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Impact of Oriental Fruit Fly Postharvest Treatments on Avocado

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Abstract

The detection in August 2015 of the Oriental Fruit Fly (*Bactrocera dorsalis* Hendel, Diptera: Tephritidae) in the Redland area in Miami-Dade County, Florida triggered a quarantine that restricted the movement of fruit fly host material in an approximately 99-square mile (256-square kilometer) area. The quarantine affected 4000 acres of fruit bearing commercial avocado groves. Approved post-harvest treatments for *B. dorsalis* and avocado included in the USDA Treatment Manual were acceptable for immediate certification and movement of fresh avocados from the quarantine area. However, it was unknown if Florida avocados would meet quality standards (US combination grade) after the treatments. Three post-harvest treatments that combine methyl bromide fumigation and cold storage periods were tested on six avocado varieties from Florida. The treatments differed in the durations of the fumigation and cold treatment periods. A seven day transit period at 8.3˚C (47˚F) was added to account for the time when the fruit leaves the packing house until it is sold by retailers. None of the six varieties had met the US combination grade after the treatments and transit period. Treated fruit exhibited both internal (pulp) and external (skin) damage. Damage was attributed to the fumigation component of the treatment, but the six varieties tolerated the cold portion of the treatment. Damage by fumigation ranged from 26% - 100%. In general, the longer the fumigation period the worse the effect. The need for alternative post-harvest treatments for Florida avocados is discussed.

Keywords

Bactrocera dorsalis, *Persea americana*, Postharvest, Quarantine, Fumigation

1. Introduction

The movement of agricultural commodities is a pathway by which exotic pests are introduced into new areas. Some exotic pests cause irreparable damage to natural ecosystems and costly economic losses due to increased crop damage, control programs and quarantine restrictions imposed on trade. Postharvest commodity treatments kill, sterilize, or eliminate regulatory pests in exported commodities [1]. Effective postharvest quarantine treatments eliminate destructive pests to allow the movement of fresh commodities to domestic and international markets. A commodity's tolerance of available treatment technologies affects the feasibility of the treatment [2].

In the US, tephritid fruit flies are among the most commonly encountered pests that lead to quarantines. The oriental fruit fly, *Bactrocera dorsalis* (Hendel), is of particular concern due to its destructive impact on fruit and vegetable production [3]. The damage occurs through oviposition punctures and subsequent larval development in fruit [4]. Native to tropical Asia, *B. dorsalis* is also established over much of sub-Saharan Africa, certain Pacific islands including Hawaii, Marianna islands and Tahiti, and is often intercepted on the mainland US, sometimes triggering eradication programs [5]. *Bactrocera dorsalis* was recently synonymized to include *Bactrocera invadens* Drew, Tsuruta & White, *Bactrocera papayae* Drew & Hancock, and *Bactrocera philippinensis* Drew & Hancock [3]. With the synonymization of *B. dorsalis*, there may be over 470 fruit and vegetable species to regulate if a quarantine is established including many commercially important crops [6].

In August 2015, 45 *B. dorsalis* males were detected in a monitoring trap in the Redland area of Miami-Dade County, Florida. This number of flies reached the trigger to start an eradication program [7], which included a 99-square mile (256-square kilometer) quarantine. The movement of more than 400 potential hosts of *B. dorsalis* was regulated including virtually all the tropical fruit crops grown in south Florida. Avocado was the most affected crop in the area with approximately 4000 acres inside the quarantine area. Many of the avocado orchards were being harvested. Avocado producers with groves in the quarantine area had two alternatives for movement of fruit: 1) a 30-day preharvest treatment combining application of insecticide baits and fly monitoring, or 2) use of approved phytosanitary postharvest treatments included in the USDA Treatment Manual. Approved postharvest treatments were acceptable and eligible for immediate certification and movement of fruit fly host material from the quarantine area.

