

# Potential use of ethyl formate treatment to control surface pests of ‘Hass’ avocado fruit

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**Abstract** Ethyl formate (EF) is a Generally Recognised As Safe (GRAS) alternative to methyl bromide, with potential to control surface pests on New Zealand avocados before export. ‘Hass’ avocados, two-spotted spider mites (TSM) and oleander scale (OS) insects were fumigated with 1.2% EF (240 g VAPORMATE™/m<sup>3</sup>) at 6°C for 1, 2 or 4 h. Fruit were then stored at 5°C for 3 weeks before external and internal fruit quality assessment. Survival of pest species was assessed 1 or 7 days later. Fumigation with 1.2% EF + 10% CO<sub>2</sub> for 2 or 4 h controlled all life stages of non-diapausing TSM and all life stages of OS, apart from crawlers (0.3–0.7% survivorship). Diapausing TSM were harder to control (17% ± 4.8% mortality; 4-h treatment). Ethyl formate treatment resulted in damage to avocado skins (41% ± 5.3%–91% ± 2.0%), and an increase in vascular browning and rots. The EF fumigations of avocado fruit at doses used here have potential to control non-diapausing mites and scale insects, but treated fruit were of unacceptable quality. Future studies could examine whether prior cool storage could reduce fruit damage.

**Keywords** VAPORMATE™, *Persea americana*, fumigation, fruit quality, GRAS, two-spotted spider mite, oleander scale.

## INTRODUCTION

There are a number of cultivars of avocado (*Persea americana*) but ‘Hass’ is the main cultivar traded internationally (FAO 2004). New Zealand exports ‘Hass’ avocados to Australia; however, the national body overseeing exports, New Zealand Avocado (NZA), has identified quarantine issues with pests on New Zealand fruit in the Australian market. Some of the pests intercepted with avocado shipments are mites, scale insects and thrips. In the past, New Zealand avocados have been inspected and cleared under the Australian pre-clearance programme (MPI 2018), but this programme is being phased out. Instead, the industry is moving to a ‘trusted trader scheme’, whereby the first 10 shipments will be inspected on arrival and, if all pass, then the importer

will move to a 1-in-2 inspection regime. If live quarantine pests are found during inspection, the shipment will be fumigated with methyl bromide at ~11°C.

New Zealand Avocado would like to investigate an onshore phytosanitary treatment using ethyl formate (EF) that can be applied before shipping to ensure that live pests are not detected at the border in Australia. Ethyl formate is a Generally Recognised As Safe (GRAS) alternative to methyl bromide, and has the potential to be used in a pest-management programme with no detectable residues. Ethyl formate is commercially available in New Zealand in combination with CO<sub>2</sub> as VAPORMATE™. Ethyl formate combined with CO<sub>2</sub> has been reported to control many pest species (Chhagan et al. 2013; De Lima 2009,

2010; Griffin et al. 2013; Jamieson et al. 2013, 2014, 2015, 2016; Pupin et al. 2013; Simpson et al. 2004, 2007; van Epenhuijsen et al. 2007).

In this study, the potential of EF (+CO<sub>2</sub>) was assessed: (a) for the control of surface pests of New Zealand 'Hass' avocado, such as mites and scale insects; (b) as a disinfestation treatment before export; and (c) for the effect it has on fruit.

## MATERIALS AND METHODS

### Pest insects

Insects for treatment were obtained from colonies maintained at The New Zealand Institute of Plant & Food Research Ltd (PFR), Auckland. Each container of insects represented one replicate within a treatment. There was a minimum of three replicates per treatment.

#### *Oleander scale (OS)*

Oleander scale (*Aspidiotus nerii*) were reared on red potatoes at 22°C, 16:8 h L:D. On the morning of treatment, potatoes infested with scale were collected from the colony and individually placed into containers (100 mm x 105 mm; 650 mL) with fine mesh (0.5 mm aperture) at either end.

