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ECO_2FUME AS A QUARANTINE FUMIGANT FOR IMPORT OF NURSERY TREES

Young-Mi Moon [1], Min-Gu Park[1], Justin Tumambing[2]
Bong-Su Kim[3], Byung-Ho Lee[3*]

[1]Animal, Plant and Fisheries Quarantine and Inspection Agency(QIA), South Korea
[2]CYTEC Australia Holding Pty Ltd. Australia
[3]Dongbu ARI, Dongbu Hannong Co. Ltd, South Korea
Corresponding author's e-mail: *byungholee@dongbu.com*

ABSTRACT

ECO_2FUME, a cylinderized gas formulation of 2% phosphine with carbon dioxide, is a potential alternative to methyl bromide (MB). In Korea, MB has been used to treat the imported nursery tree for quarantine purpose. Currently limited use of MB fumigation is due to both low efficacy at low temperatures and phytotoxic damage to imported nursery trees: mainly dracaena and palm trees in Korea. Quarantine fumigation in nursery trees is important in terms of importer's requirements not to be damaged. Phosphine gas is outstanding alternatives of MB to reduce phytotoxic symptoms on post fumigations as well higher efficacy at low temperature due to better penetration properties than other alternatives. This study was conducted to obtain the efficacy data of ECO_2FUME to target pests and quality assessment after fumigation in import nursery trees: dracaena and palm trees.

Key words: Phosphine gas, ECO_2FUME, Quarantine, Methyl bromide chemical alternatives, Import, Dracaena tree, Palm tree, Citrus mealybug, Postharvest quality

INTRODUCTION

Thirteen species of mealybugs were intercepted in quarantine at Korean ports of entry on the imported nursery trees over the past 9 years (2000-2009). They were fumigated with 24~ 56 $g \cdot m^{-3}$ of methyl bromide (MB) for 2 h depending on the temperature. MB can damage some nursery trees as well as it has been identified as an ozone-depleting chemical with restricted use according to the Montreal protocol. Currently, there are no restrictions on use of MB as a commodity treatment for pre-shipment and quarantine purpose, but in the future restrictions may be imposed, with the deadline for phase-out now advanced to 2015.

During the 1980s, a cylinder gas formulation of phosphine, Phosfume® (2% phosphine with carbon dioxide) was developed (Winks and Russell, 1994), eliminating problems of powder residues associated with solid formulations and enabling fumigation times to be reduced. Phosfume®, recently renamed ECO_2FUME®, has been used successfully in experiments to control several important pests of Australian wildflowers for export using phosphine concentrations of up to 1 $g \cdot m^{-3}$ and exposure periods of up to 16 h (Muhunthan *et al*., 1997; Williams and Muhunthan, 1998).

This study was conducted to obtain the efficacy data of ECO_2FUME to target pests and assess the quality after fumigation in import nursery trees: dracaena and palm trees.

MATERIALS AND METHODS

Material

Egg and adult stages of *Planococcus citri* (citrus mealybug) were used in this test. The colony was maintained at 26±1℃, 90% RH, and a photoperiod of 16:8(L:D) on potatoes for several years. Nursery trees, dracaena (*Dracaena fragrans*) and palm trees (*Chamaedorea elegans*), were purchased from nursery importer. ECO_2FUME (2% PH_3 + 98% CO_2) for fumigation studies was supplied by Cytec Industries via its local distributor, Dongbu Hannong Co., Ltd in Korea.

Measurement of Phosphine and Calculation of CT (concentration X time) Product

Concentrations of phosphine were measured at 0.5, 2, 6, 24 h after the first injection in fumigation chamber (12 L glass desiccators) and refrigerated container (28.6 m^3). The gas samples were stored in gas-tight Tedlar® sampling bags before analysis. The *ct*-products (concentration x time) were calculated from the arithmetic average of phosphine concentration readings during the 24 h exposure period. Fumigant concentrations were determined with a gas chromatograph (Agilent 7890A, FPD, USA), fitted with a DB-WAX, FFD at 250℃, injection temperature of 200℃ (He as carrier gas) and oven temperature of 200℃.

