applied sciences

MDPI

Article

The Efficacy, Phytotoxicity, and Safety of Liquid Ethyl Formate Used to Control the Grape (Campbell Early) Quarantine Pest *Pseudococcus comstocki*

Byung-Ho Lee [1], Ki-Jeong Hong [2] and Min-Goo Park [3,*]

[1] Institute of Quality & Safety Evaluation of Agricultural Product, Kyungpook National University, Daegu 41566, Korea
[2] Department of Plant Medicine, Sunchon University, 255 Jungang-ro, Suncheon 57922, Korea
[3] Department of Bioenvironmental Chemistry, Jeonbuk National University, Jeonju 54896, Korea
* Correspondence: pmg@korea.kr

Featured Application: *Pseudococcus comstocki*, a quarantine pest, was completely killed when fumigated with liquid ethyl formate (EF), without any phototoxicity or alterations in the internal condition of the grapes (Campbell Early), at EF 70 g/m^3 for 4 h, 145.85 g h/m^3 with a loading ratio of 10% at 5 °C. EF can be an alternative to methyl bromide (MB) in terms of safety in the workplace as well as preventing ozone depletion.

Abstract: Mealybugs found on grapes during quarantine in Korea are fumigated with methyl bromide (MB). However, MB is an ozone-depleting, highly toxic pesticide; therefore, the International Plant Protection Convention, a representative organization involved in quarantine, recommends using MB alternatives. We evaluated the feasibility of using liquid ethyl formate (EF), a new EF formulation, to control mealybugs (*Pseudococcus comstocki)* on grapes (Campbell Early). Large-scale tests and the comparative evaluation of EF and MB desorption from grapes were conducted during the simulated 72-h post-fumigation period. Dose–response tests showed that the EF concentration and time product causing 99% mortality (LCt_{99}) at 5 °C was 47.36 and 145.85 g h/m^3 for adults and eggs, respectively. EF treatment at 70 g/m^3 for 4 h at 5 °C with a loading ratio of 10% achieved an LCt_{99} of 145.85 g h/m^3 on *P. comstocki*, confirming EF efficacy on mealybugs without phytotoxic effects on grapes. EF fumigation may also be safer because EF concentrations were maintained at less than 100 ppm, the specified exposure limit of EF; meanwhile, those of MB were higher than the exposure limit (1 ppm). Therefore, liquid EF can be used as a safer alternative to MB in phytosanitary treatments of grapes to control *P. comstocki*.

Keywords: ethyl formate; post-fumigation safety; pest control; sorption rate; mealybug

Citation: Lee, B.-H.; Hong, K.-J.; Park, M.-G. The Efficacy, Phytotoxicity, and Safety of Liquid Ethyl Formate Used to Control the Grape (Campbell Early) Quarantine Pest *Pseudococcus comstocki*. *Appl. Sci.* **2022**, *12*, 9769. https://doi.org/10.3390/app12199769

Academic Editor: Ana Arnaiz

Received: 7 September 2022
Accepted: 27 September 2022
Published: 28 September 2022

1. Introduction

Grapes are a major temperate fruit group worldwide, with a total trade of $11 billion in 2020, and the top exporters and importers are Chile ($1.18 billion) and the United States ($1.36 billion) [1]. In the same year, the Republic of Korea imported 70,932 t of grapes valued at $212 million, and 2108 t valued at $31 million were exported [2]. During the export or import of grapes at Korean ports, they are usually fumigated with methyl bromide (MB), as per the present phytosanitary treatment protocols [3]. However, MB is an ozone-depleting substance and a highly toxic pesticide, making it necessary to find an effective alternative [4,5]. MB also causes acute or chronic toxicity, posing a health risk for workers [6–9]. Exposure to concentrations above the permissible 1 ppm limit causes functional degradation of the nervous system in workers [10–12]. Therefore, the International Plant Protection Convention (IPPC), as a representative organization in quarantine worldwide, recommends replacing MB or reducing its use as a phytosanitary measure [13].

Ethyl formate (EF), known as a safer fumigant for workers and consumers, was designated a "generally recognized as safe" material status by the Food and Drug Administration and is also used in the manufacturing of food flavoring and no-residue commodities [14]. Furthermore, the time-weighted average threshold limit value (TLV for a 40-h workweek/8-h workdays) as the exposure limit of EF is 100 ppm, which is considerably-higher than the 1 ppm limit of MB TLV [15]. Hence, EF is safer and more controllable than MB in terms of maintaining exposure below the limit [16].

In Korea, approximately 41 t of MB was used to treat fruits in quarantine in 2021, which is almost 10 percent of the total MB consumption. A total of 258 t of EF was used for fruits during the same period. However, grapes cannot be fumigated with EF as there are no EF guidelines for grapes [17].

From 2007 to 2011, the major quarantine pests on grapes imported into Korea were mealybugs and scale insects [18]. During the export inspection of grapes, *Planococcus* sp. and insects of the family Pseudococcidae were found [19], with reports of restrictions on exports to China, Thailand, and Australia [20]. Recently, EF has been reported as a potential fumigant to replace MB due to its insecticidal effect on different quarantine pests found on perishable commodities, such as bananas, oranges, dry dates, blueberries, and even non-food commodities with less than 4 h of shelf-life [21–25]. EF was specifically effective in disinfesting mealybugs and scale insects and had an efficacy similar to that of MB [26–30]. However, it is difficult to adapt EF liquefied with CO_2 in high-pressured heavy steel cylinders for commercial use in terms of cost and handling. Hence, liquid EF in plastic casing could be a safer and more cost-effective alternative for liquefied EF in cylinders [21,22].

In this study, the feasibility of EF as an alternative to MB for mealybugs on grapes (Campbell Early, a major variety of grapes in Korea) was evaluated as follows: (1) The efficacy of EF in the egg and adult stage of *Pseudococcus comstocki* (Kuwana) was evaluated in small-scale laboratory trials. This has not been done in previous studies. (2) The grape sorption and permeability of EF were analyzed to determine the appropriate loading ratio and box types for the grapes. (3) Commercial scale trials using 10 m^3 containers were conducted using eggs of the Comstock mealybug (*P. comstocki*) at a tolerant stage. (4) Concentrations of EF desorbed from treated grapes were monitored under simulated storage conditons of a container and warehouse for 72 h after fumigation completion for comparison with MB. This process generally takes place before the delivery of grapes to consumers. (5) The effect of EF fumigation on the abscission rate, decay rate, sugar content, hardness, titratable acidity, and weight loss of grapes was assessed. Liquid EF was used with nitrogen gas in this study for safer and easier handling [21,22].

2. Materials and Methods

2.1. Fumigants

MB was provided by the Animal and Plant Quarantine Agency (APQA) in South Korea, and liquid EF (99%, Fumate^{TM}) was provided by Safefume Inc., Gangwon-do, Korea. For large-scale experiments (10 m^3), EF was applied using an EF vaporizer (SFM-1, Safefume Inc., Gangwon-do, Korea) and injected into the containers using nitrogen gas as a carrier.

2.2. Insects

P. comstocki specimens were collected from a plant nursery in Jinju, South Korea, and reared on potato sprouts at 25 ± 1 °C with 60% relative humidity (RH) and 16:8 h (light:dark) in a culture room at Gyeongsang National University in South Korea.