#### *Non-diapausing two-spotted spider mite (TSM)*

Non-diapausing two-spotted spider mites (*Tetranychus urticae*) were reared on bean plants (*Phaseolus vulgaris*) following the methods described by Singh & Clare (1993) (16:8 h L:D at 25°C). On the morning of treatment, infested bean leaves were collected from the plants and placed into vials (107 mm x 42 mm; 120 mL) with fine mesh (0.085 mm aperture) at either end. Each vial contained at least 100 TSM of various life stages. The lid was secured with Parafilm® (Bemis NA) prior to treatment.

#### *Diapausing two-spotted spider mite (dTSM)*

Diapausing adult two-spotted spider mites were collected from a colony reared on bean plants (Singh & Clare 1993). This second colony was exposed to environmental conditions (12:12 h L:D at 15°C) during development, to induce the adults to move from the bean plants onto a cardboard-shelter card (5 cm x 6 cm) and enter

diapause. Cards of dTSM were collected from the colony and kept at 4°C until required. On the morning of treatment, dTSM on cardboard shelters were removed from 4°C and placed into vials (107 mm x 42 mm; 120 mL) with fine mesh (0.085 mm aperture) at each end. Each vial contained at least 100 dTSM. The lid was secured with Parafilm prior to treatment.

### Fruit

'Hass' avocado fruit were sourced from three commercial orchards (designated O1, O2 and O3) in the Bay of Plenty, New Zealand. Fruit were harvested on 24 October 2017, handled and packed commercially, and cool stored at 5–6°C before shipment to PFR on 31 October 2017. On arrival, fruit temperatures were 9–10°C, and fruit were immediately placed into a cool-store at 5°C and held overnight before commencing the EF treatments the following day, 8 days after harvest. Fruit were of two sizes (count size 23 and 25) and size classes were spread evenly across the treatments. On a 20-fruit sub-sample, fruit firmness, skin colour and maturity (dry matter content) were measured before treatment, using the methods described below.

### Treatments

To investigate the effect of EF on avocado fruit quality and insect survival, five treatments (designated T1-T5) were compared. Vials containing the representative mite and scale species at mixed life stages were placed with avocados into the treatment chambers for each fumigation.

Treatments were conducted in the Volatile Treatment Facility (VTF) consisting of 15 stainless steel chambers (76.8 L each) at PFR in Auckland, as described by Jamieson et al. (2014). Each replicate comprised fruit from three orchards (one tray per orchard) and there were three replicates (chambers). Thus, each chamber had 3 trays of fruit from three orchards, and there was a total of nine trays per treatment. Fruit and insects were fumigated with 1.2% EF + 10% CO<sub>2</sub> (equivalent to 240 g m<sup>-3</sup> VAPORMATE) at 6°C for 1, 2 or 4 h. Control fruit and insects were treated with CO<sub>2</sub> (10%) for 4 h (CO<sub>2</sub> control),

or held at 6°C with no change in atmosphere for the duration of the treatment (untreated control). After EF fumigation, fruit were stored at 5–6°C for 3 weeks, and external and internal fruit quality assessment was carried out when fruit were ripe. Pest samples were placed in a controlled temperature room at 20°C, 16:8 h L:D before mortality assessment.

### Assessment methods

#### *Insects/pests*

Both TSM and dTSM were assessed the day after treatment. Oleander scale were assessed 7 days after treatment, to allow for dead insects to dehydrate and thus make mortality assessment more reliable. For all mobile life stages, individuals were assessed as live if movement was seen when gently prodded with a pin. If no movement was seen, the insect was assessed as dead. Immobile OS were assessed by removal of the armoured cap and visual assessment of the body. A scale with a full, turgid body of normal colour was assessed as live and those with flaccid, dried and/or discoloured bodies were assessed as dead. All samples were assessed under a stereo microscope (5–40x magnification). For each species, counts of a maximum of 100 for each life stage were recorded.

#### *Fruit*

Before treatment with EF, fruit firmness was measured non-destructively by whole fruit compression using a Fruit Texture Analyser (model GS14; GUSS, Strand, South Africa) fitted with a flat plate. The Fruit Texture Analyser settings were: approach speed (forward) 5 mm/s; trigger force 30 gf; measure speed 5 mm/s; measure distance 2 mm; and return speed 5 mm/s. Firmness was measured twice at the widest part of each fruit, with the two measurements taken at 90° to each other; the average of both values was recorded as the mean fruit firmness value. Firmness was measured as kgf and data were converted to Newton (N), where 1 kgf = 9.81 N.

