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Efficacy of propylene oxide as a postharvest fumigant for fresh citrus pest disinfestation and its impact on citrus fruit quality

S G Gautam[1]*, David Obenland[1], Spencer Walse[2], E Grafton-Cardwell[2]

[1]Department of Entomology, University of California, Riverside, CA 92521, USA.
[2]USDA, Agricultural Research Service, San Joaquin Valley Agricultural Sciences Center, Parlier, CA 93648, USA.
*Corresponding author's email: sangautam@ucanr.edu

Abstract

Arthropods found on citrus at the time of harvest but not present in countries where the fruit is exported must be controlled to prevent their accidental introduction. Regulatory and technical logistics related to methyl bromide, a postharvest fumigant long relied upon by the California citrus industry, is upon the discretion of importing countries. We evaluated the toxicity of fumigant propylene oxide (PPO) in combination with CO_2 on California red scale (*Aonidiella aurantii* Maskell), Fuller rose beetle [(*Naupacts godmanni* (Crotch)], bean thrips [*Caliothrips fasciatus* (Pergande)]; and three mite species, namely, *Brevipalpus californicus* (Banks), *Brevipalpus lewisi* McGregor, and *Lorryia formosa* Cooreman Caged. Arthropod specimens were exposed to different concentrations of PPO during a 2 h fumigation at 15.6°C to generate dose-response mortality. Results showed that propylene oxide was toxic to all species tested but response to treatments varied greatly among species. Fuller rose beetle egg control was not achieved during the 2 h fumigation schedule; therefore, 24 h fumigation exposure was tested. Among the species tested during 2 h exposure, bean thrips adults and *B. californicus* adults were the most susceptible and the most tolerant species, respectively, and required 27.8 and 151.1 mg/hL to achieve 99% mortality. Propylene oxide at 40 mg/L did not cause phytotoxic effects on mandarins and navel oranges, but exposure to 112 mg/L resulted in higher level of decay of navel oranges. These data showed that PPO was toxic to arthropods of export concern to the citrus industry. Future research will aim at studying the time-concentration relationship, effects of fumigation schedule on fruit quality, and residues. Potential of PPO as promising fumigant alternative to methyl bromide for the California citrus industry and future studies were discussed.

Keywords: Postharvest fumigation, Export, Citrus

Introduction

California is the leading producer of fresh citrus in the U.S. with annual production exceeding 3.7 million tonnes with a farmgate value of $3.4 bn (NASS 2019). Export markets contribute nearly 1/3rd of the total revenue (CCQC 2020). Postharvest control of the arthropods that may be present

on citrus at the time of harvest but not present in importing countries is one of the challenges the citrus industry must overcome for retention and expansion of the export markets. As such, one fumigant methyl bromide (CH_3Br abbreviated as MeBr) had dominated the industry as a nearly ideal postharvest fumigant choice. However, regulatory concerns regarding the ozone depleting nature of this chemical have led to its limited use for the Quarantine and Pre-Shipment (QPS) treatments. Even QPS MeBr is on borrowed time and at the discretion of importing countries and there are concerns about future availability and cost of this fumigant (Walse, 2017). Subsequently, postharvest treatments to replace MeBr must be developed to provide biological safeguard against pests of export concern.

Current method of mitigation of export pests is system's approach or MeBr fumigation is used for all citrus arriving South Korea (Pupin et al., 2013). Many fumigants have been studied as a MeBr replacement for a phytosanitary treatment (Pupin et al., 2013; Walse, 2017; Bikoba et al., 2019). Phosphine is the only other fumigant alternative to MeBr but requires ≥ 6-fold longer exposure to control pests compared to 2 h MeBr treatments. In this study we evaluated the toxicity of propylene oxide in combination of carbon dioxide on export concern species.

Propylene oxide (PPO) is an FDA approved sterilant to kill bacteria, mold, and yeast contamination on processed spices, cocoa, and processed nutmeats except peanuts (Griffith, 1999). It is a favored treatment method for pasteurizing almonds. The major disadvantage, its flammability, can be overcome by fumigating under vacuum or in combination with CO_2 (Navarro et al., 2004). Propoxide, 8:92 (wt: wt) combination of PPO and CO_2 is a registered fumigant for controlling stored-product insect pests in the United States.

The overarching goal of this study was to determine the potential of PPO as a postharvest treatment for fresh fruits. First, we established efficacy data for citrus pests of export concern. Second, we determined PPO sorption by citrus at different chamber capacities. Next, we also evaluated the phytotoxic effects of PPO on navels and mandarins. The potential of PPO as a postharvest fumigant for fresh produce as well as the need for future studies were discussed.

