

# Susceptibilities of Five Species of Scales (Diaspididae and Coccidae) and Mealybugs (Pseudococcidae) to Fumigation with a Gas Mixture of Ethyl Formate and Carbon Dioxide under Normal Atmospheric Pressure or Vacuum

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**Abstract:** Five species of scales (*Diaspis boisduvalii*, *Aonidiella auranti*, and *Coccus hesperidum*) and mealybugs (*Planococcus citri* and *Dysimicoccus brevipes*) were fumigated with a gas mixture of ethyl formate (EF, C<sub>3</sub>H<sub>6</sub>O<sub>3</sub>) and carbon dioxide (CO<sub>2</sub>) to simulate a fumigation by using a commercial product formulation (EF 16.7% + CO<sub>2</sub> 83.3%, w/w) and their susceptibilities were investigated. All developmental stages of *D. boisduvalii*, *A. auranti*, and *C. hesperidum* tested were completely killed at a fumigation dosage of EF 25.1 mg/l + CO<sub>2</sub> 124.9 mg/l (equivalent to 150 mg/l of the gas mixture) for 3 h at 15°C. Crawler, nymph, and adult *D. brevipes* were more tolerant than *P. citri*; however, these stages of both mealybugs were 100% killed at dosages of EF 16.7 mg/l + CO<sub>2</sub> 83.3 mg/l (100 mg/l of gas mixture) for 3 h at 15°C. Among the five species tested, the least susceptible stage was *P. citri* eggs, which showed 25.3%, 54.2%, and 92.3% mortality at gas mixture dosages of 150 mg/l, 250 mg/l (EF 41.8 mg/l + CO<sub>2</sub> 208.3 mg/l), and 350 mg/l (EF 58.5 mg/l + CO<sub>2</sub> 291.5 mg/l), respectively. LD<sub>50</sub> and LD<sub>95</sub> for the *P. citri* egg stage under gas mixture fumigation were calculated as 242.0 mg/l and 408.0 mg/l. The efficacy of gas mixture fumigation was increased under vacuum [ $<18$ – $22$  kPa (ABS)]. Mortalities of *P. citri* egg were increased under vacuum of up to 61.2%, 70.2%, and 100% with a gas mixture at 150 mg/l, 250 mg/l, and 350 mg/l dosage rates, respectively.

**Keywords:** ethyl formate, scale insect, mealybug, vacuum fumigation, carbon dioxide

## Introduction

Despite its use for quarantine purposes, a transition from methyl bromide (MB) to alternative chemicals or techniques is needed worldwide. Japan imports many types of agricultural plant products, and those detected a quarantine pest by plant quarantine inspection at import are destined for MB fumigation. There has, however, been little research aimed at the development of alternatives to MB for fresh fruits and vegetables in Japan (Yamada *et al.*, 2012). A quarantine treatment that can reduce up to 50% the MB dosage on fresh vegetables has been developed (Misumi *et al.*, 2009), but will not allow a complete phasing out of MB. Techniques allowing a full transition to alternatives are necessary. MB and hydrogen cyanide are currently used in plant quarantine treatment for fresh fruits and vegetables,

but various alternative options for quarantine treatment are considered desirable because of public concerns about environment and food safety.

Ethyl formate (EF, C<sub>3</sub>H<sub>6</sub>O<sub>2</sub>) is a liquid fumigant whose boiling point is 52–54°C (ICSC, 2010) and for propelling, enhancing vaporization, and reducing explosion risk, it has been registered as an agrochemical of gas mixture with carbon dioxide (CO<sub>2</sub>) in countries including Australia, New Zealand, and South Korea. EF decomposes easily and has relatively low human toxicity. It is also approved to use for “organic” products as a naturally existing material. For fruits and vegetables, strawberries showed no differences between EF-treated and untreated fruits except for calyx damage (Simpson *et al.*, 2004), and table grapes tolerated 5.0% EF treatment for 1 or 2 h, although rachis browning was increased (Simpson *et al.*, 2007). In addition, for silo

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fumigation for wheat grain, EF has recently been examined for use in a gas mixture with methyl isothiocyanate (MITC) instead of CO<sub>2</sub> to control stored product pests such as *Sitophilus oryzae* (Ren *et al.* 2008). However, some phytotoxicity has been reported, with EF fumigation causing a deterioration in quality or injury to plant products. Yamada *et al.* (2012) reported damage to string bean leaves fumigated with high dosages of an EF/CO<sub>2</sub> gas mixture in a mortality test for leaf miners and spider mites. Phytotoxicity on lettuce treated with EF was observed at high concentration (Stewart and Mon, 1984). The explosion limits of EF are described as 2.7–16.5 vol%. The development of a fumigation schedule with lower EF dosage is desirable for safe implementation of EF fumigation in quarantine processes at ports of entry and export.