An irradiation treatment of 150 Gy (minimum absorbed dosage) is approved for *B. dorsalis* in various commodities including avocado [8]. Previous work on the radiation sensitivity of avocados is not conclusive on whether Florida avocados can tolerate this approved treatment. There are three distinct races of avocados: Mexican, Guatemalan and West Indian [9]. All previous studies on phytosanitary irradiation of avocado concluded that Guatemalan, Mexican, or Guatemalan-Mexican avocado hybrids, that dominate the international avocado trade, are sensitive to radiation [10] [11] [12]. Florida produces "tropical" West Indian and West Indian-Guatemalan avocado hybrids that have less oil content, larger size and thinner skin [9] and therefore could be more or less sensitive to radiation. Florida currently lacks an irradiation facility dedicated to phytosanitary treatments and the cost of shipping and treating avocados in other states may not be justified.

Alternatively, the USDA Treatment Manual has four approved treatments for the oriental fruit fly and avocado that involve Methyl Bromide (MB) fumigation, either alone, or combined with a cold storage period (T101-c-1, T108-a-2, T108-a-3 and T108-a-3) [8]. Previous work by Spalding *et al.* [13] determined that methyl bromide fumigation did not result in skin injury immediately after treatment but increased anthracnose decay occurred in four major avocado varieties grown in Florida. In addition, Witherell *et al.* [14] tested the tolerance of 30 major and minor avocado cultivars in commercial production in Florida to methyl bromide fumigation followed by cold storage. The study concluded that 21 (70%) cultivars successfully withstood methyl bromide fumigation (32 g/m^3 for 2.5 h at 21.1˚C) followed by 7 days of storage at 7.2˚C (T101-a-2). In addition, 14 cultivars tolerated treatment T101-c-1 consisting of methyl bromide fumigation at 32 g/m^3 for 4 h (at 21.1˚C), followed by 3 days of storage at 7.2˚C [14]. However, these studies did not account for the strict fruit quality standards currently used by the Florida avocado industry and thus provided no guidance on the feasibility of using USDA approved treatments. In addition, the effect of standard fruit handling procedures in the market supply chain with quarantine treatments is unknown. The Avocado Administrative Committee requested assistance to determine if South Florida avocados can withstand approved postharvest treatment of methyl bromide and methyl bromide plus cold treatments and remain commercially acceptable.

2. Materials and Methods

More than 25 avocado varieties are grown in south Florida, with a harvest season extending throughout the year. Six avocado varieties that were harvested during the 2015 *B. dorsalis* outbreak (Loretta, Black Prince, Beta, Choquette, Leonas, and Booth 7) were used to test three postharvest treatments included in the USDA Treatment Manual (**Table 1**). Fruit for the experiments came from commercial groves located outside the quarantine area. The selected fruit were based on the picking and shipping schedule specified in the USDA Marketing Order No. 915 [15] for avocados grown in south Florida. This order specifies minimum fruit weight, size (diameter), quality and maturity. Eighty fruits of each tested variety were harvested early in the morning by a representative the Avocado Administrative Committee and delivered to the USDA, APHIS, PPQ, S&T, CPHST Miami facility (13601 Old Cutler Rd, Bldg 63, Miami, FL 33158) to conduct the methyl bromide fumigation component of the treatment. One variety was fumigated per day and the fumigation period varied according to the specific treatment (**Table 1**).

Table 1. Avocado cultivars subjected to different phytosanitary treatments against *B. dorsalis* included in the USDA treatment manual.