2.3. EF Concentration and Determination of the Ct (Concentration × Time) Product in Scaled-Up Fumigation

During fumigation, the concentration of EF was measured by a gas chromatography–flame ionization detector (GC–FID) after separation on an HP-5 Column (J&W Sci. 19091J-

413). The oven temperature was 150 °C, and the injector and detector temperatures were 240 °C. EF concentration was calculated on the basis of the peak area against external EF gas standards. The concentrations of EF were checked at timed intervals of 0.5-, 1.0-, 2.0-, and 4.0-h exposure periods in the fumigation chambers. The Ct product was calculated as described by Ren et al. [31]:

$$Ct = \sum \frac{(C_i + C_{i+1})(t_{i+1} - t_i)}{2}, \quad (1)$$

where C = concentration of the fumigant (mg/L), t = time of exposure (h), i = order of measurement, and Ct = the concentration × time product (g h/m^3).

2.4. Efficacy of EF against P. comstocki in Laboratory Trials

Pseudococcus comstocki was fumigated with EF in glass desiccators (Duran®, 6.9 L) with a mini fan placed at the bottom of each desiccator for air circulation. Insect samples were inoculated in breeding dishes and placed inside desiccators without grapes. After the desiccators were sealed using grease, liquid EF was injected using a gas-tight syringe (SGE Analytical Science, Melbourne, Australia) with a scheduled dose. The dosage range of EF was from 10 to 70.0 g/m^3. Desiccators with EF were placed in a fumigation room at 5 ± 1 °C for 4 h. Next, the desiccators were opened and aerated for 1 h in a fume hood. The treated insect samples were removed from the desiccators and reared under 25 ± 2 °C and 75 ± 5% RH. The mortality of the adults was determined at 72 h after fumigation treatment. The mortality of eggs was determined by checking for the emergence of nymphs 14 d post-fumigation. The number of mealybugs used was 50 adults and 50 eggs per replication. All experiments were replicated three times, including controls without fumigation.

2.5. EF Sorption under Different Loading Ratios of Grapes

EF fumigation of grapes (Campbell Early) with various loading ratios (10, 15, and 20% *w*/*v*) was performed in 1 m^3 metal chambers with a fan at the top of each chamber for inner air circulation. After the chambers were sealed, scheduled doses of 70.0, 80.0, and 90.0 g/m^3 of EF were applied at 5 ± 1 °C, and the chambers were fumigated for 4 h. Gas samples were obtained at timed intervals (0.1, 0.5, 1.0, 2.0, 3.0, and 4.0 h) by withdrawing them through an air pump into a gas bag (SKC Tedlar bag, 1 L). The concentrations of EF were monitored with GC–FID, as described above. The treatments were performed in triplicate.

2.6. Permeability of EF Gas through Packaging Film Used for Grapes

Grapes are packaged in shipping boxes wrapped with linear low-density polyethylene (LDPE) film with small holes. To check the permeability and distribution of EF gas through LDPE film, the EF concentration inside and outside of fruits packaged with LDPE was measured in 10 m^3 chambers, using gas sampling lines that were placed at two different locations (inside and outside the fruit packaging). The gas samples were obtained at timed intervals (0.1, 0.5, 1.0, 2.0, 3.0, and 4.0 h) by withdrawing the gas through an air pump into a gas bag (SKC Tedlar bag, 1 L). The concentrations of EF were monitored with GC–FID, as described above. Monitoring was performed in triplicate.

2.7. Large-Scale (10 m^3) Fumigation Using Liquid EF on P. comstocki

Large-scale trials were conducted in 10 m^3 chambers at an APQA site in Gimchon. Each fumigation chamber was fitted with a fan in the inner-top portion for air circulation. Insect eggs and adults were inoculated separately in each breeding dish and placed inside boxes in chambers with various loading ratios (10 and 20%, *w*/*v*) of grapes (Campbell Early) purchased from a local retailer (Gimchon). One female and five males were inoculated in the breeding dish to obtain eggs. We confirmed with a microscope that an average of 400 eggs were laid at 25 °C [32]. Then, the eggs were inserted into the boxes after the adults were removed from a mealybug colony with a small brush. The dose (70.0 g/m^3) of EF

used in this study was injected in the chambers using an EF vaporizer (SFM-1) at 5 ± 1 °C, and the samples were fumigated for 4 h. The emergent nymphs were investigated for 7–14 d post-fumigation. The corrected mortalities were recalculated based on Abbott's formula [Mort. of treatment − Mort. of control)/(1 − Mort. of control) × 100]. Untreated samples were used as the control.

2.8. Assessment of Phytotoxic Damage on Grapes Post-EF-Fumigation

The phytotoxicity of grapes (Campbell Early) was evaluated after 2, 4, and 6 weeks of cold storage (2 ± 1 °C) based on abscission rates, decay rates, hardness, sugar content, titratable acidity, and weight loss after 4 h of fumigation with 70 g/m^3 EF. A fruit firmness tester (53,205 Digital fruit firmness tester, TR Turoni, Forli, Italy), combined with an 8 mm steel plunger, was used to measure hardness The maximum values obtained during the measuring procedure were recorded. Hardness was monitored three times per fruit for a total of 10 fruits. The results were expressed in kgf. The soluble sugar content was monitored using a portable refractometer (Hand refractometer ATC-1E, Atago Co., Ltd., Tokyo, Japan). Whole fruits were ground using a tissue grinder and filtered with a funnel covered with filter paper. The filtered liquid (0.5 mL) was dropped on the refractometer, and the sugar content was monitored by observation through the scope of the refractometer. Ten fruits were measured, and the results were expressed as % soluble sugar. Titratable acidity was calculated using a fruit acidity meter (GMK-708, Jiwon Hitek, Seoul, Korea). Abscission was evaluated at the whole cluster level by detaching the cluster naturally during the storage period. Desiccation was measured using a procedure modified from Lichter et al. [33] and assessed on an index rating of 1 to 5. Desiccation ratings were as follows: 1 = rachis and pedicels green and full as at harvest; 2 = slight browning; 3 = browning of rachis and pedicels but no shriveling; 4 = browning and some shriveling; and 5 = both rachis and pedicels dry and brown.

2.9. Desorption of EF and MB from Fumigated Grapes

We designed this experiment based on current MB guidelines as well as the EF guidelines presented in this study for imported grapes in South Korea as follows: (1) Fumigation: Grapes were filled at a 20% (*w*/*v*) loading ratio in a shipping container and fumigated using MB (64 g m^{-3} for 2 h, at >5 °C) and EF (70 g m^{-3} for 4 h, at >5 °C); (2) Post-fumigation process until delivery to the end-user: <2 h ventilation with an open door using circulation fans. Further passive ventilation was conducted for 18–24 h with a small ventilation hole at the rear of the container under closed door conditions; in this case, circulation fans were automatically turned on and off to adjust the temperature (5 °C). The container was transported and the grapes were loaded to the cooling storage adjusted to 2–5 °C. Finally, the grapes were unpacked at the work place at temperatures of 10–15 °C. We monitored the levels of MB and EF released from the grapes during the post-fumigation periods. All experiments were conducted in mini-shipping containers (0.65 m^3) used to simulate the post-fumigation process. Park et al. [22] was used as a reference for the design of this experiment. The methodology to develop phytosanitary fumigation guidelines is illustrated in Figure 1.

2.10. Statistical Analysis

The dose–response effects of EF on *P. comstocki* were estimated through probit analysis [34]. The indices of EF toxicity measurements derived from these analyses were LCt_{50} and LCt_{99}, which are the median lethal concentrations that cause 50% and 99% mortality, respectively, of exposed *P. comstocki*. Differences in the sorption of EF on grapes depending on the loading ratio were analyzed using Duncan's multiple range test (SAS Institute 2009). Differences in the permeability of EF on grapes inside and outside the packaging film were analyzed using a *t*-test (SPSS ver. 23). Assessments of quality parameters such as weight loss, hardness, sugar content, and surface color change were calculated with Fisher's least significant difference (SAS Institute 2009) (LSD, $p = 0.05$).

