Ethyl Formate: A Potential Disinfestation Treatment for Eucalyptus Weevil (*Gonipterus platensis*) (Coleoptera: Curculionidae) in Apples

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ABSTRACT Export of Pink Lady apples from Australia has been significantly affected by infestations of adult eucalyptus weevils (*Gonipterus platensis* Marelli). These weevils cling tenaciously to the pedicel of apple fruit when selecting overwintering sites. As a result, apples infested with live *G. platensis* adults lead to rejection for export. Since the Montreal Protocol restricted use of methyl bromide as postharvest treatment, it was necessary to consider alternative safer fumigants for disinfestation of eucalyptus weevil. Laboratory experiments were conducted using concentrations of 5, 10, 15, 20, 25, 30, 40, and 80 mg/liter of ethyl formate. Complete control (100% mortality) was achieved at 25–30 mg/liter of ethyl formate at 22–24°C for 24-h exposure without apples. However, with 90–95% of the volume full of apples, complete control was achieved at 40 mg/liter of ethyl formate at 22–24°C for 24-h exposure. No phytotoxicity was observed and after one day aeration, residue of ethyl formate declined to natural levels (0.05–0.2 mg/kg). Five ethyl formate field trials were conducted in cool storages (capacity from 250–900 tons) and 100% kill of eucalyptus weevils were achieved at 50–55 mg/liter at 7–10°C for 24 h. Ethyl formate has great potential for preshipment treatment of apples. Its use is considerably cheaper and safer than already existing fumigants like methyl bromide and phosphine.

KEY WORDS ethyl formate, apple, eucalyptus weevil, fumigation, fumigant

Australia is a major exporter of fresh horticultural produce of which apples especially Pink Lady apples are becoming popular worldwide, but recently export of Pink Lady apples from Australia have been significantly affected by infestations of adult eucalyptus weevil (*Gonipterus platensis* Marelli). This species is native to Tasmania where it feeds on leaves of eucalypts (*Mapondera et al. 2012*). The insect was accidentally introduced to Western Australia where it inhabits blue gum (*Eucalyptus globulus*) plantations, but in autumn some adult weevils seek shelter in apple orchards for the harsher weather.

Weevil legs carry a multitude of tiny hooks which allow the adults to grip very tightly to the pedicel of the fruit and are not dislodged during harvest. They even remain attached after fruit is water-flumed into a grading shed, blow-dried, packed, and cool stored until late winter. They can be removed from pedicel only after concerted effort. The weevils do not damage apple trees or fruit, but rest at the pedicel when selecting overwintering sites. As a result, apples infested in autumn remain infested in late winter when removed from storage. When subjected to quarantine inspection in Australia prior to overseas export, such fruit would be rejected.

It is considered that the only practical method for shipping apples free of live weevils is to develop a disinfestation process. Due to restrictions governing use of methyl bromide as mandated by the Montreal Protocol (United Nations Environment Program [UNEP] 2006), use of naturally occurring plant volatiles as potential fumigants for postharvest treatment of insect pests was considered a priority for investigation. One such compound is ethyl formate (EF) which has a long history as a fumigant for stored products and for dried fruit in particular (Cotton and Roark 1928, Roark and Cotton 1929, Simmons and Fisher 1945, Vincent and Lindgren 1972, Banks and Hilton 1996). For the past few years, ethyl formate has been re-evaluated as an alternative fumigant for grain stored in unscaled farm bins (Annis 2002, Ren et al. 2003, Ren and Mahon 2006). It is currently registered as a fumigant for dried fruit in Australia and has a history of being a safe food additive. Ethyl formate occurs naturally in soil, water, vegetation, and a range of raw and processed foods including vegetables, fruit, grain, beer, grapes, wine, and animal products like milk and cheese (Hiroyasu et al. 1972, Desmarchelier 1999). Threshold limit value (TLV) for ethyl formate is 100 ppm, whereas TLV for methyl bromide and phosphine is 3 ppm and 0.3 ppm, respectively; as a result,

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methyl bromide and phosphone are almost 100 and 1,000 times more toxic than ethyl formate.

Unlike other fumigants, ethyl formate kills insects rapidly and its residue breaks down to naturally occurring products, formic acid and ethanol (Desmarchelier et al. 1998). It is a colorless liquid with a low boiling point (54.1°C) and has a pleasant aromatic odor. Its flammable limit is 85 mg/liter. The US Food and Drug Administration reviewed its use as a flavoring agent and characterized it as safe (US Food and Drug Administration 1984). Experiments have been conducted using ethyl formate as a postharvest fumigant for some pests of strawberries, table grapes, thrips in onion and lettuce (Stewart and Aharoni 1983; Stewart and Mon 1984; Simpson et al. 2004, 2007; Van Epenhuisen et al. 2007).

Here we report the effectiveness of various concentrations of ethyl formate in controlling G. platensis adults both in laboratory scale and commercial scale tests and its phytotoxicity data along with preliminary residue studies.