Avocado Variety	Treatment	Hours of Methyl Bromide Fumigation, dosage rate (32 g/m^3)	Cold Treatment (days)	Transit Period (days)	Shelf period (days at 21.1˚C)
Loretta	T108-a-2	2.5	4 (4.4˚C)	7 (4.4˚C)	5
Black Prince	T108-a-2	2.5	4 (4.4˚C)	7 (4.4˚C)	5
Beta	T108-a-3	3	3 (8.3˚C)	7 (8.3˚C)	5
Choquette	T108-a-3	3	3 (8.3˚C)	7 (8.3˚C)	5
Leonas	T101-c-1	4		7 (4.4˚C)	5
Booth 7	T101-c-1	4		7 (4.4˚C)	5

Methyl Bromide Fumigation

Groups of 10 fruit were placed into one of 6 fumigation chambers (modified Labconco® 28.32-L vacuum chambers) held inside two fume hoods (3 chambers in each hood) maintained at 23.8˚C. Ten additional fruit were used as non-fumigated controls held in each of the hoods (23.8˚C) through the duration of the fumigation and removed prior to fumigant aeration. The fruit pulp temperature was determined with a pulp thermometer (Smart Reader Plus 8 temperature recorder, ACR Systems Inc.) prior to injection of the fumigant. The fumigant was injected into each chamber with a gas-tight syringe (1500 ml, Model S-1500, Hamilton Co., Reno, Nevada) in the required amount of methyl bromide gas to obtain a dose of 32 g/m^3 (2.0 lbs/1000ft^3). The circulation fan in each of the chambers was then activated indicating the start time of the fumigation. Thirty min. after injecting the fumigant, the fan was turned off and a gas sample (40 ml) was taken using a 100 ml glass syringe with a Luer Lock tip. The MB concentration of the gas sample was determined using a gas chromatography (GC-ECD). Additional gas samples from each chamber were taken at 2, 2.5, 3 and/or 4 h after injection of fumigant and were analyzed with GC-ECD to ensure compliance with the minimum concentration readings specified in USDA Treatment Manual [8]. Upon completion of the fumigation period, the chambers' doors were opened and circulation fans were turned on again to vent inside the hood. A photoionization detector (PID, MiniRAE 3000, RAE Systems, San Jose, CA) was used to determine if aeration process had finished (MB readings bellow 5 ppm). Then the fruit was removed from the chambers and held at 23.8˚C until collected by a representative of the Avocado Administrative Committee and transported to the Brooks Tropicals packing house.

3. Cold Treatment

Upon arrival at the packing house, the pulp temperature was determined by Brooks Tropicals quality control personnel using a pulp thermometer (Smart Reader Plus 8, ACR Systems, Inc.) before transferring the fruit to refrigerated rooms kept at 4.4˚C or 8.3˚C depending on the test treatment (Table 1). Fruit temperature was monitored every two hours until completion of the cold period of each treatment (Table 1). Fruit quality was evaluated by certified FDACS and USDA fruit-inspectors at several time intervals: 1) immediately after finishing the fumigation and cold treatments specified in the USDA treatment manual, 2) seven days after treatment considered an average transit period from when the fruit leaves the packing house until it reaches the retailer, and 3) five days after the transit period or the shelf period, the time from when the fruit reaches the retailer until the consumer is ready to consume it. During the transit period, the fruit was held at the same temperature as used in the cold treatment (Table 1). During the shelf period the fruit was held at 21˚C. Immediately after treatment and after the transit period inspectors evaluated fruit external quality using the U.S. combination grading system [15]. Each fruit was assigned a grade: 1 = avocados free from decay, anthracnose, and freezing injury, and free from damage caused by mechanical or other means, 2 = avocados free from decay and freezing injury and free from serious damage caused by anthracnose, mechanical or other means, and 3 = avocados free from decay and free from serious damage caused by anthracnose and free from very serious damage caused by freezing injury, or mechanical or other means. Avocados sold outside the production area must meet the US Combination Grade, which is a combination of at least 60 percent of US No. 1 grade and 40% or less US No. 2 grade avocados in each container [15]. Consequently, a combination grade (CG) of 1.4 or higher, (considered significant when 95% confidence intervals did not overlap with CG = 1.4), was used to determine whether the fruit was not acceptable for commercialization. In addition, after the shelf period all fruit were dissected to record the occurrence of internal freezing injury and damage caused by anthracnose, another indicator of fruit quality. The control fruit was subjected to cold treatments only at 4.4˚C or 8.3˚C depending on the treatment (Table 1). Cold storage at this range of temperatures is a regular practice used by avocado handlers, packinghouses and transporters.