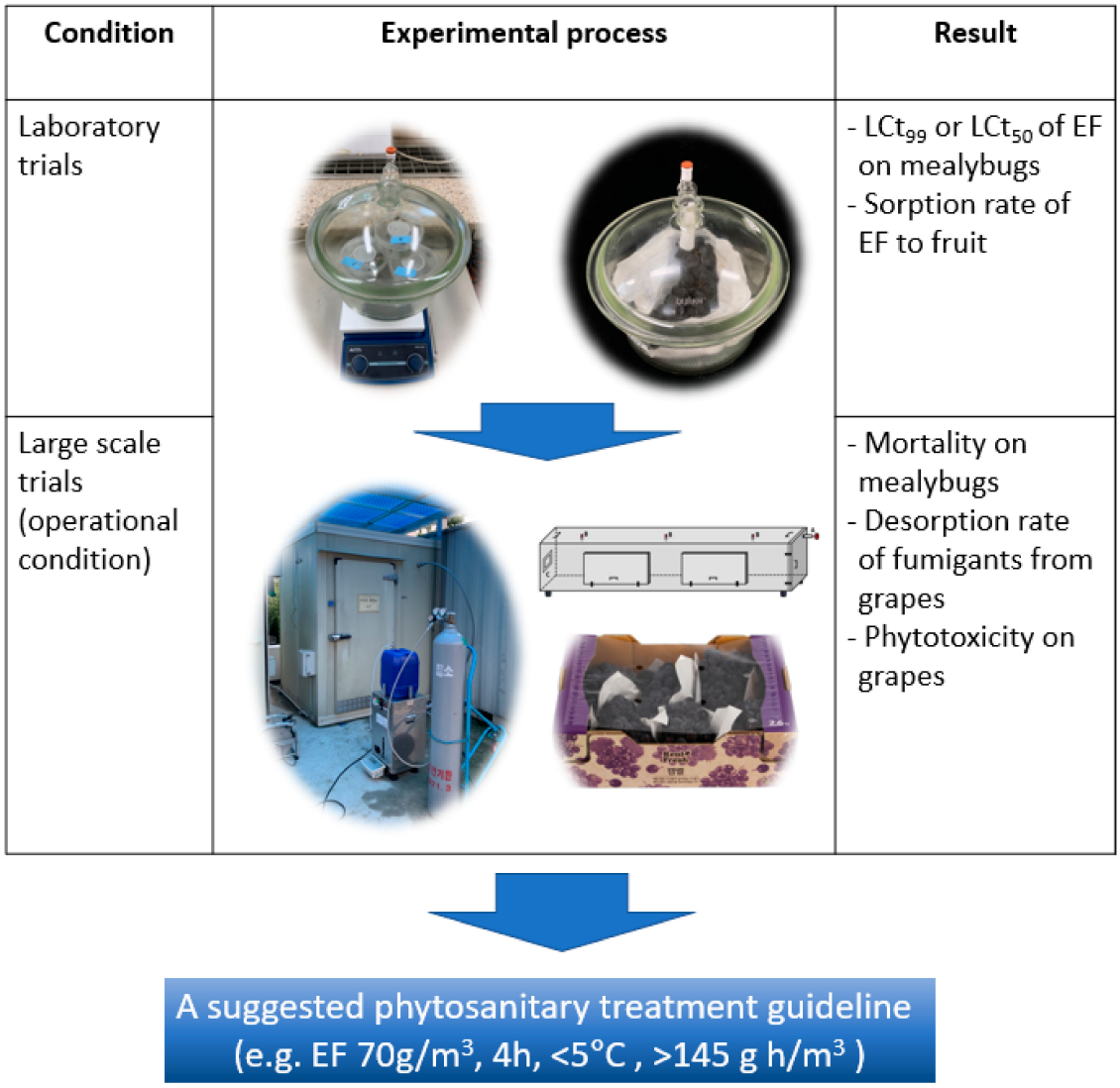

Figure 1. Illustrated methodology to develop a phytosanitary fumigation schedule and a suggested phytosantiry treatment guideline.

3. Results

3.1. Efficacy of EF against P. comstocki in Laboratory Trials

The efficacy of EF in different stages of *P. comstocki* is listed in Table 1. For adults, the LCt_{50} and LCt_{99} values of EF were 29.41 and 47.36 g h/m^3, respectively, at 5 °C. For eggs, the LCt_{50} and LCt_{99} values of EF were 52.0 and 145.85 g h/m^3 at 5 °C, respectively.

Table 1. Efficacy of fumigation with ethyl formate for 4 h during different developmental stages of *Pseudococcus comstocki* (Kuwana) in a small-scale test (6.9 L).

Insect	Stage	Temp (°C)	$LCt_{50\%}$ (95% CL, g h m^{-3})	$LCt_{99\%}$ (95% CL, g h m^{-3})	Slope ± SE	*df*	X^2
Pseudococcus comstocki	Adult	5	29.41 (28.19–30.60)	47.36 (43.88–52.70)	11.24 ± 1.05	6	24.46
	Egg		52.00 (48.72–55.33)	145.85 (127.96–172.73)	5.19 ± 0.37	10	22.43

3.2. Sorption of EF with Respect to Different Loading Ratios of Grapes

The Ct product of EF at various dosages showed significant differences depending on the loading ratios (Table 2). The dosages of EF were applied at 70 to 90 g/m^3 to achieve an LCt_{99} of 145.85 g h/m^3 on *P. comstocki*. It was confirmed that the Ct product corresponding to the EF LCt_{99} value of *P. comstocki* differed depending on the loading ratio (Figure 2). The higher the volume loading ratio, the lower the Ct product of EF.

Table 2. The Ct product of EF at various dosages depending on the loading ratios of grapes (10, 15, 20%).

Loading Ratio (*w*/*v* %)	70 g/m^3 (g h m^{-3})	80 g/m^3 (g h m^{-3})	90 g/m^3 (g h m^{-3})
10	148.33 ± 1.8 a [1]	167.91 ± 1.3 a	188.63 ± 1.0 a
15	125.65 ± 1.2 b	149.48 ± 2.1 b	161.66 ± 1.4 b
20	115.69 ± 1.1 c	133.08 ± 1.2 c	149.86 ± 1.2 c

Data are presented as the mean ± standard deviation. [1] The different letters indicate significant differences among different loading ratios of grapes at $p < 0.001$ based on Ducan's multiple range test (SAS Institute 2009).