Fruit skin colour was assessed after removal from storage and warmed overnight at 20°C and also when ripe (soft fruit flesh depresses with

light pressure, no dent) using a 0 to 100 scale, where: 0 = bright glossy green; 30 = olive green; 60 = wood brown; 80 = purple brown; and 100 = dull black (New Zealand Avocado Industry Council Fruit Assessment Manual; Dixon 2003).

Fruit flesh dry-matter content was determined by taking two 18-mm diameter core samples at 90° to each other from the widest part of the fruit. After the skin, seed coat, and seed were removed and discarded, the four pieces of fruit flesh were sliced into ~1-mm discs and weighed. Fruit discs were dried at 65°C for 24 h, and then reweighed, and the dry matter expressed as the percentage mass remaining after 24 h of drying.

Assessments of fruit quality (rots and disorders) were made according to the criteria in the New Zealand Avocado Industry Council Fruit Assessment Manual (Dixon 2003). Each disorder was scored as present or absent and, when present, severity was rated on a 0 to 100 scale for the area of the fruit affected. After removal from storage and being kept overnight or for three days at 20°C, the incidence of any external visible skin damage was recorded. Fruit were then held at 20°C to ripen. Fruit were assessed daily for firmness by hand. Once the fruit had reached eating ripeness, determined by hand to be equivalent to a compression firmness reading of ~15–20 N, they were first assessed for external rot (ER) and then cut longitudinally to assess stem end rots (SER), body rots (BR) and vascular browning (VB) internally.

### Statistical analysis

Statistical separation among sample means for treatment effects was assessed by analysis of variance, with blocking on orchards using Genstat Release 17.1 [(PC/Windows 7) © 2014; VSN International Ltd, Hemel Hempstead, UK]. Where significant treatment effects were identified, separation among treatments was assessed by Fisher's Protected Least Significant Distance (LSD) test;  $P=0.05$ . Fruit disorder incidence is presented for fruit with  $\geq 5\%$  severity; data were angular transformed before analysis, with the untransformed incidence data presented in the tables.

**RESULTS**

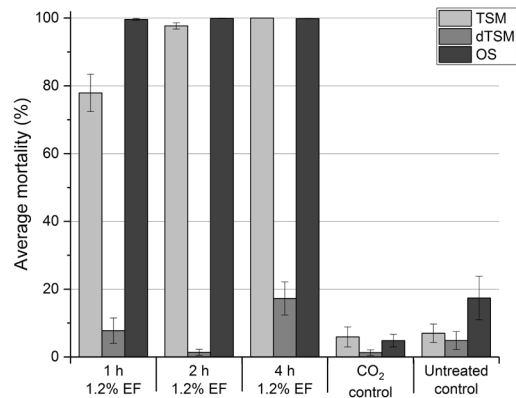
**Pest mortality**

Treatment for 1 h at 1.2% EF + CO<sub>2</sub> controlled 99.6% ± 0.28% of OS across all life stages, with the most tolerant life stage being crawlers (98.67% ± 1.33% mortality) (Fig. 1). Treatments of 2- and 4-h duration provided 100% mortality of all OS life stages (Fig. 1). The TSM were slightly more tolerant of EF (+CO<sub>2</sub>) and less than 80% mortality was achieved with 1 h of treatment. This rose to 97% ± 0.9% at 2 h, and 100% mortality of all mobile life stages was achieved after a 4-h treatment. Diapausing TSM were the most tolerant pest tested, with only 17% ± 4.8% mortality after 4 h of treatment with 1.2% EF (+CO<sub>2</sub>) (Fig. 1).

**Fruit quality**

Before treatment with EF, fruit from the three orchards had an average of 27.0% dry matter, a firmness of 84.4 N, a skin colour of 0.0 and an average fruit mass of 230.2 g (Table 1). Dry-matter values indicated fruit from O1 as just mature, according to NZA harvest maturity criteria, while fruit from the other orchards had higher dry matter contents with moderate maturity.

