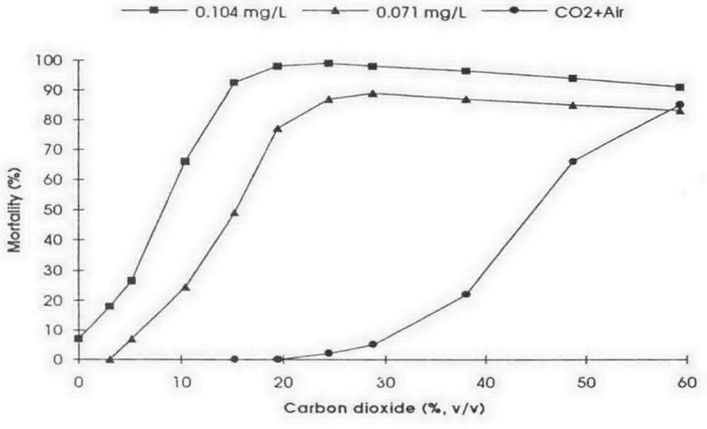

Fig. 4. Toxic effect of phosphine on *C. turcicus* adults (24 hours exposure) under different concentrations of carbon dioxide.

Table 1. Toxicity of phosphine to four representative resistant strains of *Rhyzopertha dominica* and *Cryptolestes turcicus*[a]

Strain[b]	Site of collection	Slope ± S.E.[c]	LD_{50}(95%CL) mg/L 20 hours, 25°C[c]	Resistance ratio
RdS	Susceptible strain	2.30 ± 0.63	0.008 (0.006–0.010)	1
Rd2	Nanping, Zhuhai	1.90 ± 0.12	0.141 (0.139–0.143)	18
Rd3	Nanping, Zhuhai	1.82 ± 0.04	0.068 (0.066–0.070)	8
Rd8	Guxiang, Chaozhou	2.26 ± 0.39	4.852 (4.850–4.854)	606
Rd10	Hudong, Lufeng	1.37 ± 0.08	0.182 (0.180–0.184)	23
CT	Hunan, Xiangtan	–	–	46

[a] Data from Guangdong Grain Storage Research Institute and determined by FAO, 20-hour exposure method.

[b] Rd = *R. dominica*, CT = *C. turcicus*

[c] Plotted as per Finney (1952).

Table 2. The relationship between pump strokes and concentration of CO_2.

No. pump strokes	1	2	3	4	5	6	7	8
Concentration (%) of CO_2 (v/v)	10	19	27	34	41	47	52	57

clearly a mild fumigant (Fig. 4) it is also clear that the effect of carbon dioxide on the toxicity of phosphine is an apparent synergistic effect.

Discussion

As stated above, some researchers have reported that carbon dioxide can effectively increase toxicity of the fumigant PH_3 and, although they have discussed its possible action, a satisfactory explanation has not been provided. Nor has the lower efficacy in higher concentrations of CO_2 been shown previously. As can be seen in comparing Figures 2 and 4, there is a relationship between toxicity of phosphine and respiration of insects under different concentrations of carbon dioxide, both showing a maximum at 25–30% CO_2 for *C. turcicus* exposed to 0.1 mg/L phosphine. Comparison of mortalities in Figure 4 at two levels of phosphine show that while the optimum CO_2 concentration to produce a maximal kill (LC_{90}) at 0.07 mg/L is 30% (±5%), the optimum CO_2 for 0.1 mg/L phosphine (LC_{99}) is 20% (±5%). In CO_2 atmospheres above 30%, phosphine is less effective. It can be reasoned that the increase in phosphine toxicity with carbon dioxide is due to stimulation of insect respiration, as shown by increased oxygen consumption, causing increased phosphine interaction at the target site.

It is well known that the toxicity of phosphine to insects is dependent on respiration or oxygen consumption (Bond and Monro 1967). For example phosphine is not toxic to weevils treated at °C or in 100% nitrogen, i.e., under conditions where the weevils do not respire (Sato and Suwanai 1973). In this respect the observation of increasing oxygen consumption with increasing carbon dioxide concentration (Fig. 2) was not unexpected. However, the fact that the oxygen consumption peaks at about 30% CO_2 and the phosphine intake only increases slightly above 20% CO_2 requires explanation.

From Figure 4 it can be seen that at about 30% and above, CO_2 alone has an increasing lethal effect. It may be postulated that, although CO_2 causes an increase in oxygen consumption by stimulating respiration, above 30% the toxic effect causes a consequent lowering of oxygen consumption. The phosphine intake increases with CO_2 concentration (and hence with oxygen consumption) (Fig. 2) until the 20% CO_2 level, where the intake steadies at about 100 µg/g(insect) over 24 hours. Further increases in CO_2 afford only slight increases in phosphine uptake rate. It seems likely that the ability of the insect to absorb and detoxify phosphine is saturated at about this level. Thus, phosphine would be expected to be most toxic with concentrations of CO_2 between 20 and 30%. In fact this is reflected in the LC_{90} synergy ratio in Fig. 3 which shows that concentrations of CO_2 between 15 and 35% produce the greatest enhancement of the phosphine toxicity. From Figure 3 it can also be seen that although the LC_{50} continues to fall with increasing CO_2 concentration, the LC_{90} reaches a minimum at about 35%. This indicates a change in slope of the probit mortality–dosage lines with changing CO_2, possibly resulting from heterogeneity in the population of *C. turcicus* used in these experiments.

Some researchers have suggested that higher CO_2 concentrations may also assist fumigant intake by keeping the spiracles open (Bond and Monro 1967). On the other hand many compounds can penetrate the integument independently of the spiracles and the role of either spiracles or integument with respect to the toxicity of phosphine remains uncertain.

There is no apparent correlation between phosphine uptake and oxygen consumption above 30% CO_2. Increased uptake, even at a lower respiration rate, indicates that the mechanism of phosphine uptake by insects depends not only on respiration, but also on factors such as diffusion, and binding to components of insect tissue.

From these studies on the effect of carbon dioxide on the toxicity of phosphine, it is clear that there is an advantage in adding 15–35% CO_2. In fact for an LC_{99} using 0.1 mg/L phosphine, the optimum CO_2 concentration may be near 20%. Within the 15–35% range it is apparent that CO_2 enhances the effect of the phosphine. The only advantage of co-applications of CO_2 above 35% may be the ability of CO_2 to promote a faster penetration of the PH_3 through the grain mass (Leesch 1990). These results may facilitate the determination of the cost effectiveness of adding CO_2 in phosphine fumigations. Carbon dioxide concentrations for controlled atmosphere applications are generally 40% or greater (Annis 1987). However, with combined phosphine and CO_2, lower levels of CO_2 are effective.

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