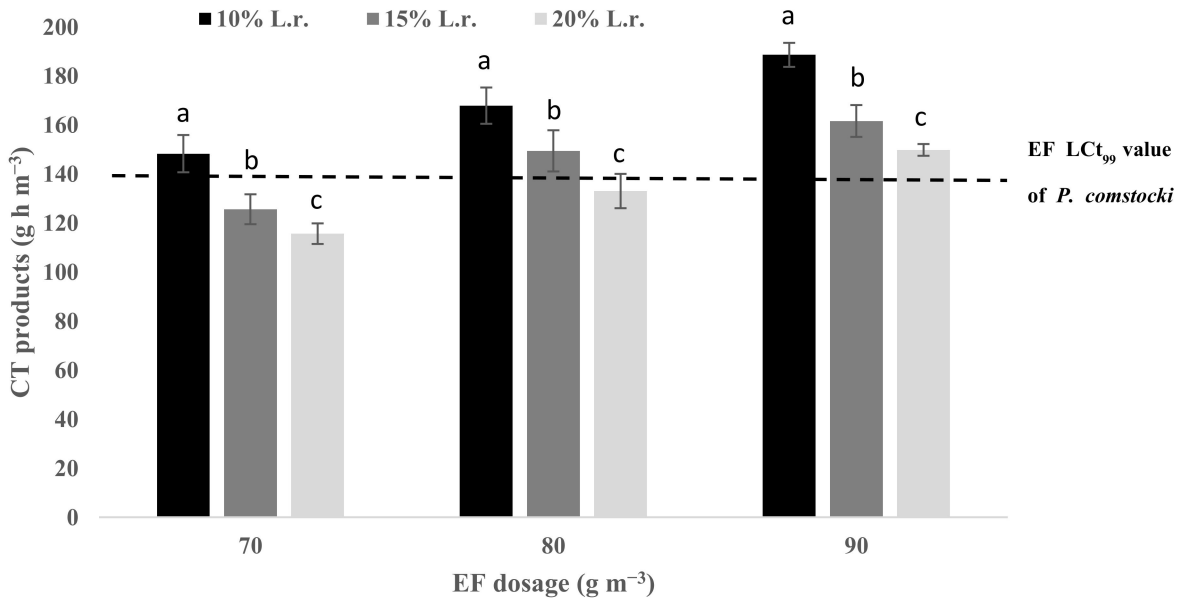

Figure 2. Ct product depending on the concentration and various loading ratios of grapes (Campbell Early) after fumigation with ethyl formate (EF) for 4 h at 5 °C. Lowercase letters above the bars (a, b, and c) show significant differences at $p < 0.05$ based on Ducan's multiple range test.

3.3. Permeability of EF Gas through the Grape Packaging Film

Wrapping the pallet with LDPE film containing small punched holes and lining it with a sulfur dioxide pad on the inside allows for the rapid cooling of grapes and greater air space and also prevents decay due to gray mold during storage [30]. No significant differences were observed between the Ct product inside and outside the packaging film ($t = 0.842$, $p = 0.447$), as shown in Figure 3.

3.4. Large-Scale (10 m^3) P. comstocki Fumigation with Liquid EF

Based on small laboratory trials for sorption and permeability, 70 g/m^3 of EF was applied with a loading ratio of 10 and 20% to achieve an LCt_{99} of 145.85 g h/m^3 on *P. comstocki* eggs. Large-scale trials are listed in Table 3. The concentrations of EF versus time intervals during each fumigation are shown in Figure 4. While 4-h fumigation was performed in individual treatments, the concentration of EF systematically decreased because of the adsorption of EF on grapes based on the weight. The concentration of EF was set at 30% of the initial dose at the end of the fumigation (Figure 3). At a load-

ing ratio of 20%, the Ct product of EF was 114.8 ± 12.36 g h/m^3 and the mortality of *P. comstocki* eggs was 96.12 ± 0.28%. At a loading ratio of 10%, the Ct product of EF was 148.2 ± 12.36 g h/m^3, and 100% mortality was achieved with *P. comstocki* eggs. The large number (>1000) of insects used in these experiments basically replicates the conditions of quarantine guidelines in South Korea [3]. This large-scale trial confirmed that the loading ratio of grapes affects the Ct product. In the ventilation process, the EF concentration in LDPE-packed grapes rapidly decreased. The EF concentration decreased to less than 100 ppm (TLV-TWA of EF) within 1 h; however, it required more than 2 h to decrease completely to less than the TLV-TWA due to the desorption of fumigants from fumigated grapes.

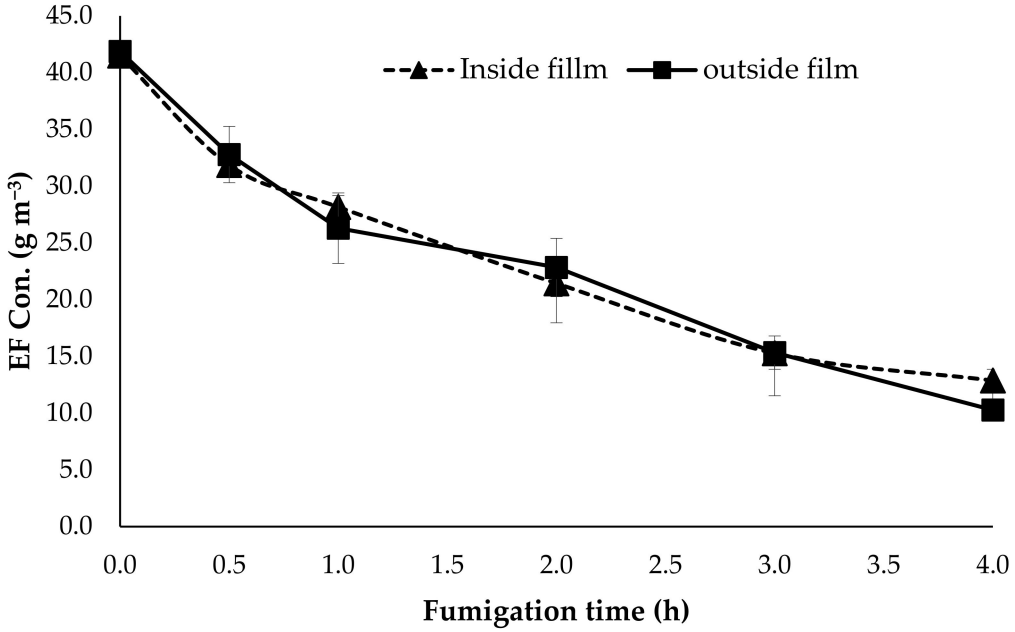

Figure 3. Concentration of EF inside and outside the packaging of grapes. The packaging consisted of low-density polyethylene film with small holes.

Table 3. Large-scale (10 m^3) liquid ethyl formate fumigation (70 g/m^3 for 4 h at 5 °C) of grapes (Campbell Early) to target *Pseudococcus comstocki* eggs.

CT Product (g h/m^3)	Loading Ratio of Grapes (*w*/*v*)	No. of Eggs Used	No. of Emerging Nymphs	Corrected Mortality * (%)
114.8 ± 12.36	20	1870	60	96.12 ± 0.28
148.2 ± 12.36	10	1800	0	100.00 ± 0.00
Untreated	-	1863	1822	2.50 ± 0.63

* Corrected Mortality (%) = (Mort. of Trt. − Mort of Cont.)/(1 − Mort. Cont.) × 100.

3.5. Evaluation of EF and MB Desorption from Fumigated Grapes

The concentration of fumigants in the mini-shipping containers (0.65 m^3) declined promptly from 325.6 to 60.9 ppm for MB and from 834.0 to 177.0 ppm for EF during the first 2 h of ventilation in the 72 h after fumigation completion (Figure 5). After the grapes were transferred to a cooled warehouse, the concentrations were monitored to be between 47.2 and 13.4 ppm for MB and between 47.0 and 13.3 ppm for EF. That is, while the MB concentrations were higher than their TLV-TWA of 1 ppm, the EF concentrations were less than their TLV-TWA of 100 ppm after the fumigated grapes were transferred to cold storage.