We investigated the fumigation efficacy of a gas mixture of EF and CO<sub>2</sub> on scales and mealybugs at normal atmospheric pressure, aimed at introduction into a quarantine treatment schedule for fresh fruits and vegetables. We also tested vacuum (VAC) fumigation using the same gas mixture, with the purpose of optimizing fumigation with a reduced EF dosage rate for safety and efficient use of EF gas in commercial fumigation.

## Materials and Methods

### 1. Test insects

*Diaspis boisduvalii*, an orchid scale, was collected from a pot plant of an orchid reared at the pest identification laboratory of Yokohama Plant Protection Station (YPPS) in November 2009 and reared for successive generations on Japanese squash in the research division of YPPS was used. Eggs were oviposited under the shells of adult females and were investigated separately from other stages in the evaluation of fumigation efficacy.

*Aonidiella aurantii* individuals were derived from a colony in the citrus grove of an agrochemical manufacturer in Shizuoka prefecture. Branches and leaves of lemon infested with mixed stages of *A. aurantii* were collected in August 2011 and used for fumigation.

*Coccus hesperidum* nymphs were collected from a lemon tree in the back yard of the research division of YPPS in July 2011 and reared with lemon seedlings in a rearing room at 25°C. When the numbers had been increased enough for the study, lemon seedling pots infested with mixed stages of *C. hesperidum* were fumigated.

*Planococcus citri*, a citrus mealybug was collected from a banana tree (*Musa* sp.) at a glass house of the Tukuba Botanical Garden, National Museum of Nature and

Science, Ibaraki prefecture, in August 2010 and reared for successive generations on Japanese squash in the research division of YPPS was used. Three-day-old eggs and mixed stages of nymph and adult were prepared for fumigation. Eggs were collected in a method that adult females were allowed to lay eggs for one day in laboratory dishes lined with black-dyed filter paper. Eggs on the filter paper, which was cut into pieces, and other stages were placed on a piece of squash used for rearing, whose flesh coated with paraffin was fumigated. According to the results of a developmental period study for each stage (Misumi, unpublished), the size or length of the body was used as a factor in distinguishing a hatched nymph from other stages and the stages were separately counted for the evaluation of fumigation.

*Dysimicoccus brevipes*, a pineapple mealybug, was collected from pineapples purchased at a retail store in Nago, Okinawa prefecture in April 2009 and reared for successive generations on Japanese squash in the research division of YPPS was used. Because a pineapple mealybug is recognized as an ovoviviparous insect, mixed stages from hatched nymph to adult on a piece of squash prepared in the same way as for citrus mealybug were fumigated. According to a study on the developmental period of each stage (Misumi, unpublished), the insects were classified into three groups: hatched nymph, 2<sup>nd</sup>–3<sup>rd</sup> nymph, and 3<sup>rd</sup> nymph and adult, based mainly on the body size at the evaluation.

For both species of mealybugs, the method described by Sawamura and Narai (2008) was used to determine the developmental period of days required for each stage under the rearing conditions.

All five species tested were reared at a constant temperature of 25°C at 50–70% RH under a photoperiod of light 16 h: dark 8 h in rearing rooms, and each insect stage of the species was moved to a temperature-controlled fumigation room on the day before fumigation, or at least 4 h before fumigation. Japanese squash (variety Kurokawa) was used for rearing and at fumigation was harvested in a glasshouse in Miyazaki prefecture.