Results

"Loretta" and "Black Prince" fruit were subjected to T108-a-2 (2.5 h methyl bromide fumigation + 4 d cold storage at 4.4˚C). Loretta fruit showed no significant external damage immediately after completion of the fumigation and cold storage components of the treatment. The rating of 1.18 CG was below the acceptable higher limit of 1.4 CG. However, after the transit period, fruit showed serious damage caused by anthracnose and reached a rating of 1.81 CG (Figure 1). In addition, 88% of the treated fruit showed internal damage after the shelf period clearly indicating that treated fruits were unmarketable. By contrast, non-fumigated Loretta control fruit remained marketable showing 1.25 and 1.16 CG ratings after the treatment and transit periods (Figure 1), and an 8% internal damage rate after the shelf period. Similarly, "Black Prince" fruit showed ratings of 1.70 CG after treatment and 2.25 CG after the transit period. The external fruit quality did not meet the U.S. combination immediately after treatment. By

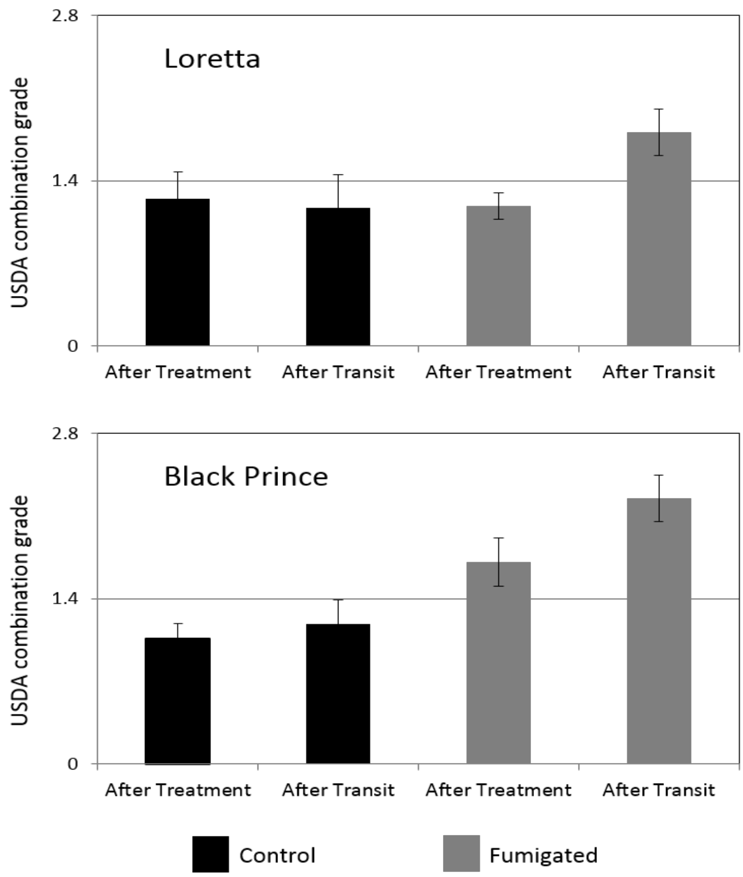

Figure 1. "Loretta" and "Black Prince" avocados subjected to treatment T108-a-2 (2.5 h methyl bromide fumigation + 4 d cold storage at 4.4˚C). A combination grade of 1.4 or lower indicates whether the fruit is acceptable for commercialization. Error bars represent 95% confidence intervals.

contrast, control fruit showed CGs of 1.06 after the treatment period and 1.18 after the transit period, which met meeting market standards (Figure 1). After the shelf period, 22% and 6% of treated and control "Black Prince" fruit showed internal damage, respectively.