Materials and methods

Insects and rearing

California red scale, *Aonidiella aurantii* Maskell (Hemiptera: Diaspididae), used for the experiment, was initiated from insects collected from an insecticide free backyard lemon tree, *Citrus limon* L., in Porterville, CA in 1991 and maintained on green lemons at 24 ± 3°C and 12:12 (L:D). Fuller rose beetle, *Naupactus godmanni* (Crotch) (Coleoptera: Curculionidae), eggs were obtained from adults collected from citrus orchards near Parlier, California. Bean thrips, *Caliothrips fasciatus* (Pergande) (Thysanoptera: Thripidae), adults for the experiments were collected from an organic alfalfa at KARE. *Brevipalpus californicus* (Banks) (Arachnida: Acari: Tenuipalpidae) and *Brevipalpus lewisi* McGregor (Arachnida: Acari: Tenuipalpidae) were obtained from laboratory colonies maintained on Valencia oranges at 26 ± 1°C and 60 ± 5% RH. *Lorryia formosa* for the experiment was obtained from laboratory colony maintained on rough lemon plants. Experimental setups for fumigation were prepared as shown in Fig. 1.

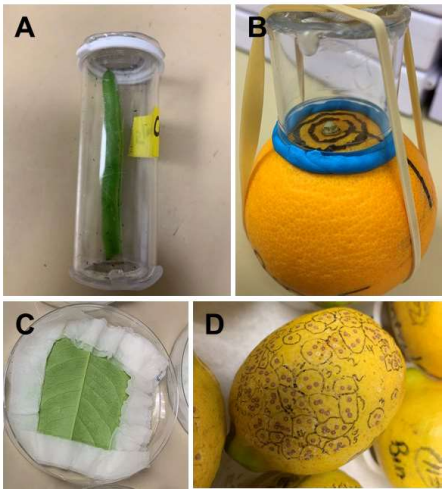

Fig. 1. Experimental setup for fumigation to control bean thrips (A), *B. californicus* (B), *L. formosa* (C), and California red scale (D).

Fumigation

Laboratory-scale exploratory fumigations were conducted in 28.3-L Labconco® vacuum desiccators (Labconco® # 5530000) (referred to as chambers hereafter) housed in a 17 m^3 environmental room with programmable temperature and humidity control at the SJVASC, USDA-ARS, Parlier, CA.

Pure liquid propylene oxide (≥ 99.5%; #82320 Aldrich, Sigma-Aldrich Co. St. Louis, MO) was drawn from a 50-mL conical flask with a glass stopper under a certified fume hood using a 0.1 mL gas syringe (Hamilton, Foxboro/Analabs, North Haven, CT) or 1-, 2-, or 5- mL gas syringes (Precision syringe, Dynatech Precision Sampling, Baton Rouge, LA) befitting the applied dosages and injected through a modified septum onto a petri dish lined with filter paper. Carbon dioxide was drawn from a cylinder (13.4 x 45.7 cm) containing compressed CO_2 using 500-, 1,000-, or 1,500-mL gas syringes or weighted out using a scale and injected through stopcock before injecting PPO.

The range of concentrations of propylene oxide tested for California red scale, fuller rose beetle, bean thrips, *B. californicus*, *B. lewisi*, and *L. formosa* were 4-32, 16-80, 4-32, 4-96, 2-96, and 4-96 mg/L, respectively. For each species, at least seven different concentrations within the range were tested. Each treatment was replicated at least 3-5 times. Prior to injecting liquid PPO into the chambers, a pressure of 150 mmHg was created to ensure space for CO_2 and PPO volatilization while preventing the development of positive pressure in the chamber. To simulate 8:92 mixture of PPO:CO2, volume of CO_2 equivalent to that which would have been administered into each chamber in a scenario where the gas introduced was premixed in the same ratio. After the PPO volatilized (within 2-5 min), the pressure inside the chamber was normalized by permitting air in from the stopcock. This marked the start of a 2 h (or 24 h in case of Fuller rose beetle) fumigation exposure period.

Concentrations of PPO inside each chamber were determined at the start (after the pressure was normalized) and at end of the experiment. Gas samples were taken using a 100 mL gas syringe (Becton, Dickinson and Co., Franklin Lakes, NJ) to withdraw 40 mL of gas through the stopcock. Concentrations of PPO in fumigation chambers were quantitatively monitored and analyzed using a gas chromatograph (GC) (Model 3800, Varian Inc., Walnut Creek, CA). Doses of PPO expressed as concentration × time (CT) product (mg h/L) were calculated by the method of Bond (1984). The temperature set point for the exploratory fumigations was 15.6°C (60°F).