After EF treatment, storage for 3 weeks at 5–6°C and overnight at 20°C, there were significant differences in external skin damage between treatment groups. Fumigation with EF led to external skin damage of avocado fruit (Fig. 2, Table 2). Increase in EF treatment duration



**Figure 1** Mean mortality (%) of pest insects in each ethyl formate (EF) treatment (delivered with CO<sub>2</sub>). Vertical lines represent the standard error of the means. TSM, two-spotted mite; dTSM, diapausing two-spotted mite; OS, oleander scale.

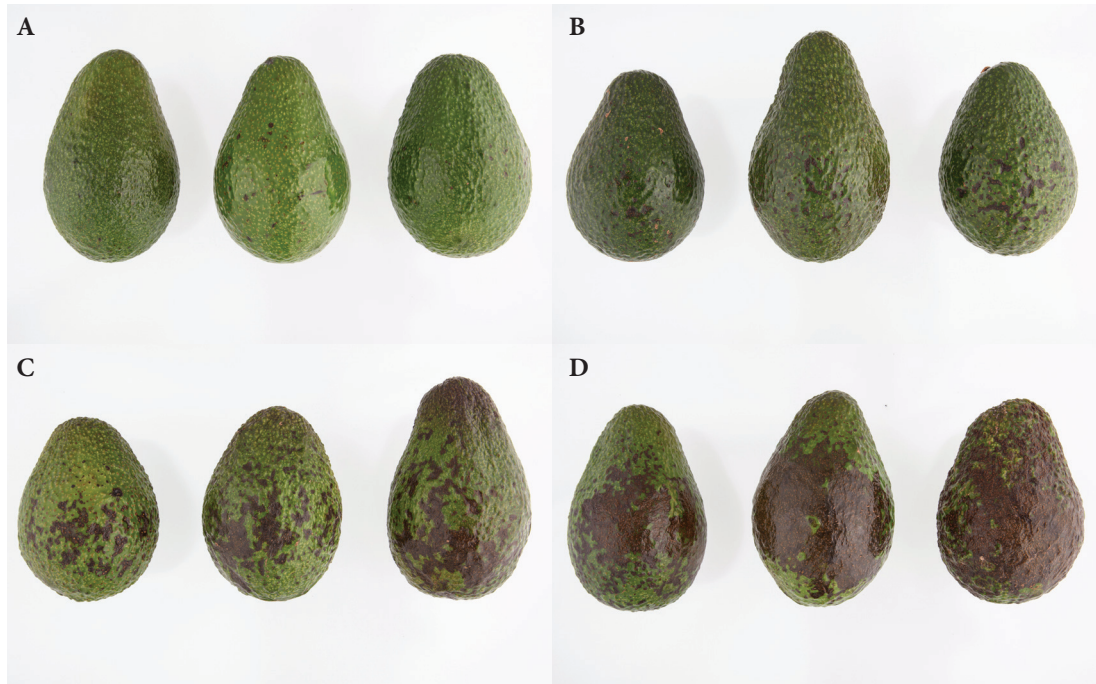
resulted in a consistent increase in skin damage severity. No skin damage occurred in any of the control fruit. The rounded “back” of the fruit generally showed more damage than the flatter “front” side of the fruit, where the peduncle/stem button is located.

There were also significant differences in firmness and skin colour between treatments (Table 3). After 3 weeks of storage and 3 days at 20°C, control fruit were slightly softer than the treated fruit and slightly more advanced in skin colour.

**Table 1** Fruit mass, firmness, skin colour and dry matter of ‘Hass’ avocado fruit from three orchards (O1–O3) immediately before ethyl formate (EF)+ CO<sub>2</sub> treatment. Values are means of 20 fruit per orchard.

Orchard	Fruit mass (g)	Firmness (N)	Skin colour (0–100 scale)	Dry matter (%)
O1	234.5 a	85.6 a	0.0	24.2 a
O2	229.3 a	84.4 a	0.0	28.3 b
O3	226.9 a	83.1 a	0.0	28.7 b
Mean	230.2	84.4	0.0	27.0
Statistical analysis: ANOVA P values				
Orchards	0.208	0.538		<0.001

Values in a column not sharing a common letter differ at P<0.05 by Fisher’s Protected LSD.