"Beta" and "Choquette" cultivars were treated with T108-a-3 (3 h methyl bromide fumigation + 3 d cold storage at 8.3˚C). "Beta" fruit had a CG rating of 1.38 immediately after treatment, which was marginally acceptable for the market, but after the transit period, fruit was unmarketable with a CG of 2.06 (Figure 2). Control fruit remained marketable having 1.06 and 1.31 CG ratings after the treatment and transit periods, respectively. In addition, 100% of the "Beta" treated fruit showed internal damage whereas no damage was observed on the control fruit after the shelf period. "Choquette" fruit showed significant external damage immediately after the treatment and transit periods, reflected in CG ratings of 1.52 and 2.41, respectively (Figure 2). In addition, 19% of the treated fruit showed internal damage after the shelf period clearly indicating that treated fruit were unmarketable. By contrast, non-fumigated "Choquette" fruit remained marketable with 1.0 CG ratings after the treatment and transit periods with no internal damage.

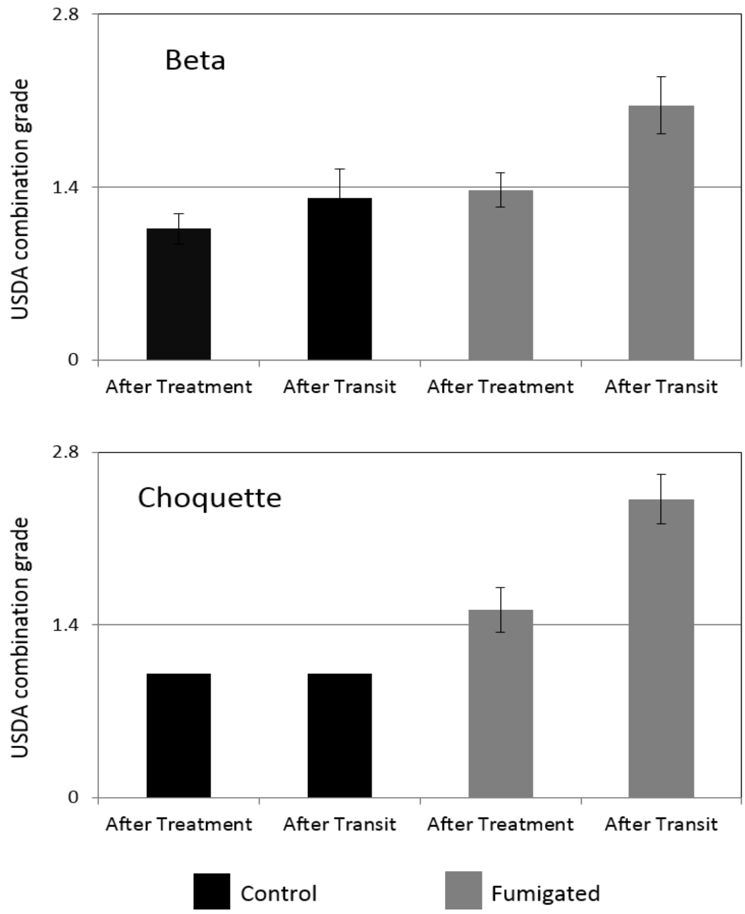

Figure 2. "Beta" and "Choquette" fruit subjected to treatment T108-a-3 (3 h methyl bromide fumigation + 3 d cold storage at 8.3˚C). A combination grade of 1.4 or lower indicates whether the fruit is acceptable for commercialization. Error bars represent 95% confidence intervals.

"Booth 7" and "Leonas" cultivars were subjected to a 4 h fumigation period (T101-c-1). Even though no cold storage is required for this postharvest treatment, fruit were exposed to a 7 d transit period at 4.4˚C. Both varieties showed no external damage after the fumigation treatment showing CG ratings of 1.08 and 1.0' respectively, which were similar to the non-fumigated control fruit (Figure 3). However, these fruits deteriorated after the transit period showing CG ratings of 2.40 and 2.14, respectively. The percentages of fruits with internal damage after the shelf period were 100 and 89 for "Booth 7" (Figure 4) and "Leonas", respectively.