For all experiments, PPO concentrations in the chamber headspace were measured using a gas chromatograph (GC) (Model 3800, Varian Inc., Walnut Creek, CA) equipped with a 1 cc gas sampling loop and a flame ionization detector (FID).

Mortality evaluation and effects of PPO on navel oranges and mandarins

After fumigation, adult mites, thrips, California red scale, and Fuller rose beetle eggs were incubated and mortality evaluations were conducted 3, 3, 14, and 28 d, respectively. All mortality assessments were conducted using a stereoscope and survivors were diagnosed based on their ability to move one body length when prodded (adults), except CRS gravid females which were determined visually (discoloration). Eggs were evaluated as hatched or unhatched.

For determining the effects of PPO on fruit quality, commercially packed, export grade navel oranges and mandarins sourced from local packinghouses were fumigated. Two different concentrations of PPO, 40 and 112 mg/L during a 2 h fumigation at 15.6°C were tested. Fruit was repacked into modified cardboard boxes (to fit inside the fumigation chamber) for fumigation. Fumigation was conducted in 9 ft^3 stainless steel chambers and the fumigation procedure was the same as that described above. Following fumigation, fruit was stored for 3 wk at 2.8°C (37°F) to simulate sea shipment plus 1 wk at 20°C (68°F) to simulate handling and shelf life. Evaluations were conducted 4 wk after storage. Fruit was checked weekly during 4 wk storage (3 wk at 2.8°C and 1 wk at 20°C) and those showing symptoms of decay were removed. Final evaluations were conducted at the end of the 4 wk storage period for peel color, overall external appearance, decay, stem end rot, firmness, and juice content.

Data Analysis

Dose response data for each species tested were subjected to probit analysis using PoloPlus (LeOra Software, Petaluma, CA; LeOra Software, 2005) to estimate lethal concentrations to kill 50, 95, and 99% of the individuals and their 95% confidence intervals and to predict probit 9 values. For determining the phytotoxic effects of PPO on navel oranges and mandarins, data were analyzed separately for percentage decay, firmness, fruit color, and juice content using one-way ANOVA analysis (Statgraphics, 2018).

Results and discussion

Propylene Oxide Toxicity

Propylene oxide was toxic to all species tested at 15.6°C for 2 h; however, the tested species responded differently to propylene oxide (Table 1).

The number of specimens tested, slope of regression estimate, estimated CT exposures to cause 50, 95, 99, and 99.9968% mortalities and the upper (UL) and lower limits (LL) of the 95% confidence limits, chi-square, degrees of freedom, and regression heterogeneity (H) are presented in Table 1.

Among the species tested, bean thrips were the most susceptible species and required 27.3 mgh/L (13.6 mg/L) to kill 99% of the individuals. Fuller rose beetle was the most difficult to kill, requiring 1,694.5 mgh/L for 99% mortality. California red scale required 51.4 mgh/L.

Table 1. Probit analyses parameters of citrus export concern species following exposure to propylene oxide at 15.6°C. Lethal exposure values (LEs) are expressed as mgh/L

Species	Number tested	Slope ± SE	LE_{50} (95% CI)	LE_{99} (95% CI)	P_9 (95% CI)	X^2 (df) [H*]
California red scale	1,939	12.8 ± 0.81	33.8 (32.3-35.2)	51.4 (47.9-57.4)	69.5 (61.3-84.9)	53.1(17) [3.1]
Bean thrips	1,033	12.7 ± 0.95	18.3 (17.6-18.8)	27.8 (26.3-30.3)	37.7 (33.6-43.9)	60.5(38) [1.6]
Fuller rose beetle eggs*	6,542	4.02 ± 0.13	447.2 (374.0-513.3)	1,694.5 (1,322.5-2,529.8)	4,418.8 (2,869.29,116.2)	591.6(40) [14.8]
<u>*Mites*</u>						
B. californicus	2,632	2.8 ± 0.11	22.7 (19.1-26.3)	151.1 (113.6-225.9)	590.3 (360.1-1,206.0)	131.7(21) [6.3]
B. lewisi	2,513	9.3 ± 0.43	22.1 (20.9-23.1)	39.2 (36.3-43.6)	62.6 (51.1-92.7)	77.6(21) [3.7]
L. formosa	800	3.4 ± 0.27	15.7 (13.6-17.7)	77.1 (63.9-98.5)	242.4 (175.0-375.9)	21.1(30) [0.7]

*Heterogeneity factor
**Exposure period for Fuller rose beetle was 24 h.