### 2. Fumigation

Fumigations were conducted using an acrylic fumigation box (W22×D22×H18 cm external dimensions, 6 l internal volume; equipped with gas injection and sampling ports, temperature probe, stirring bar and gas exhaust valve) placed in a temperature-controlled fumigation room at 15°C. A larger acrylic fumigation box with internal volume 29.5 l was also used only for a fumigation test of *C. hesperidum*. To simulate commercial fumigation in actual

plant quarantine, a dosage rate of EF and CO<sub>2</sub> was determined based on commercial gas cylinders (Vapormate™, EF 16.7% and CO<sub>2</sub> 83.3%, w/w). Because Vapormate™ is not available in Japan, dosing was conducted by the following procedure; EF liquid (purity over 98%, Alfa Aesar) was collected from a glass vial using a syringe and injected into the fumigation box through an injection port with a septum, and subsequently CO<sub>2</sub> from a gas cylinder was quickly introduced into the box. The pressure in the fumigation box was decreased to 20–21 kPa (ABS) just before dosing of EF and CO<sub>2</sub> and then restored to atmospheric pressure at 10 min after dosing. A mercury manometer (Sansho Co., Ltd.) or a digital manometer (HT-1500NH, Hodaka Co., Ltd.) was used to measure pressure inside the fumigation boxes. For gas circulation inside the box, a magnetic stirrer was used throughout the exposure period of fumigation. Forced aeration (3 l/min) was applied for 1 h after 3 h of fumigation. The fumigation tests were replicated two or three times for each stage.

EF and CO<sub>2</sub> gas concentrations in the space inside the fumigation box during fumigation were measured at 30, 60, 120, and 180 min after dosing, by gas chromatographs (EF: Shimadzu GC-2014 with FID, CO<sub>2</sub>: Shimadzu GC-2014 with TCD) to calculate CT (concentration × time) product, and a temperature recorder (Chino graphic logger CR 1016-A) was used to monitor temperatures during fumigation. A NDIR gas analyzer (UR-126G Komyo-rikagaku kogyo) was also used for measuring CO<sub>2</sub> concentration. Untreated control insects were placed in another fumigation box in the same temperature-controlled fumigation room and underwent the same fumigation procedures except for dosing.

### 3. Evaluation of mortality

Each test insect treated was moved to a temperature-controlled rearing room after fumigation. For the egg stages of scales, egg hatch was counted daily from 3 to at least 14 days after fumigation. Mortalities of other stages of scales were evaluated based on freshness and shriveling of the body under the stereoscopic microscope from 7 to 10 days after fumigation. For scales whose mortality was difficult to determine by body freshness and shriveling alone, a biological microscope was used and the pulsation and/or flowing of body fluids were assessed as indicators of vital status. Larval and adult stages of the citrus mealybug and pineapple mealybug and the egg stage of citrus mealybugs were each maintained in the rearing room from 3 to 5 days and from 7 to 14 days, after which their mortality was evaluated under the stereoscope by motion of insects or egg hatching.

Probit analysis was adopted for estimating the lethal dosage values of EF/CO<sub>2</sub> gas mixtures (Polo Plus, version 2.0; LeOra Software 2002).

## Results and Discussion

The mortalities of *Diaspis boisduvalii* and *Aonidiella aurantii* are presented in Table 1 and those of *Coccus hesperidum* in Table 2.

All stages of *D. boisduvalii* and *A. aurantii* fumigated with two dosages of gas mixture at EF 25.1 mg/l and CO<sub>2</sub> 124.9 mg/l and at EF 41.8 mg/l and CO<sub>2</sub> 208.3 mg/l showed 100% mortality at each dosage rate for 3 h at 15°C. Aharoni *et al.* (1987) reported that an exposure of *A. aurantii* for 3 h at EF concentration 1.5% resulted in 100% mortality of all stages. A dosage of EF 25.1 mg/l is approximately 0.8% and is approximately half of the concentration investigated by Aharoni *et al.* (1987). In our study, the efficacy of EF may have been enhanced by mixing with CO<sub>2</sub>. A gas concentration of EF at the initial phase of fumigation of *A. aurantii* was measured as 22.3 mg/l at the dosage rate of EF 25.1 mg/l, and had decreased by approximately 11% at the termination of the exposure. In contrast, in the test for *D. boisduvalii*, the EF gas concentration initially was 23.3 mg/l for fumigation at the EF 25.1 mg/l dosage rate, but the loss of gas concentration was approximately 16%. *D. boisduvalii* was fumigated on an arch-shaped section of Japanese squash coated with paraffin, a condition that may account for the lower EF gas concentration during the fumigation of *D. boisduvalii*. When a whole squash was fumigated with the EF/CO<sub>2</sub> gas mixture, little EF gas was absorbed by the squash and the EF gas concentration did not decrease during fumigation at a loading of about 0.1 kg/l (Misumi *et al.*, 2013). All tested stages of *C. hesperidum* showed complete mortality under the same fumigation conditions as for the Diaspididae species, though CT products estimated from actual EF concentrations in the mortality test for *C. hesperidum* were slightly lower than those for the Diaspididae species. Sorption of EF to the seedling pots of lemon infested with *C. hesperidum* may have contributed to lowering the CT products. In a preliminary test, we had confirmed that *Ceroplastes rubens*, red wax scale, on mandarin branches could be killed completely under the same fumigation conditions but using dosage rates of EF 58.5 and CO<sub>2</sub> 291.5 mg/l (Misumi, unpublished).