**Figure 2** Skin damage of 'Hass' avocado fruit after treatment with ethyl formate (EF) (+CO<sub>2</sub>) for 1-, 2- or 4-h duration (B, C and D), compared with untreated control fruit (A), after storage for 3 weeks at 5°C and then 3 days at 20°C.

**Table 2** Mean external skin damage of 'Hass' avocado fruit after treatment with or without ethyl formate (EF) + CO<sub>2</sub>, storage for 3 weeks at 5–6°C and overnight at 20°C. Values are the means of three orchards: three trays, each of 23–25 fruit per orchard.

Treatment	Treatment details	Duration (h)	Mean external skin damage (%)
T1	1.2% EF + CO <sub>2</sub>	1	41.4 b
T2	1.2% EF + CO <sub>2</sub>	2	80.0 c
T3	1.2% EF + CO <sub>2</sub>	4	91.3 d
T4	CO <sub>2</sub> control	4	0.0 a
T5	Untreated control	4	0.0 a

Statistical analysis: ANOVA P values

Treatments <0.001

Values not sharing a common letter differ at  $P < 0.05$  by Fisher's Protected LSD.

There were no statistically significant treatment effects on the time taken for fruit to ripen to eating firmness at 20°C after storage (Table 4), with fruit needing 6 to 7 days on

average to ripen. The skin colour of ripe fruit was similar (range 80.1–80.8) in all treatments after 3 weeks of storage and ripening at 20°C.

**Table 3** Firmness and skin colour of ‘Hass’ avocado fruit after treatment with or without ethyl formate (EF) + CO<sub>2</sub>, storage for 3 weeks at 5–6°C and 3 days at 20°C. Values are the means of three orchards, with five fruit per orchard.

Treatment	Treatment details	Duration (h)	Firmness (N)	Skin colour (0 to 100 scale)
T1	1.2% EF + CO <sub>2</sub>	1	19.0 abc	72.3 c
T2	1.2% EF + CO <sub>2</sub>	2	19.8 bc	63.2 a
T3	1.2% EF + CO <sub>2</sub>	4	20.3 c	64.9 ab
T4	CO <sub>2</sub> control	4	17.2 a	68.3 abc
T5	Untreated control	4	18.1 ab	71.4 bc
Statistical analysis: ANOVA P values				
Treatments			0.031	0.036

Values not sharing a common letter differ at P<0.05 by Fisher’s Protected LSD.

**Table 4** Time taken to ripen and skin colour when ripe of ‘Hass’ avocado fruit after treatment with or without ethyl formate (EF) + CO<sub>2</sub>, and storage for 3 weeks at 5–6°C, followed by ripening at 20°C. Values are the means of three orchards: three trays, each of 23–25 fruit per orchard.

Treatment	Treatment details	Duration (h)	Time to ripen (d)	Skin colour (0 to 100 scale)
T1	1.2% EF + CO <sub>2</sub>	1	6.5	80.5
T2	1.2% EF + CO <sub>2</sub>	2	6.7	80.5
T3	1.2% EF + CO <sub>2</sub>	4	6.6	80.1
T4	CO <sub>2</sub> control	4	6.6	80.6
T5	Untreated control	4	6.7	80.8
Statistical analysis: ANOVA P values				
Treatments			0.380	0.178

Values not sharing a common letter differ at P<0.05 by Fisher’s Protected LSD.

Fruit in the control groups had a lower SER incidence (12.3% to 15.2%) and BR incidence (29.7% to 30.5%) than EF-treated fruit, and there was no difference between the two control groups. Ethyl formate (+CO<sub>2</sub>) treatment resulted in an increased rot and disorder expression with longer duration increasing the incidence of SER, BR and VB (Table 5). The incidences of SER and BR were higher in fruit treated for 2 h (79% and 87.9%) or 4 h (82.5% and 90.8%), than for fruit treated for only 1 h (47.1% and 50.5%). There were no differences between treatments for ER. The incidence of VB was higher in EF-treated fruit (61% to 87.9%) than in control fruit (22.2% to 23.8%). Similarly, fruit treated for 2 h and 4 h

had a higher incidence of VB than fruit treated for only 1 h.