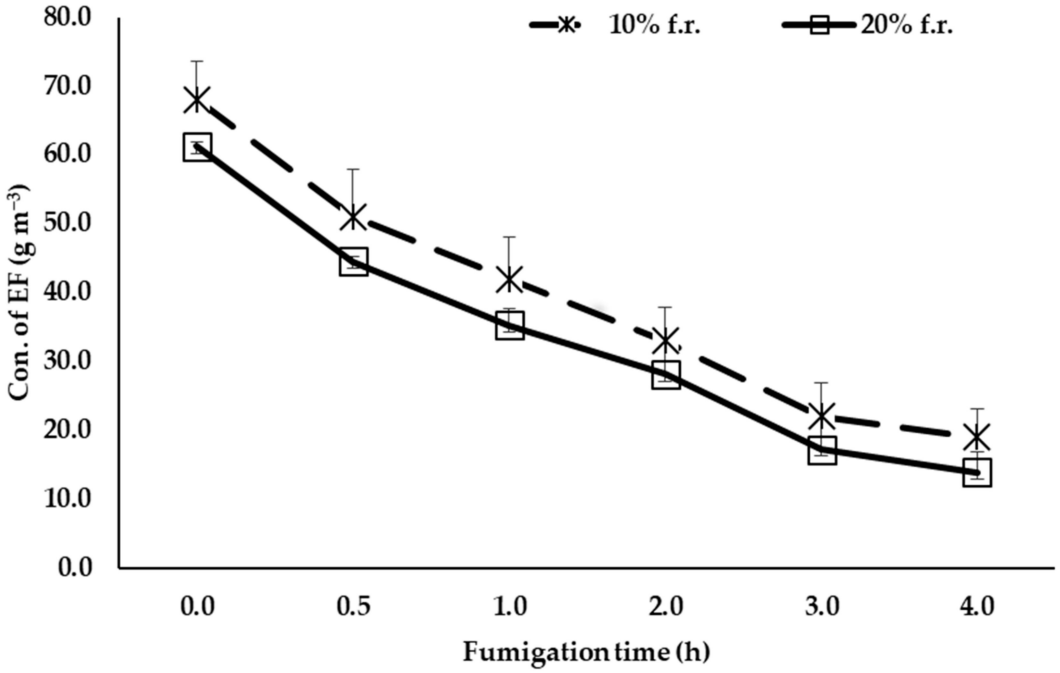

Figure 4. Concentration of EF during large-scale (10 m^3) fumigation.

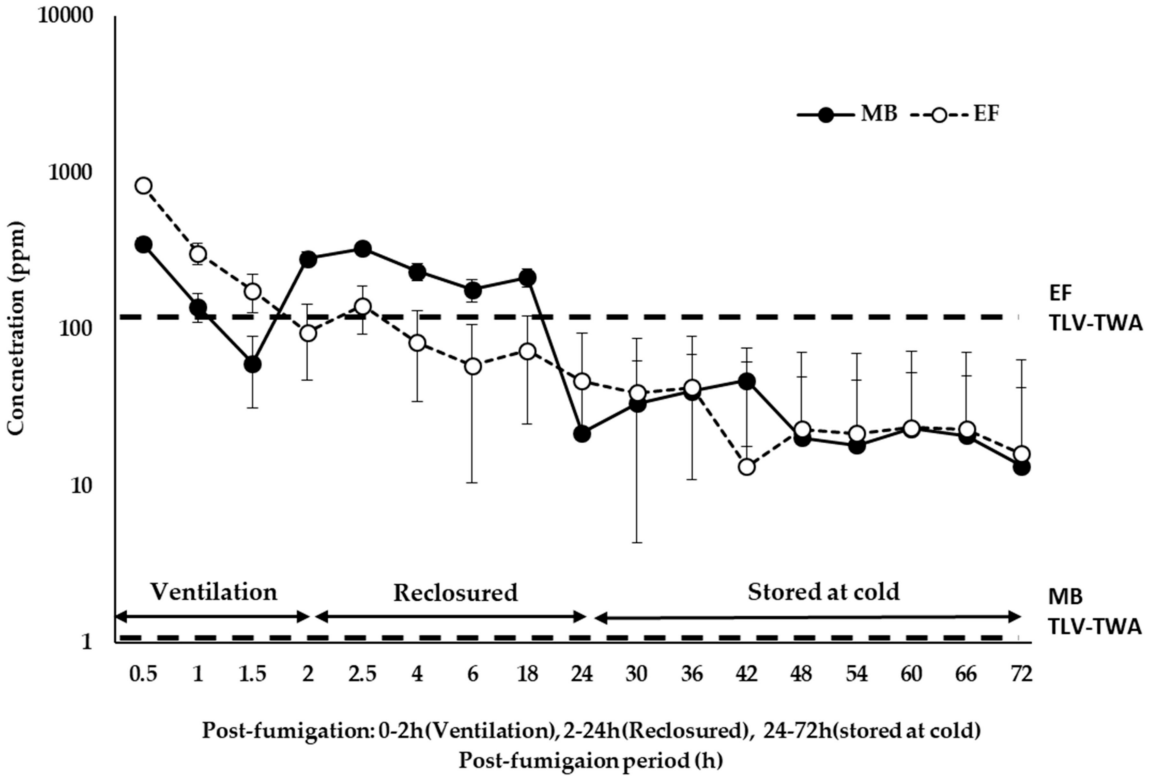

Figure 5. Methyl bromide (MB) and EF levels in the post-fumigation period (96 h) in the container and cooled warehouse. In Korea, the current permissible level of MB in the workplace is 1 ppm, and that of EF is 100 ppm.

3.6. Assessment of Phytotoxic Damages on Grapes Post-EF Fumigation

In these experiments, the abscission of grapes occurred after four weeks of storage at 2 ± 1 °C (Figure 6a). The mean abscission rates in untreated and EF-treated grapes six weeks post-fumigation were 1.21 and 2.21%, respectively. There was no significant difference

between the two treatments with respect to abscission rates (%) based on the LSD value (2.21, $p = 0.05$). The decay rate of grapes in cold storage was investigated only after four weeks (Figure 6b). The mean decay rates in untreated and EF-treated grapes six weeks post-fumigation were 0.07 and 0.17%, respectively. There was no significant difference between the two treatments with respect to the decay rate (%) based on LSD (0.38, $p = 0.05$). The mean titratable acidity in untreated and EF-treat grapes six weeks post-fumigation was 1.01 and 0.99%, respectively. There was no difference between the two treatments with respect to titratable acidity (g/L) (0.06, $p = 0.05$; Figure 6c). Figure 6d,e shows the changes in the sugar content and hardness, respectively. The sugar content (% brix) and hardness (kgf/cm^2) of grapes stored at 2 ± 1 °C were investigated six weeks post-fumigation. The mean sugar content and hardness of untreated and EF-treated grapes were 13.8 and 14.5% brix and 0.82 and 0.95 kgf/cm^2, respectively. There was no significant difference between the two treatments in terms of the sugar content (1.13, $p = 0.05$) and hardness (0.16, $p = 0.05$) based on the LSD values. Figure 6f shows the weight loss at six weeks of storage, post-fumigation. The mean weight loss of untreated and EF-treated grapes was 37.3 and 34.4%, respectively. There was no significant difference between the two treatments with respect to weight loss (%) based on the LSD (12.84, $p = 0.05$).