Based on the results of mortality tests for Diaspididae and Coccidae, all insects tested were completely killed by gas mixtures at lower dosage rates of EF 25.1 mg/l and CO<sub>2</sub> 124.9 mg/l for 3 h at 15°C, indicating that both insect

Table 1. Mortalities of two species of Diaspididae, *Diaspis boisduvalii* and *Aonidiella aurantii*, fumigated with gas mixtures at two dosage rates of ethyl formate (EF) and carbon dioxide (CO<sub>2</sub>) for 3 hours at 15°C.

Dosage rate <sup>1)</sup> (mg/l)		Dura- tion (h)	No. tested (N)	Stage tested <sup>2)</sup>	CT products (mg · h/l)		Mortality (mean% ± SD)	
EF	CO <sub>2</sub>				<i>Diaspis boisduvalii</i>	<i>Aonidiella aurantii</i>	<i>Diaspis boisduvalii</i>	<i>Aonidiella aurantii</i>
25.1	124.9	3	929,238	Egg or Crawler	54.9	53.5	100	100
			676,823	Mixed stages			100	100
41.8	208.3	3	229,158	Egg or Crawler	86.2	94.0	100	100
			223,648	Mixed stages			100	100
Control		3	307,113	Egg or Crawler	—	—	11.5 ± 13.6	7.7 ± 1.53
			713,603	Mixed stages			25.0 ± 11.5	75.0 ± 0.53

<sup>1)</sup> Both dosage rates of EF and CO<sub>2</sub> are designed to simulate a commercial gas mixture (EF 16.7%+CO<sub>2</sub> 83.3%, w/w).<sup>2)</sup> *D. boisduvalii* is oviparous and *A. aurantii* is viviparous.Table 2. Mortalities for *Coccus hesperidum* fumigated with gas mixtures at two dosage rates of ethyl formate (EF) and carbon dioxide (CO<sub>2</sub>) for 3 hours at 15°C.

Dosage rate <sup>1)</sup> (mg/l)		Dura- tion (h)	No. tested (N)	Stage tested <sup>2)</sup>	CT products (mg · h/l)	Mortality (mean% ± SD) <i>Coccus hesperidum</i>
EF	CO <sub>2</sub>					
25.1	124.9	3	255	Crawler	48.3	100
			321	Mixed stages		100
41.8	208.3	3	170	Crawler	84.3	100
			257	Mixed stages		100
Control		3	165	Crawler	—	26.3 ± 26.4
			314	Mixed stages		27.8 ± 19.8

<sup>1)</sup> Both dosage rates of EF and CO<sub>2</sub> are designed to simulate a commercial gas mixture (EF 16.7%+CO<sub>2</sub> 83.3%, w/w).<sup>2)</sup> *C. hesperidum* is ovoviviparous. Both male and female adults and nymphs were involved in the mixed stages.

families are be susceptible to gas mixture fumigation by EF and CO<sub>2</sub>.

Mortalities of each stage of *Planococcus citri* and *Dysmicoccus brevipes* for fumigation with gas mixtures at dosages of EF 8.4 mg/l and CO<sub>2</sub> 41.7 mg/l, EF 16.7 mg/l and CO<sub>2</sub> 83.3 mg/l, EF 25.1 mg/l and CO<sub>2</sub> 124.9 mg/l, EF 41.8 mg/l and CO<sub>2</sub> 208.3 mg/l, and EF 58.5 mg/l and CO<sub>2</sub> 291.5 mg/l, all for 3 h at 15°C, are presented in Tables 3 and 4.