For the mites, 99% mortality was achieved in the range of 31.7 to 151.1 mgh/L. The most tolerant species was *B. californicus*. Efficacy of PPO for controlling insect pests during short exposures such as 2 or 4 h, has been reported for many stored-product species (Isikber et al., 2006; Gautam, 2013). Prior to our study, the effects of PPO on arthropod pests infesting fresh fruit produce had not been explored. Among other postharvest fumigants being studied as a MeBr alternatives, phosphine and ethyl formate are effective (Bikoba et al., 2019; Walse et al., 2016). However, phosphine, the only other fumigant registered for use in citrus, requires longer exposures, ca. ≥ 48 h to control mites.

Tolerance of PPO by navel oranges and mandarins

Two concentrations of PPO, 40 mg/L (effective on California red scale) and 112 mg/L (effective on mites), were well tolerated by navel oranges and mandarins (Figs. 2 and 3). Exposure to 40 mg/L did not affect firmness, brix, acidity, and percentage decay in both varieties compared to control. At higher concentration, 112 mg/L, percentage decay in navels was significantly higher, but not on mandarins. At 112 mg/L, the button end mandarin was dry and darker in color compared to control. There were no significant differences between brix and acidity between treated and the control fruit suggesting no likely effect on fruit flavor as a result of exposure to PPO. Effects of PPO on fruit quality parameters have not been explored prior to this report. Effects of other fumigants such as ethyl formate, methyl bromide and phosphine on citrus fruit quality have been reported by other researchers (Pupin et al., 2013; Arpaia et al., 2021). Fruit tolerance likely varies with commodity, dose, and time after fumigation (Pupin et al., 2013). Our results on fruit sourced from three different packinghouses suggests that recommended 40 mg/L doses of PPO have minimal effects on citrus.

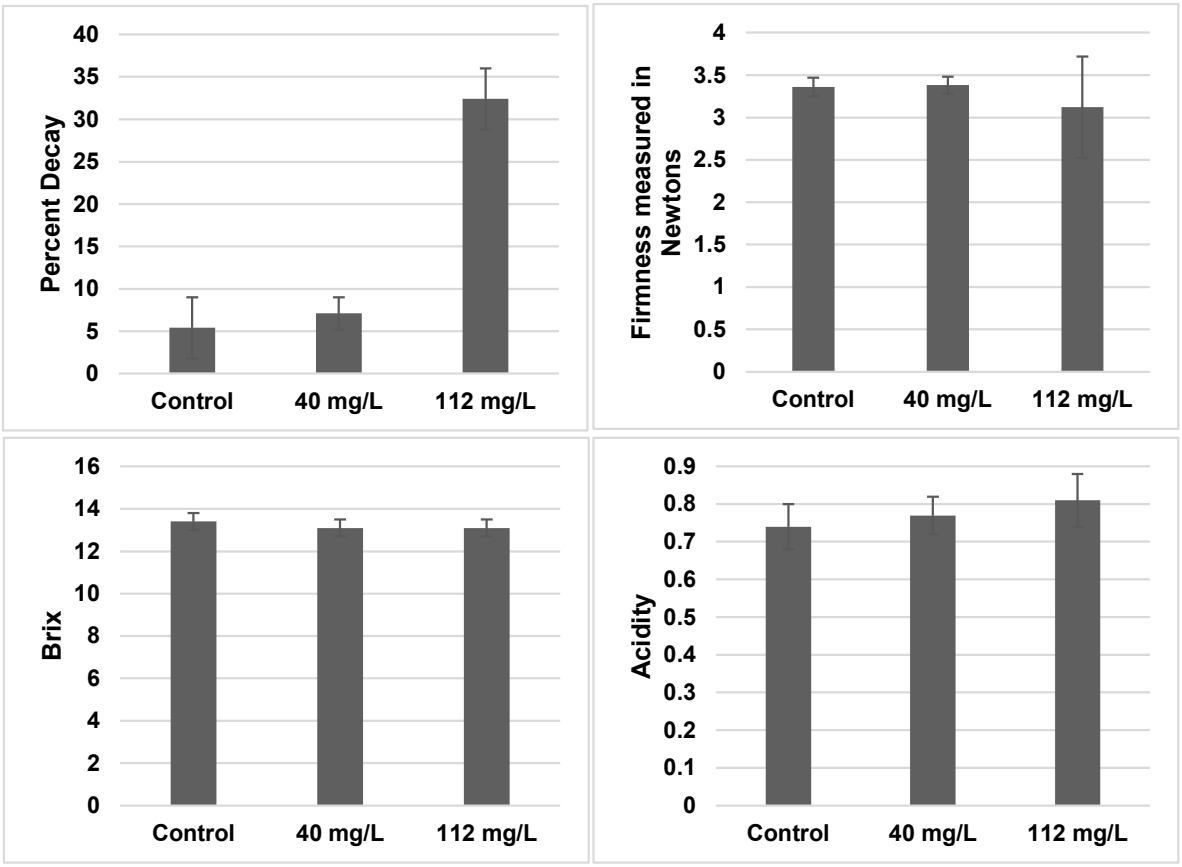

Fig. 2. General fruit quality parameters of navel oranges: percent decay (A), firmness (B), Brix (C), and acidity (D) of navels oranges exposed to propylene oxide fumigation.