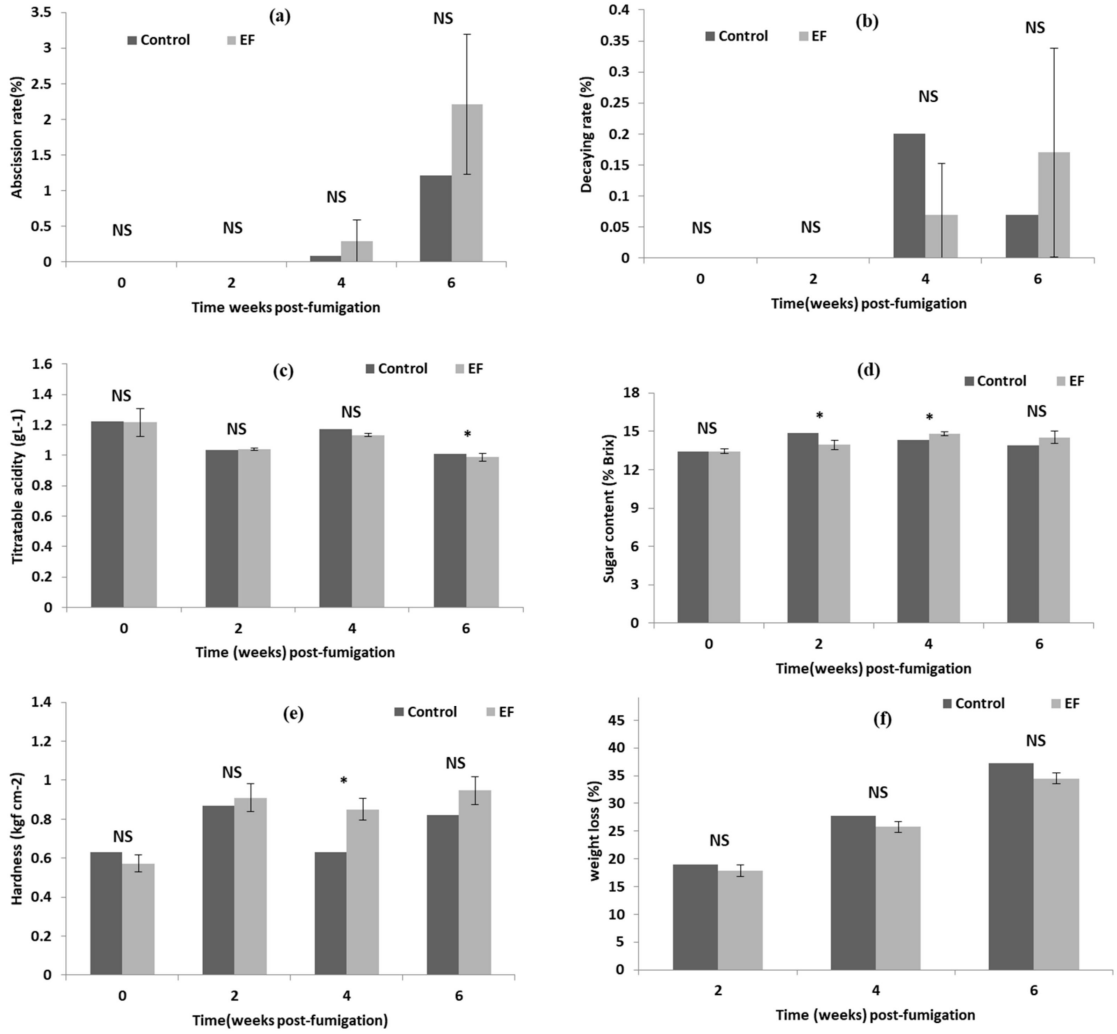

Figure 6. Phytotoxicity assessment of grapes (Campbell Early) post-fumigation, after treatment with 70 g/m^3 EF for 4 h at 5 °C. All data were analyzed using Fisher's least significant difference ($p = 0.05$). NS indicates no significant differences between the EF treatment and untreated control; * indicates significant difference between the EF treatment and untreated control.

4. Discussion

The applications of EF for controlling various pests such as scales [28], mealybugs [27,35], thrips [36], aphids [37], mites [38], and flies [39] have been studied. However, determining the LCt_{99} of EF for *P. comstocki* during quarantine was first done in this study. To develop the phytosanitary disinfection measures associated with new pests using fumigants, several tests are necessary, including efficacy data under laboratory and operational conditions [40]. Based on the LCt_{99} value of EF on *P. comstocki*, the sorption rate of EF to grapes, and large-scale tests, we revealed that *P. comstocki* was completely killed using EF at 70 g/m^3 for 4 h, 5 °C, 145.85 g h/m^3, with a loading ratio of 10%, without any phototoxic effects on grapes (Campbell Early).

The sorption rate of a commodity depends on the fumigants used. Therefore, it is critical for determining the dosage of fumigants for disinfection of target pests [21,31,41,42]. In the present study, a higher loading ratio (20%) showed more sorption on the grapes than a lower loading ratio (10%), similar to the results of a previous study involving the use of EF on fruits and nursery plants [30,43,44]. Nevertheless, the 10% loading ratio did not prevent the attainment of the LCt_{99} on the target pest in the large-scale (commercial) trials (Table 2). This suggests that a loading ratio of 10% on grapes is appropriate for commercial application.

Packing materials can affect the efficacy of the fumigant for pests by blocking access to the commodity. For example, the EF concentrations inside bags of bananas were considerably lower than those outside the bags [21]. The mortality of *Planococcus citri* eggs also decreased significantly under bagging. However, the EF concentration did not significantly differ inside and outside the packaging film in grapes (Figure 3), as the film is permeable enough to allow EF gas to penetrate. The current packaging of grapes was applied in the large-scale trials to evaluate commercial feasibility.

A few previous studies have investigated the efficacy of EF on mealybugs. The LCt_{99} of EF on *Planococcus citri* eggs at 8 °C was calculated to be 211 g h/m^3, whereas that on nymph and adults was 49.5 and 124.6 g h/m^3 [30]. For grape mealybugs (*Pseudococcus marituimus*), the EF LCt_{99} value on eggs and adults was estimated at 160.2 and 58.1 g h/m^3 at 24 °C [26]. In the present study, the LCt_{99} of EF was 145.85 g h/m^3 on *P. comstocki* eggs and 47.36 g h/m^3 on *P. comstocki* adults. This result is consistent with a previous study that showed that the eggs of mealybugs were more tolerant towards EF fumigation, compared with mealybugs at other stages. The Ct product in scale-up trials with a loading ratio of 20% was 114.8 g h/m^3, which is lower than the LCt_{99} on *P. comstocki* eggs; therefore, it is justified that not all *P. comstocki* specimens were killed. We confirmed that EF application at 145.85 g h/m^3 with a loading ratio of 10% can disinfect mealybugs completely.

The assessment of phytotoxic effects of EF on Thompson Seedless 30 d post-fumigation (5% EF for 2 h, 10% loading ratio, expected Ct product >100 g h/m^3) showed that there was no stem browning, bleaching, abscission rates, or decaying, except for small differences in rachis browning [26]. In the present study, similar conditions were provided during assessment except for temperature (25 °C), and the results were also similar in terms of physical quality factors such as abscission and decaying. We found that internal factors such as the sugar content and titratable acidity were not altered by EF fumigation. Recent reports on the use of EF treatment on sweet persimmons showed similar results with respect to the lack of phytotoxic damage and internal quality factors [45]. Thus, EF can be effectively used for fumigation without the concern of phytotoxic damage or alterations in internal quality factors.