A gas concentration of EF during fumigations for crawlers, nymphs, and adults of both *P. citri* and *D. brevipes* changed as showing a similar curve to that measured for the test of *D. boisduvalii* because both were fumigated on an arch-shaped section of Japanese squash. In contrast, *P. citri* eggs were fumigated on a piece of filter paper placed in a laboratory dish, EF gas concentration at the initial stage of exposure was almost identical at all of the dosage rates tested, and thereafter EF concentrations changed negligibly until the end of the exposure time.

Mortalities of crawlers, nymphs, and adults of *P. citri* reached 100% in fumigations with the lowest gas mixture dosage of EF 8.4 and CO<sub>2</sub> 41.7 mg/l. The egg stage (3-day-

old egg) however, showed incomplete mortalities following fumigation at every dosage of EF and CO<sub>2</sub> applied in this study. The egg mortalities were 25.3% and 54.2% at dosage rates of EF 25.1 and CO<sub>2</sub> 124.9 mg/l and of EF 41.8 and CO<sub>2</sub> 208.3 mg/l, respectively, and even fumigation at dosage rates of EF 58.5 and CO<sub>2</sub> 291.5 mg/l achieved only 92.3% mortality. Susceptibility of the egg stage is expected to be especially low compared to other stages. Although exposure time and temperature were 4 h and 21°C, LC<sub>50</sub> and LC<sub>95</sub> for nymph and adult stages of *P. citri* under EF fumigation were estimated at 2.89–5.19 mg/l and 5.58–8.25 mg/l, respectively (Park *et al.*, 2007). Given that the dosage differences between the estimated LC<sub>50</sub> and LC<sub>95</sub> ranged from 2.69 mg/l to 3.06 mg/l, it appears that this slight dosage increase could change the mortality rate from 50% to 95%. The EF dosage rate must thus be a prior factor in the mortality of *P. citri*. The nymph and adult stages of *P. citri* had high susceptibilities to EF fumigation.

*D. brevipes* showed from 41.8 to 65.3% mortalities among crawler, nymph, and adult at gas mixture dosages of EF 8.4 and CO<sub>2</sub> 41.7 mg/l, but complete mortalities were

Table 3. Mortalities of egg, nymphal, and adult stages of *Planococcus citri* fumigated with gas mixtures at five dosage rates of ethyl formate (EF) and carbon dioxide (CO<sub>2</sub>) for 3 hours at 15°C.

Dosage rate <sup>1)</sup> (mg/l)		Duration (h)	No. tested (N)	Stage tested	CT products (mg·h/l)	Mortality (mean% ± SD)
EF	CO <sub>2</sub>					
8.4	41.7	3	303	Hatched nymph	19.6	100
			225	Mixed nymph & adult		100
16.7	83.3	3	385	Hatched nymph	35.8	100
			369	Mixed nymph & adult		100
25.1	124.9	3	593	3-day-old egg	60.1	25.3 ± 11.4
			285	Hatched nymph	52.6	100
			189	Mixed nymph & adult		100
41.8	208.3	3	604	3-day-old egg	98.2	54.2 ± 4.98
58.5	291.5	3	600	3-day-old egg	147.6	92.3 ± 1.34
Control		3	574	3-day-old egg	—	7.1 ± 6.37
			259	Hatched nymph	—	10.9 ± 0.33
			276	Mixed nymph & adult	—	8.4 ± 1.04

<sup>1)</sup> Both dosage rates of EF and CO<sub>2</sub> are designed to simulate a commercial gas mixture (EF 16.7%+CO<sub>2</sub> 83.3%, w/w).

Table 4. Mortalities of nymphal and adult stages of *Dysmicoccus brevipes* fumigated with gas mixtures at three levels of dosage rates of ethyl formate (EF) and carbon dioxide (CO<sub>2</sub>) for 3 hours at 15°C.