Fumigation Methods for Dose Response (12 L desiccators)

The calculated amount of phosphine was injected in gas-tight 12 L glass desiccators which were known their volume for 24 h at 15±1℃. The desiccators were sealed with glass stoppers containing a septum through which the mixture of gas was dosed. Several doses and gas samples were taken for analysis by gas chromatography. The dosage and the required volume for the fumigant concentration were calculated from Ren et al (2011). After 24 h of fumigation, the desiccators were opened in the fume hood for aeration. Mortality of adult stage of *P. citri* was assessed under a microscope after incubation for 1 and 3 days. The egg hatch rate of *P. citri* was examined after 7 days of incubation.

Commercial Scale Fumigation Method (Refrigerated container)

Nursery trees imported to China and Southeast Asia were stored and transported at 15℃ for 1 day and normally displayed at room temperature for sale purpose. This experiment was carried out at commercial scale temperature conditions. Fumigations using 1.5 $g.m^{-3}$ of phosphine for 24 and 48 h were carried out with 100 dracaenas and palms trees in a 28 m^3 refrigerated container. For comparative studies of MB phytotoxicity were carried out in a 0.5 m^3 stainless fumigation chamber at the same temperature condition. Gas monitoring was carried out on samples taken from two points (front and rear) inside the refrigerated container to assess the distribution of the gas. Concentration of phosphine gas was determined by taking gas samples using a pump and then analyzing with GC-FPD at initial time and at the end of the fumigations. The *ct*-products were calculated as indicated by Ren et al (2011).

After fumigation, the chambers were aerated using a fan for 2 h, and then samples taken from the commodities were incubated at 15±1℃ for 1 day. This was followed by storage at 21±1℃ for 3 weeks for assessment of quality and phytotoxicity. Assessment of quality and phytotoxicity such as diameter, chlorophyll contents, color and damage of leaves was conducted for 1, 7, 14 and 21days after fumigation. Individual assessment was more than 10 replicates of each nursery trees.

Diameter was measured at the widest side with Veniera-califers tester (Coolant proof IP 67, Japan). Chlorophyll was measured using chlorophyll-meter (SPAD-502 Plus, Japan). The color was measured using colorimeter (SpectroDens A711073, Germany). Injury of leaves was subjectively scored as zero (none), one (slight, <5% affected), two (moderate, <25% affected), or three (severe, >25% affected). Mortality of adult and nymph of the insects was assessed under a microscope after incubation for 1 and 3 days. Hatching rate of eggs was investigated after incubation for 5~7 days.

RESULTS

1. Dose Response of Phosphine to *P. citri*

The *ct*-products (g h m^{-3}) of phosphine that achieved more than 99% kill of eggs and adults of *P. citri* was 25.23 and 1.08 g h m^{-3}, respectively. Table 1 shows the *ct*-products of phosphine on egg stage of *P. citri*.

Table 1. Calculated *ct*-products (Concentration × time) of phosphine at 50% (Lct_{50}) and at 99% (Lct_{99}) mortality of eggs of *P. citri* at 15±1°C

Temperature	No. of insects	L*ct* (95% C.l.)		Slope	Degrees of Freedom	X^{2b}
		Lct_{50}[a]	Lct_{99}[a]			
15℃	14,812	0.04 (0.001 ~ 0.267)	25.23 (13.99 ~ 50.73)	0.84	53	215.95

[a]. Unit of Lct_{50}, Lct_{99}= g h m^{-3} for 24 h
[b]. χ^2 is based on pooling of data with low expectation

2. Results of Refrigerated Container Fumigation

2-1. Efficacy of Phosphine to *P. citri*

The *ct*-products of phosphine for three individual trials were to 34.8 g h m^{-3} for 24 h fumigation (1st trial), 57.6 g·h·m^{-3} for 24 h fumigation (2nd trial) and 58.4 g h m^{-3} for 48 h fumigation (3rd trial). For MB fumigations, *ct*-products was g h m^{-3} for 2.0 h fumigation (Table 2).