This study had some limitations. Efficacy tests were not performed for all stages of mealybug or different types of pests. In addition, the effect of fumigation on different cultivars of grape was not explored. Future research should include the identification of the dose and Ct product for complete disinfection in all stages of mealybug and other pests of various grapes. Nevertheless, this study identified the Ct product that can control a newly identified pest, *P. comstocki*, on a new grape variety (Campbell early). The applicability of the results from this study, including the sorption and phytotoxicity of EF on grapes

using liquid EF in the field, was also confirmed. This liquid EF formulation will thus be more actively applied to improve the safety and effectiveness of fumigation as it overcomes the cost and handling limitations of EF liquefied with CO_2 in high-pressured heavy steel cylinders. Moreover, this study demonstrated the level of EF that is safe for workers, i.e., less than the TLV limit of 100 ppm, during ventilation periods after fumigation completion, whereas MB concentrations were always higher than the TLV limit of 1 ppm during the same period. The results of this study will help set a standard for phytosanitary treatment using EF and will further accelerate the replacement of MB with this safer formulation.

5. Conclusions

Under laboratory conditions, the LCt_{99} values of EF with respect to the adults and eggs of *P. comstocki* were 47.36 and 145.85 g h/m^3, respectively, at 5 °C. Under operational conditions (commercial/large-scale), applying EF at 70 g/m^3 for 4 h at 5 °C with a loading ratio of 10% proved effective towards *P. comstocki* control without having any phytotoxic effects on grapes. We also found that EF fumigation could be safer in the workplace because EF concentrations were maintained at less than the EF TLV of 100 ppm, whereas those of MB were more than the specified limit of 1 ppm. Therefore, liquid EF can be used as a technically feasible alternative to MB as a fumigant for grapes.

Author Contributions: B.-H.L. conceived and designed the manuscript. M.-G.P. wrote the first draft of this paper. B.-H.L. and M.-G.P. reviewed this paper. K.-J.H. contributed to data acquisition. B.-H.L. and K.-J.H. analyzed and interpreted the data. All authors have read and agreed to the published version of the manuscript.

Funding: This study was supported by the Animal and Plant Quarantine Agency (Z-1543086-2021-23-06). This paper was also supported by research funds for newly appointed professors of Jeonbuk National University in 2022.

Data Availability Statement: All data supporting the findings of this study are available from the corresponding authors upon reasonable request.

Acknowledgments: We would like to thank Mee Jang, an officer in pest control division of APQA, for providing the information on regulation and MB usage for quarantine purpose.

Conflicts of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

References

1. OEC. Grapes. Available online: https://oec.world/en/profile/hs/grapes (accessed on 24 August 2022).
2. KATI. Grape. Available online: https://m.kati.net/product/basisInfo.do?lcdCode=MD154 (accessed on 19 July 2022).
3. APQA. Phytosanitary Disinfestation Guidelines. Available online: http://www.qia.go.kr/plant/disinpect/listXdclbzWebAction.do (accessed on 10 June 2022).
4. UNEP. *Handbook for the Montreal Protocol on Substances that Deplete the Ozone Layer*, 12th ed.; United Nations Environment Programme: Nairobi, Kenya, 2018; p. 918.
5. MBTOC. *2010 Report of the Methyl Bromide Technical Options Committee*; 9789966200006; United Nations Environment Programme: Nairobi, Kenya, 2010; pp. 1–398.
6. Suwanlaong, K.; Phanthumchinda, K. Neurological Manifestation of Methyl Bromide Intoxication. *J. Med. Assoc. Thai.* **2008**, *91*, 421–426. [PubMed]
7. Baur, X.; Budnik, L.T.; Zhao, Z.; Bratveit, M.; Djurhuus, R.; Verschoor, L.; Rubino, F.M.; Colosio, C.; Jepsen, J.R. Health risks in international container and bulk cargo transport due to volatile toxic compounds. *J. Occup. Med. Toxicol.* **2015**, *10*, 19. [CrossRef] [PubMed]
8. Preisser, A.M.; Budnik, L.T.; Baur, X. Health effects due to fumigated freight containers and goods: How to detect, how to act. *Int. Marit. Health* **2012**, *63*, 133–139. [PubMed]
9. Deschamps, F.J.; Turpin, J.C. Methyl bromide intoxication during grain store fumigation. *Occup. Med.* **1996**, *46*, 89–90. [CrossRef]
10. Park, M.-G.; Choi, J.; Hong, Y.-S.; Park, C.G.; Kim, B.-G.; Lee, S.-Y.; Lim, H.-J.; Mo, H.-h.; Lim, E.; Cha, W. Negative effect of methyl bromide fumigation work on the central nervous system. *PLoS ONE* **2020**, *15*, e0236694. [CrossRef]
11. Park, M.-G.; Hong, Y.-S.; Park, C.G.; Gu, D.-C.; Mo, H.-H. Variations in methyl bromide concentration with distance and time during quarantine fumigation. *Environ. Monit. Assess.* **2021**, *193*, 397. [CrossRef]