Dosage rate <sup>1)</sup> (mg/l)		Duration (h)	No. tested (N)	Stage tested	CT products (mg·h/l)	Mortality (mean% ± SD)
EF	CO <sub>2</sub>					
8.4	41.7	3	17	Hatched nymph	15.9	43.3 ± 33.0
			502	2 <sup>th</sup> –3 <sup>rd</sup> nymph		65.3 ± 13.2
			311	3 <sup>rd</sup> nymph-adult		41.8 ± 23.0
16.7	83.3	3	16	Hatched nymph	34.9	100
			288	2 <sup>th</sup> –3 <sup>rd</sup> nymph		100
			417	3 <sup>rd</sup> nymph-adult		100
25.1	124.9	3	37	Hatched nymph	50.0	100
			243	2 <sup>th</sup> –3 <sup>rd</sup> nymph		100
			435	3 <sup>rd</sup> nymph-adult		100
Control		3	29	Hatched nymph	—	0.0 ± 0.00
			423	2 <sup>th</sup> –3 <sup>rd</sup> nymph		1.7 ± 0.42
			436	3 <sup>rd</sup> nymph-adult		0.7 ± 0.03

<sup>1)</sup> Both dosage rates of EF and CO<sub>2</sub> are designed to simulate a commercial gas mixture (EF 16.7%+CO<sub>2</sub> 83.3%, w/w).

achieved at EF 16.7 and CO<sub>2</sub> 83.3 mg/l. Comparing mortalities for both stages of nymph and adult between *P. citri* and *D. brevipes*, susceptibilities of *D. brevipes* were recognized lower than those of *P. citri* except in the egg stage. A dosage rate of EF 16.7 mg/l that caused 100% mortality in almost all stages is equivalent to 0.53% at 15°C. This concentration is acceptably far below the explosion limit for EF of 2.7%.

Because the egg stage of *P. citri* was the most tolerant of the stages tested in five species of scales and mealybugs,

further investigation, employing a dose–response test to estimate LD values, was carried out. LD<sub>50</sub> and LD<sub>95</sub> of *P. citri* eggs are presented in Table 5. LD values were estimated for the gas mixtures of EF and CO<sub>2</sub>.

The LD<sub>50</sub> of a gas mixture estimated for a range of 239.1–245.6 mg/l is equivalent to a range of 39.9–41.0 mg/l of EF dosage rate as a constituent of a gas mixture. The egg stage of *P. citri* showed low susceptibility. An EF dosage rate in the range 39.9–41.0 mg/l completely killed other scales and other stages of mealybugs, whereas for *P. citri*



Table 5. LD<sub>50</sub> and LD<sub>95</sub> for egg stage of *P. citri* estimated from a dose-response test with gas mixture<sup>1)</sup> of ethyl formate and carbon dioxide for 3 hours at 15°C.

Stage	No. tested (N)	Replication	LD values (95% confidence limits)		Slope
			LD <sub>50</sub>	LD <sub>95</sub>	
3-day-old eggs	3134	1	239.1 (143.2–284.3)	411.2 (346.9–667.6)	0.01
	3662	2	245.6 (204.9–279.2)	404.2 (349.3–550.6)	0.01
	6796	1 and 2	242.0 (187.3–278.6)	408.0 (350.5–575.8)	0.01

<sup>1)</sup> Dosage rate of gas mixture (ethyl formate 16.7% + carbon dioxide 83.3%, w/w).

Table 6. Mortalities for the egg stage of *P. citri* fumigated with gas mixture at three levels of dosage rates of ethyl formate (EF) and carbon dioxide (CO<sub>2</sub>) for 3 hours at 15°C under vacuum [ $<18$ –22 kpa (ABS)].

Dosage rate <sup>1)</sup> (mg/l)		Dura- tion (h)	No. tested (N)	Stage tested	Mortality (mean% $\pm$ SD)
EF	CO <sub>2</sub>				
25.1	124.9	3	827	3-day-old egg	61.2 $\pm$ 11.35
41.8	208.3	3	765	3-day-old egg	70.2 $\pm$ 10.98
58.5	291.5	3	854	3-day-old egg	100
Control		3	775	3-day-old egg	10.9 $\pm$ 7.78

<sup>1)</sup> Dosage rate of gas mixture (ethyl formate 16.7% + CO<sub>2</sub> 83.3%, w/w).

eggs it would produced only 50% mortality. Thus, though gas mixture fumigation with EF and CO<sub>2</sub> is effective against those scales and mealybugs, a treatment schedule capable of killing the egg stage of *P. citri* will be necessary for plant quarantine treatment. A standard dosage rate of gas mixture of EF and CO<sub>2</sub> usually requires a 120% increase with respect to the estimated LD<sub>95</sub> dosage rate. The LD<sub>95</sub> for gas mixture in this study corresponded to approximately 68.1 mg/l dosage of EF, so that the EF dosage required for a quarantine treatment standard would then be 120% of this LD<sub>95</sub> partial dosage, or 81.7 mg/l. This EF dosage represents a 2.61% concentration at 15°C. Because this concentration is below the explosive limits of EF, it may be the basis of a practical and safe fumigation schedule.