4. Discussion

Four out of the six avocado cultivars that were being harvested during the 2015 *B. dorsalis* quarantine in south Florida met the US combination grade immediately after being subjected to fruit fly disinfestation treatments. These results and those reported by Witherell *et al.* [14] suggested that Florida avocados cultivars differ in tolerance to methyl bromide fumigation, and that some cultivars could withstand the treatments in the USDA Treatment Manual. However, our

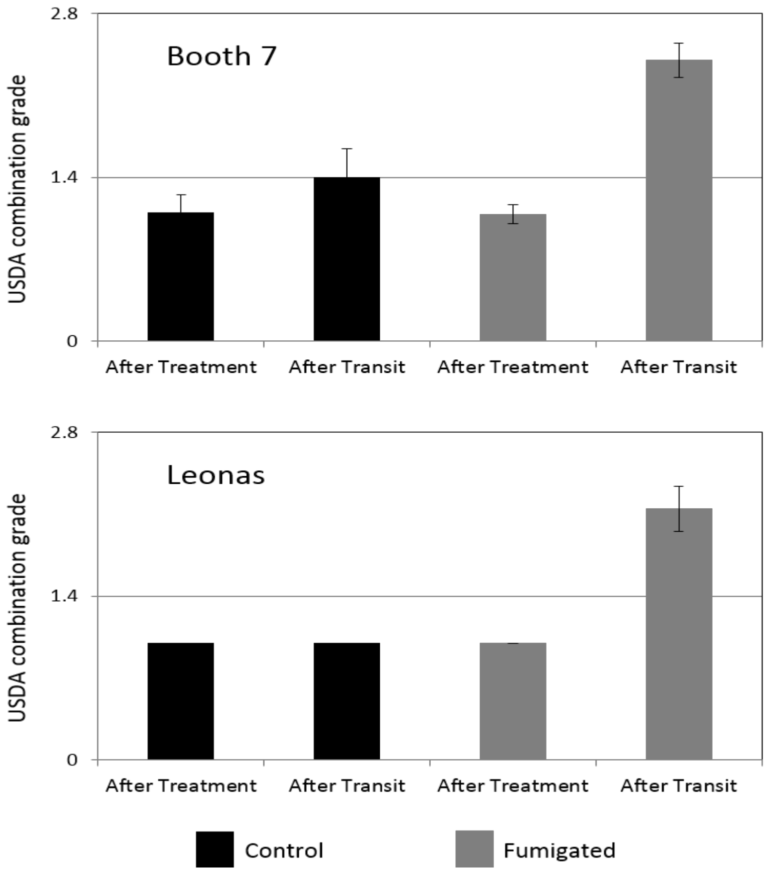

Figure 3. "Booth 7" and "Leonas" fruit subjected to treatment T101-c-1 (4 h methyl bromide fumigation). A combination grade of 1.4 or lower indicates whether the fruit is acceptable for commercialization. Error bars represent 95% confidence intervals.

Figure 4. (A) "Booth 7" fruitshowed no internal damage after the transit (4.4˚C for 7 days) and shelf (5 days at 21.1˚C) periods. (B) "Booth 7" fruit subjected to treatment T101-c-1 (4 h methyl bromide fumigation) showed 100% internal damage after the transit (4.4˚C for 7 days) and shelf (5 days at 21.1˚C) periods.

study indicates fruit deteriorates rapidly after treatment showing damage caused by anthracnose, freezing injury and abnormal ripening. Under these conditions, fruit will be rejected by retailers and consumers, which could negatively affect the reputation that Florida avocados have in the market. A more thorough screening of more avocado cultivars grown in Florida would be required to ascertain their tolerance to *B. dorsalis* disinfestation treatments. However, our results and those reported by Spalding *et al.* [16] suggest that West Indies and Guatemalan-West Indies hybrid avocados do not tolerate the fumigation with methyl bromide alone or combination with cold storage at the levels and times included in the USDA Treatment Manual. Damage was attributed to the fumigation component of the treatment, whereas the six varieties tolerated the cold storage.