12. Choi, J.; Hong, Y.-S.; Cha, W.; Mo, H.-H.; Park, M.-G. Heart rate variability analysis in workers exposed to methyl bromide as a quarantine treatment. *J. Occup. Environ. Med.* **2021**, *63*, e32. [CrossRef]
13. IPPC. *Reccomendation on: Replacement or Reduction of the Use of Methyl bromide as a Phytosanitary Measure*; FAO: Rome, Italy, 2008; pp. 1–8.
14. Haritos, V.; Dojchinov, G. Cytochrome c oxidase inhibition in the rice weevil *Sitophilus oryzae* (L.) by formate, the toxic metabolite of volatile alkyl formates. *Comp. Biochem. Physiol. Part C Toxicol. Pharmacol.* **2003**, *136*, 135–143. [CrossRef]
15. American Conference of Governmental Industrial Hygienists. *2005 TLVs and BEIs*; American Conference of Governmental Industrial Hygienists: Cincinnati, OH, USA, 2005.
16. Yang, J.O.; Kim, H.M.; Park, Y.J.; Park, M.G.; Ren, Y.L.; Lee, B.H. New Quarantine Trials for Using Liquid Ethyl Formate with Nitrogen Application on Imported Citrus Fruits_Cost Effectiveness and Worker Safety. In Proceedings of the International Symposium and Annual Meeting of the Korean Society of Pesticide Science (KSPS), Yeosu, Korea, 6–7 April 2017; p. 245.
17. Apqa. *Regulations for Phytosanitray Treatment of Import and Export Plant*; Apqa: Gimcheon, Korea, 2019.
18. Suh, S.-J.; Yu, H.M.; Hong, K.-J. List of Intercepted Scale Insects at Korean Ports of Entry and Potential Invasive Species of Scale Insects to Korea (Hemiptera: Coccoidea). *Korean J. Appl. Entomol.* **2013**, *52*, 141–160. [CrossRef]
19. APQA. Available online: http://www.qia.go.kr/animal/prevent/listwebQiaCom.do?type=3_52jynb&clear=1 (accessed on 20 July 2022).
20. APQA. Available online: http://www.qia.go.kr/bbs/lawAnn/listLawWebAction.do?type=0&key=%ED%8F%AC%EB%8F%84&firstkorkey=&nextkorkey=&lawQuery.jbbk_fl=all&pager.maxPageItems=10&pager.offset=0 (accessed on 20 July 2022).
21. Park, M.-G.; Park, C.-G.; Yang, J.-O.; Kim, G.-H.; Ren, Y.; Lee, B.-H.; Cha, D.H. Ethyl Formate as a Methyl Bromide Alternative for Phytosanitary Disinfestation of Imported Banana in Korea with Logistical Considerations. *J. Econ. Entomol.* **2020**, *113*, 1711–1717. [CrossRef]
22. Park, M.-G.; Lee, B.-H.; Yang, J.-O.; Kim, B.-S.; Roh, G.H.; Kendra, P.E.; Cha, D.H. Ethyl Formate as a Methyl Bromide Alternative for Fumigation of Citrus: Efficacy, Fruit Quality, and Workplace Safety. *J. Econ. Entomol.* **2021**, *114*, 2290–2296. [CrossRef]
23. Bessi, H.; Bellagha, S.; Lebdi, K.G.; Bikoba, V.; Mitcham, E.J. Ethyl formate fumigation of dry and semidry date fruits: Experimental kinetics, modeling, and lethal effect on carob moth. *J. Econ. Entomol.* **2015**, *108*, 993–999. [CrossRef]
24. Lee, B.-H.; Park, C.-G.; Park, M.-G.; Roh, G.-H.; Kim, D.; Riddick, E.W.; Chen, J.; Cha, D.H. Ethyl formate fumigation for the disinfestation of red imported fire ants Solenopsis invicta Buren. *J. Asia-Pac. Entomol.* **2019**, *22*, 838–840. [CrossRef]
25. Kwon, T.H.; Park, C.G.; Lee, B.-H.; Zarders, D.R.; Roh, G.H.; Kendra, P.E.; Cha, D.H. Ethyl formate fumigation and ethyl formate plus cold treatment combination as potential phytosanitary quarantine treatments of Drosophila suzukii in blueberries. *J. Asia-Pac. Entomol.* **2021**, *24*, 129–135. [CrossRef]
26. Simpson, T.; Bikoba, V.; Tipping, C.; Mitcham, E. Ethyl formate as a postharvest fumigant for selected pests of table grapes. *J. Econ. Entomol.* **2007**, *100*, 1084–1090. [CrossRef]
27. Misumi, T.; Ogawa, N.; Yamada, K.; Shukuya, T. 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. *Res. Bullutine Plant Prot. Jpn.* **2013**, *49*, 1–9.
28. Pupin, F.; Bikoba, V.; Biasi, W.B.; Pedroso, G.M.; Ouyang, Y.; Grafton-Cardwell, E.E.; Mitcham, E.J. Postharvest Control of Western Flower Thrips (Thysanoptera: Thripidae) and California Red Scale (Hemiptera: Diaspididae) With Ethyl Formate and Its Impact on Citrus Fruit Quality. *J. Econ. Entomol.* **2014**, *106*, 2341–2348. [CrossRef]
29. Jamieson, L.E.; Griffin, M.J.; Page-Weir, N.E.M.; Chhagan, A.; Redpath, S.P.; Connolly, P.G. Developing ethyl formate treatment for disinfesting pipfruit. *N. Z. Plant Prot.* **2014**, *67*, 96–102. [CrossRef]
30. Yang, J.; Park, Y.; Hyun, I.-H.; Kim, G.-H.; Kim, B.-S.; Lee, B.-H.; Ren, Y. A combination treatment using ethyl formate and phosphine to control Planococcus citri (Hemiptera: Pseudococcidae) on pineapples. *J. Econ. Entomol.* **2016**, *109*, 2355–2363. [CrossRef]
31. Ren, Y.L.; Lee, B.H.; Padovan, B. Penetration of methyl bromide, sulfuryl fluoride, ethanedinitrile and phosphine into timber blocks and the sorption rate of the fumigants. *J. Stored Prod. Res.* **2011**, *47*, 63–68. [CrossRef]
32. Cho, M.-K.; Kwon, H.R.; Yu, Y.M.; Youn, Y.N. Development by Temperatures and Copulation Flight of Comstock mealybug, *Pseudococcus comstocki*. *Korean J. Appl. Entomol.* **2018**, *57*, 105–115.
33. Lichter, A.; Zutahy, Y.; Kaplunov, T.; Lurie, S. Evaluation of table grape storage in boxes with sulfur dioxide-releasing pads with either an internal plastic liner or external wrap. *HortTechnology* **2008**, *18*, 206–214. [CrossRef]
34. Finney, D.J. *Probit Analysis*, 3rd ed.; Cambridge University Press: Cambridge, UK, 1971.
35. Kim, K.; Park, M.-G.; Lee, Y.H.; Jeon, H.-J.; Kwon, T.H.; Kim, C.; Park, J.; Lee, B.-H.; Yang, J.O.; Lee, S.-E. Synergistic Effects and Toxic Mechanism of Phosphine with Ethyl Formate against Citrus Mealybug (*Planococcus citri*). *Appl. Sci.* **2021**, *11*, 9877. [CrossRef]
36. Bikoba, V.; Pupin, F.; Biasi, W.; Rutaganira, F.; Mitcham, E. Use of Ethyl Formate Fumigation to Control Adult Bean Thrips in Navel Oranges. *J. Econ. Entomol.* **2019**, *112*, 591–596. [CrossRef]
37. Lee, B.H.; Kim, H.M.; Kim, B.S.; Yang, J.O.; Moon, Y.M.; Ren, Y. Evaluation of the synergistic effect between ethyl formate and phospine for control of Aphis gossypii (Homoptera: Aphididae). *J. Econ. Entomol.* **2016**, *109*, 143–147. [CrossRef]
38. Kim, K.; Lee, Y.H.; Kim, G.; Lee, B.-H.; Yang, J.-O.; Lee, S.-E. Ethyl formate and phosphine fumigations on the two-spotted spider mite, Tetranychus urticae and their biochemical responses. *Appl. Biol. Chem.* **2019**, *62*, 50. [CrossRef]

39. Kwon, T.H.; Kim, D.B.; Kim, K.W.; Park, M.G.; Roh, G.H.; Lee, B.H. Scaled-up ethyl formate fumigation to replace methyl bromide on traded mushroom to disinfest mushroom fly (*Lycoriella mali*). *Appl. Biol. Chem.* **2021**, *64*, 64. [CrossRef]
40. IPPC. *Phytosanitary Treatments for Regulated Pests*; ISPM 28; FAO/IPPC: Rome, Italy, 2007; pp. 1–11.
41. Bond, E.J. *Manual of Fumigation for Insect Control*, 2nd ed.; FAO: Rome, Italy, 1984; pp. 134–135.
42. Park, M.G.; Ren, Y.; Lee, B.H. Preliminary study to evaluate ethanedinitrile (C2 N2) for quarantine treatment of four wood destroying pests. *Pest Manag. Sci.* **2021**, *77*, 5213–5219. [CrossRef]
43. Kyung, Y.; Kim, H.K.; Cho, S.W.; Kim, B.-S.; Yang, J.-O.; Koo, H.-N.; Kim, G.-H. Comparison of the efficacy and phytotoxicity of phosphine and ethyl formate for controlling *Pseudococcus longispinus* (Hemiptera: Pseudococcidae) and *Pseudococcus orchidicola* on imported foliage nursery plants. *J. Econ. Entomol.* **2019**, *112*, 2149–2156. [CrossRef]
44. Lee, J.S.; Kim, H.K.; Kyung, Y.; Park, G.-H.; Lee, B.-H.; Yang, J.-O.; Koo, H.-N.; Kim, G.-H. Fumigation activity of ethyl formate and phosphine against *Tetranychus urticae* (Acari: Tetranychidae) on imported sweet pumpkin. *J. Econ. Entomol.* **2018**, *111*, 1625–1632. [CrossRef]
45. Lee, B.-H.; Park, C.-G.; Yang, J.-O.; Kim, K.; Lee, S.-E. Concurrent application of ethyl formate and 1-methylcyclopropene to control *Tetranychus urticae* on exported sweet persimmons (*Diospyros kaki* Thunb. 'Fuyu'). *Entomol. Res.* **2018**, *48*, 198–203. [CrossRef]