## DISCUSSION

This work is the first published information on response of avocado fruit to EF (+CO<sub>2</sub>) treatment, and while a full matrix of temperatures and durations was not applied, the treatments that were applied provide direction for further research.

Use of EF (+CO<sub>2</sub>) was found to control 80–100% of non-diapausing TSM and OS at 1- to 2-h durations; however, diapausing TSM were more tolerant, with only 20% mortality after 4 h of EF treatment. In this trial, TSM and OS were used

**Table 5** Incidence of stem end rot (SER), body rot (BR), external rot (ER) and vascular browning (VB) in ripe ‘Hass’ avocado fruit after treatment with or without ethyl formate (EF) + CO<sub>2</sub>, storage for 3 weeks at 5–6°C, followed by ripening at 20°C. Values are the mean of three orchards: three trays, each of 23–25 fruit per orchard.

Treatment	Treatment details	Duration (h)	SER(%)	BR(%)	ER(%)	VB(%)
T1	1.2% EF + CO <sub>2</sub>	1	47.1 b	50.5 b	3.3	61.0 b
T2	1.2% EF + CO <sub>2</sub>	2	79.0 c	87.9 c	6.5	85.5 c
T3	1.2% EF + CO <sub>2</sub>	4	82.5 c	90.8 c	4.9	87.9 c
T4	CO <sub>2</sub> control	4	12.3 a	29.7 a	0.5	22.2 a
T5	Untreated control	4	15.2 a	30.5 a	3.1	23.8 a
Statistical analysis: ANOVA P values						
Treatments			<0.001	<0.001		<0.001

Values not sharing a common letter differ at  $P < 0.05$  by Fisher’s Protected LSD.

as representative pests of avocado and were held within containers and treated with fruit. Future work should examine the response of other pest species that are commonly intercepted on avocados (e.g. other mites in the orders Acaridae and Winterschmidtidae plus the genera *Tyrophagus* spp. and *Tarsonemus* spp.). Previous trials indicate EF treatment for 1 or 2 h at 1.2% is likely to kill mobile life stages of thrips, mealybugs and scale insects on various commodities such as apples, apricots and kiwifruit (Brown et al. 2018; Chhagan et al. 2013; Griffin et al. 2013; Jamieson et al. 2014), which may also be found on avocado fruit. However, eggs of some species (e.g. leafrollers) may survive treatment at 1.2% EF (Griffin et al. 2013). The response of TSM eggs to EF (+CO<sub>2</sub>) was not investigated in this trial, but Jamieson et al. (unpublished results) found that a 1-h exposure to 1.06% EF (+CO<sub>2</sub>) resulted in 28% egg hatch. There is sufficient evidence that EF treatment at 1.2% would control most pest species found on avocados, however, there is reduced efficacy against more tolerant life stages such as diapausing mites and eggs of various species.

The key limitation to avocado quality of EF (+CO<sub>2</sub>) treated fruit was external skin damage, which also resulted in increased SER, BR and VB incidence. However, EF (+CO<sub>2</sub>) treatment had no effect on skin colour or time taken for fruit to

ripen to eating firmness after 3 weeks of storage and ripening at 20°C. The damage became evident after only 1 week in cool-store at 5°C. The fact that no skin damage was noticed in fruit undergoing control treatments indicates that the observed damage was not due to handling or to CO<sub>2</sub>.

In conclusion, shorter duration EF (+CO<sub>2</sub>) treatment has the potential to control non-diapausing mites and scale insects so may be useful during certain periods of the year. However, EF (+CO<sub>2</sub>) treatment increased avocado skin damage and this is likely to be the key limitation along with increased rot expression, particularly at longer treatment durations. Given that mites appear to be the main pests intercepted, we suggest examining a range of lower-concentration EF treatments around the 1- to 2-h treatment duration window. Future work could also examine the effect of storage duration before EF (+CO<sub>2</sub>) treatment since we have found prior storage can reduce EF damage in other fruit (unpublished results).

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