Phytosanitary post-harvest treatments are designed and tested in areas with established populations of *B. dorsalis*. Postharvest treatments for *B. dorsalis* were designed for "Hass" type hard skinned avocados (Guatemalan and Mexican races) imported from Hawaii, Israel or the Philippines into the continental US. Our results suggest that these treatments are not suitable for avocados grown in Florida. Avocado is the main fruit crop in south Florida and there are no postharvest treatments locally available for this fruit to use under quarantine conditions.

Armstrong [17] reviewed fruit fly disinfestation strategies beyond methyl bromide and concluded that there are no alternative fumigants available for quarantine treatments. According to Sao *et al.* [18], low temperature fumigation with hydrogen phosphide (phosphine, PH_3) affectively eliminated *B. dorsalis* infestations from 10 Hawaiian varieties of avocados, but fumigated avocados ripened faster than nonfumigated avocados. Phosphine is being used extensively in Chile as a quarantine treatment for avocado targeting several species of fruit flies including *B. dorsalis* [19]. Low temperature phosphine fumigation is being considered as a phytosanitary treatment against *Bactrocera tau* [20].

Gamma irradiation is a potential treatment against fruit flies infesting avocado. All previous studies on phytosanitary irradiation of avocado concluded that "Hass" type avocados are sensitive to gamma radiation [10] [11] [12]. Florida avocados have less oil content, larger size and thinner skin [9] than "Hass" avocados and therefore could be less sensitive to gamma radiation. Research is needed to determine whether irradiation could be used as a fruit fly disinfestation quarantine treatment for Florida avocados. Since irradiation stimulates the production and release of ethylene, studies should be conducted in which this ripening hormone is rapidly removed from the time of treatment until the treated fruit enters the retail market. Irradiation of climacteric fruit in an advanced stage of maturity may avoid the spike in ethylene production [21]. The possibility that gamma irradiation in nitrogen or other atmospheres may reduce the damage to avocado tissues to a greater extent than to tephritid eggs and larvae within the fruit should be explored. Also the use of electron beam irradiation for phytosanitary treatments has come into commercial use during the past decade, and it should be investigated for use on avocado. As noted by Hallman *et al.* [21]: "The comparative advantage electron beam irradiation is that it the desired dose is achieved with extreme rapidity. Large pallets of products cannot be irradiated in 1 operation, and individual packages of commodities (with dimensions of typically of 10 cm each) must pass through the electron beam. However the technology offers the advantage of the radiation being electrically generated; electron beams can be switched off and do not present any radioactive hazard." Electron beam irradiators can be portable and readily transported to a quarantined area. The National Center for Electron Beam Research at Texas A&M University is involved in phytosanitary treatment processing of both imported and exported fruit.

Heat treatments in the form of hot-water immersion, vapor heat, or forced hot air are other potential treatments against *B. dorsalis* and other fruit flies that have not been tested on avocados. Cold treatments against *B. dorsalis* require prolonged storage periods at low temperatures (*i.e.*, T107-j, 18 days at 1.38˚C) that are unlikely to be suitable for avocados (Alan Flinn, personal communication).

The recent *B. dorsalis* outbreak in the Redland area in south Florida revealed that currently no post-harvest phytosanitary treatments are available for avocado to facilitate the movement of this fruit out of quarantined areas. Specific actions are needed towards making available phytosanitary postharvest treatments on avocado against *B. dorsalis* and other quarantine pests. For a realistic assessment, this study highlights the importance that future evaluations of postharvest treatments include transportation and storage time associated with the marketing of the fruit.

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