Table 2. Efficacy of phosphine fumigation of nursery trees on egg and adult stages of *P. citri* at 15±1°C in refrigerated container

ct-products of fumigant ($g \cdot h \cdot m^{-3}$)	Fumigation Time (h)	*Planococcus citri*			
		Egg		Adult	
		No. of tested insects[1]	Corrected mortality (%)[2]	No. of tested insects[1]	Corrected mortality (%)[2]
Untreated	-	1,500	-	100	-
Phosphine (34.8)	24.0	3,587	100	60	100.0
Phosphine (57.6)	24.0	2,735	100	420	100.0
Phosphine (58.4)	48.0	997	100	60	100.0
MB (72.0)	2.0	3,014	100	60	100.0

[1] Sum of three replicates
[2] (Mortality for the treated-mortality for the control) / (100-mortality for the control)*100

2-2. Post Fumigation Damage of Dracaena and Palm Trees

Dracaena fragrans was not damaged by *ct*-product of phosphine at 34.8 $g \cdot h \cdot m^{-3}$ carried out at 15°C, and after fumigation stored for 3 weeks at 20°C. Phosphine at 57.6 $g \cdot h \cdot m^{-3}$ and MB at 72.0 $g \cdot h \cdot m^{-3}$ caused phytotoxicity damage. However, none of *Chamaedoreae elegans* was damaged in this test (Table 3).

Table 3. Phytotoxic effect of phosphine and MB on nursery stocks at 15±1 °C.

-	*ct*-products of fumigant ($g \cdot h \cdot m^{-3}$)	Fumigation time (h)	Chlorophyll			Leaf browning[1] (%)	Damage[2]
			Mean ± SD	t	*p*		
Dracaena tree	Untreated	24.0	50.2±8.8	-	-	17.7	0
	Phosphine 34.8	24.0	48.0±8.9	1.7	0.09	14.1	0
	Untreated	24.0	50.0±9.5			10.1	0
	Phosphine 57.6	24.0	42.9±13.1	1.7	0.16	17.7	1
	MB 72.0	2.0	30.8±16.6	4.4	0.01	54.7	3
Palm tree	Untreated	24.0	46.9±6.4	-	-	12.1	0
	Phosphine 34.8	24.0	41.9±7.0	5.5	0.00	39.5	2
	Untreated	24.0	48.5±4.0	-	-	23.3	0
	Phosphine 58.4	48.0	38.7±2.9	4.3	0.01	35.0	2
	Phosphine 57.6	24.0	37.3±4.3	3.4	0.03	56.7	2
	MB 72.0	2.0	38.7±3.9	3.5	0.03	60.0	2

[1]Leaf browning = (Leaf browning/Total Leaf)*100
[2] Damage score: zero (none), one (slight), two (moderate), three (severe)

DISCUSSION

Phosphine fumigation for imported nursery plants in Korea was practically demonstrated in this study. The *ct*-products of phosphine at >30 $g \cdot h \cdot m^{-3}$ which is equivalent to 2 $g \cdot m^{-3}$ application for 24 and 48 h in well-sealed fumigation model at 15℃ could be enough for controlling egg stages of *P. citri* and without phytotoxic effect to dracaena and palm trees compared to current MB applications. These results show the similarity of previous work by Horn et al (2005) where high mortality of *Pseudococcus kraunhiae* was reported at 2.1 g m^{-3} of phosphine for 48 h fumigation.

Phosphine from ECO_2FUME could be a positive alternative fumigant to MB for various perishable commodities such as fruits and vegetables not only in terms of 100% efficacy at low temperature but without or minimal phytotoxic injury.

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