Susceptibility of grape mealybug (*Pseudococcus maritimus*) on table grapes has been investigated, although under different temperature and exposure times than those used in our study. For the egg stage, the LC<sub>99</sub> is estimated as EF 4.85% applied for 1h at 24°C, and the EF concentration could be reduced to 3.48% when EF was applied with 10% CO<sub>2</sub> (Simpson *et al.*, 2007). Bean thrips (*Caliothrips fasciatus*) on navel oranges were also completely controlled by a 1-h commercial-scale fumigation with Vapormate™ at an EF concentration of 31 mg/l in a 20-ft marine container (Mitcham *et al.*, 2011). For plant quarantine fumigation with a mixture of EF and CO<sub>2</sub>, the dosage rate of the gas mixture may be calculated as 489 mg/l (including 81.7mg/ or 2.61% of EF). Thus a fumigation with gas mixture of EF and CO<sub>2</sub>

at a dosage rate of 489 mg/l for 3 h at 15°C would also be effective against grape mealybugs and bean thrips. When applied as a gas mixture with CO<sub>2</sub>, EF fumigation will be more effective and flammability risk will be greatly reduced.

When EF fumigation without CO<sub>2</sub> was applied against dried fruit beetle, *Carpophilus hemipterus*, on prunes, the use of VAC conditions (at 800 mb pressure) reduced both EF dosage rate and exposure time while maintaining control efficacy against the adult stage of the beetle (Rouzes *et al.*, 2008). To investigate the effect of VAC fumigation and to achieve 100% mortality of the egg stage of *P. citri*, individuals were fumigated with a gas mixture of EF and CO<sub>2</sub> for 3 h at 15°C under VAC conditions and mortality was recorded. The pressure in a fumigation box containing 3-day-old eggs of *P. citri* was reduced to 8 kPa just before dosing with the gas mixture, and three levels of dosage rates (EF 25.1 mg/l and CO<sub>2</sub> 124.9 mg/l, EF 41.8 mg/l and CO<sub>2</sub> 208.3 mg/l, and EF 58.5 mg/l and CO<sub>2</sub> 291.5 mg/l) were tested. The fumigation was carried out as described above, and the pressure inside the box was confirmed at the end of the exposure time with a mercury or digital manometer. These results are shown in Table 6.

Even though the fumigation conditions including dosage rate, temperature, and exposure time were identical between treatments under VAC and normal atmospheric pressure (NAP), increased mortality was observed with VAC fumigation: from 25.3% to 61.2% for the mixture EF 25.1 mg/l and CO<sub>2</sub> 124.9 mg/l and from 54.2% to 70.2% for

the mixture EF 41.8 mg/l and CO<sub>2</sub> 208.3 mg/l, although estimated mortality rates for those fumigations varied widely, owing to large standard deviations. Furthermore, 100% mortality was achieved by VAC fumigation with a mixture of EF 58.5 mg/l and CO<sub>2</sub> 291.5 mg/l. All pressures following exposure ranged from 18 to 22 kPa.

From the results of the above tests, we conclude that a mixture of EF 16.7% and CO<sub>2</sub> 83.3% is effective as a plant quarantine treatment for pests of fresh fruits and vegetables under NAP, though the confidence limits of the LD<sub>95</sub> values for *P. citri* eggs were not sufficiently narrow. In addition, fumigation efficacy will be enhanced when this formulation is applied under VAC conditions. For improved quarantine security, it is thus advisable to fumigate with an EF and CO<sub>2</sub> gas mixture under VAC instead of NAP when higher risk insect pests are detected in plant quarantine inspection at ports of entry.