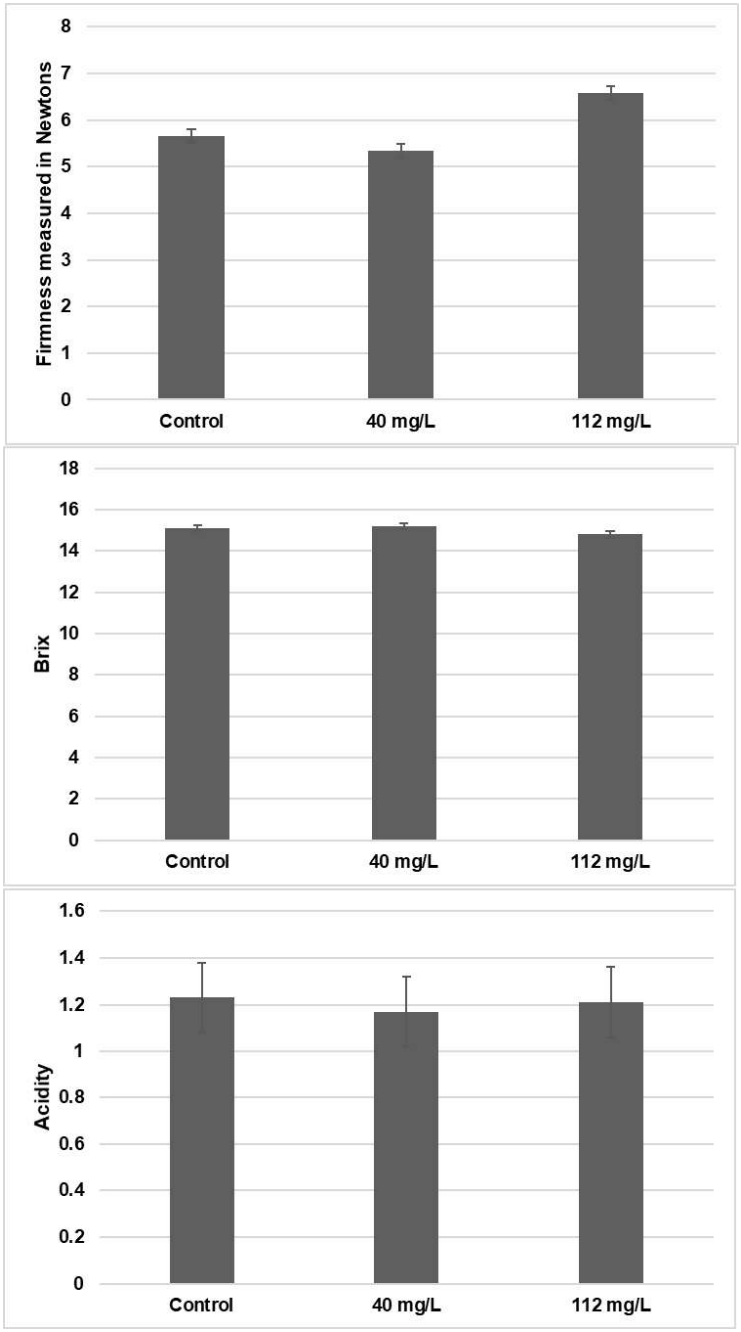

Fig. 3. General fruit quality parameters: firmness (A), Brix (B) and acidity (C) of mandarins exposed to propylene oxide fumigation. Fruit decay was not observed in mandarins.

Conclusions

Results showed evidence that propylene oxide was toxic to all pests of postharvest concern in citrus. Recommended concentrations to achieve probit-9 mortalities, 40 mg/L of two important pests, bean thrips and California red scale, did not have any negative effects on fruit quality. However, residues of PPO on different citrus varieties might also need to be considered to develop a treatment schedule for export of citrus fruit. Future studies will focus on developing time-temperature relationship, sorption, residues, and validating results during commercial scale trials.

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