However, for establishing an effective quarantine treatment schedule for insect pests on fresh fruits and vegetables, a certain amount of gas loss by EF sorption to fumigated material should be anticipated and the resulting fumigation efficacy should be confirmed. For the final stage of the development of a fumigation schedule, on-site fumigation tests to evaluate gas sorption and penetration into fumigated materials are necessary. In addition, it is obvious that VAC fumigation of imported fruits and vegetables will be difficult to apply in the conventional fumigation with using common warehouses at ports of entry. Moreover, introducing a substantial volume of a gas mixture of more than 350 mg/l dosage rate (EF 58.5 mg/l and CO<sub>2</sub> 291.5 mg/l, equivalent to 1.87% of EF at 15°C) requiring a pressure of more than 1.8 kPa inside a warehouse will not be practicable. Further study of gas dosing methods for cylinderized gas mixtures of EF and CO<sub>2</sub> into a warehouse is also required.

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## 和 文 摘 要

ギ酸エチル及び二酸化炭素混合ガスの常圧及び減圧くん蒸に対する5種  
カイガラムシ類（マルカイガラムシ科、カタカイガラムシ科及びコナカイガラムシ科）の感受性三角 隆・小川 昇<sup>1)</sup>・山田邦彦<sup>2)</sup>・宿谷珠美<sup>3)</sup>

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5 種のカイガラムシ類（ランシロカイガラムシ [*Diaspis boisduvalii*] 及びアカマルカイガラムシ [*Aonidiella auranti*]）、ヒラタカタカイガラムシ (*Coccus hesperidum*) 及びコナカイガラムシ（ミカンコナカイガラムシ [*Planococcus citri*] 及びパイナップルコナカイガラムシ [*Dysimicoccus brevipes*]）をギ酸エチル (EF) と二酸化炭素 (CO<sub>2</sub>) の混合製剤 Vapormate™ の混合比 (EF 16.7% 及び CO<sub>2</sub> 83.3%, w/w) を想定して混合ガスくん蒸を実施し、それらの感受性について調査した。

ランシロカイガラムシ、アカマルカイガラムシ及びヒラタカタカイガラムシのすべての発育態は混合ガス単位薬量 150 mg/l (EF 25.1 mg/l 及び CO<sub>2</sub> 124.9 mg/l)、15℃、3 時間のくん蒸で完全殺虫された。パイナップルコナカイガラムシの幼虫と成虫はミカンコナカイガラムシのそれらよりも耐性であったが、混合ガス単位薬量 100 mg/l (EF 16.7 mg/l 及び CO<sub>2</sub> 83.3 mg/l)、15℃、3 時間のくん蒸で完全殺虫された。

供試された5種カイガラムシ類のうち、当該くん蒸に対して最耐性の態はミカンコナカイガラムシの卵態であり、殺虫率は混合ガス単位薬量 150 mg/l で 25.3%、250 mg/l で 54.2%、350 mg/l で 92.3% であった。また、プロビット分析

により LD<sub>50</sub> 値は混合ガスとして、242.0 mg/l 及び LD<sub>95</sub> 値 408.0 mg/l と算出された。

今後は実用化試験を実施し、当該基準が有効かどうか検証する必要があるが、仮にくん蒸基準として LD<sub>95</sub> 値の 20% 増の単位薬量を設定する場合で計算すると、単位薬量は EF 及び CO<sub>2</sub> 混合製剤として約 489 mg/l となる。よって、ギ酸エチルと空気の混合気体における爆発限界の下限値よりも低い濃度でくん蒸基準を設定することが可能である。

我々はさらに、くん蒸による殺虫効果をより向上させることを目的として、EF 及び CO<sub>2</sub> の混合ガスくん蒸を、減圧条件下 (18 ~ 22kPa[ABS] 以下) で実施したところ、殺虫効果が向上し、最耐性のミカンコナカイガラムシ卵は、混合ガス単位薬量 150 mg/l で 61.2%、250 mg/l で 70.2% まで殺虫率が上昇し、350 mg/l では 100% 殺虫が達成できた。

以上の結果から、ギ酸エチルと二酸化炭素の混合ガスくん蒸は、青果物害虫に対する検疫処理として有効であると考えられ、また、それら混合ガスくん蒸を減圧くん蒸で実施した場合、処理強度が向上し、より検疫の安全度を高めることができると考えられた。しかしながら、実用くん蒸では、くん蒸される青果物への薬剤収着等によるガス濃度の低下を考慮してくん蒸基準を設定する必要がある。

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