Materials and Methods

Fruit and Insect Samples. For both laboratory study and field trials, Cripps Pink apples supplied by Harvey Giblett from Newton Brothers’ orchard in Manjimup, Australia (−34.2418403, 116.1455942) were used. The fruit samples were stored at 5°C in a cool room until laboratory trials. G. platensis adults collected from blue gum (Eucalyptus globulus) plantations in Manjimup, Australia, were used for bioassay.

Reagents and Apparatus. Ethyl formate used for laboratory study (standard preparation and laboratory bioassays) was supplied by Sigma Aldrich, reagent grade, 97% purity. For commercial-scale fumigation, food grade ethyl formate, Eranol, supplied by Orica Australia and SRL India was used. One-liter Erlenmeyer flask (Crown Scientific, Code FE1003) equipped with cone and screw-thread adapter with 1.1-cm blue septa (Grace Davison Discovery Sciences, catalog: 6518) were used as fumigation chambers; 120-ml glass bottles (Grace Davison Discovery Sciences, catalog: 6518) equipped with cone and screw-thread adapter (Crown Scientific, Code ST 5313) with 1.1-cm blue septa (Grace Davison Discovery Sciences, catalog: 6518) were used as fumigation chambers; 120-ml glass bottles (Plasdene Glass Pak, Perth, Australia) were used to monitor weevils after fumigation; and 4-liter glass jars (Plasdene Glass Pak) were used for sting insect mortality and the insects were transferred to new bottles (120 ml) containing fresh blue gum leaves at 25°C to check for their recovery. The end point for assessment was 2 d after removal to account for any possible recovery of insects.

Analysis of Ethyl Formate. Ethyl formate was analyzed using a DPS portable GC companion 600 (from DPS instruments, Inc., 11201 5th street, Unit L203 Rancho Cucamonga, CA) equipped with a flame ionization detector (FID) after isothermal separation on a 30 m x 0.33 mm (i.d.) 3 μm, metallic column, Restek 800-356-1685 phase MXTI-S (Catalogue no. 70285, serial no. 702152) at oven and detector temperature 90°C and 150°C, respectively, with carrier Helium flow rate at 55 KPa. The concentrations of fumigants were monitored at time intervals over the exposure period and were used to calculate the Concentration x time product (C×t) (Ren et al. 2012). Gas standards were prepared by taking out calculated amount of air from an Erlenmeyer flask (1 liter) and then injecting calculated amount of liquid ethyl formate into the Erlenmeyer flask. All the samples and standards were injected in duplicate. The concentrations of ethyl formate were calculated on the basis of peak areas which were calibrated periodically using the external gas standards and data were recorded in Microsoft Excel.

Laboratory-Scale Trials. Laboratory Bioassays. Fumigation was carried out in Erlenmeyer flasks (250 ml) without apples at 5, 10, 15, 20, 25, 30, 40, and 80 mg/liter of ethyl formate. Four replications of each concentration with 25 adult weevils in each were taken.

For bioassays with apples, 4-liter glass jars were loaded 90–95% full with apples and 100 adult weevils were added in each. The jars were sealed with airtight lids equipped with septa as an injection port and a cone-shaped filter paper inside to absorb the liquid ethyl formate to avoid liquid ethyl formate directly contacting the fruit. Three jars each were treated with 10, 20, 30, 40, and 80 mg/liter of ethyl formate and one served as an untreated control. The bioassay was conducted at temperature between 22–24°C. The concentration of ethyl formate was measured by gas chromatography (GC-FID) at intervals over the exposure period of 24 h as described above. After 24-h fumigation, jars or jars were opened at 22–24°C to assess insect mortality and the insects were transferred to new bottles (120 ml) containing fresh blue gum leaves at 25°C to check for their recovery. The end point for assessment was 2 d after removal to account for any possible recovery of insects.

Laboratory Phytotoxicity and Residual Studies. For phytotoxicity and residual studies, eight apples were placed in each of the seven 4-liter glass jars. The jars were sealed with lids with septa as injection ports and a cone-shaped filter paper inside to absorb the liquid ethyl formate. Three jars were treated with 40 mg/liter, another three with 80 mg/liter of ethyl formate, and one served as an untreated control. After 24-, 48-, and 96-h fumigation at temperature between 22–24°C, one jar each of 40 and 80 mg/liter were opened and the apples were checked for morphological and physiological changes compared with unfumigated fruit. For analysis of residues, one apple each from 24-, 48-, and 96-h exposure with no aeration, one day, two days, and four days aeration, and the untreated control were taken out and kept in a freezer prior to determination.
of levels of ethyl formate. Fortified standards in duplicate of 44.33 mg/kg and 88.66 mg/kg concentration were prepared using 10 mg of sodium chloride + 20 ml of distilled water + 20 mg of untreated finely cut apples in Erlenmeyer flask (100 ml) and then adding 1 μl of ethyl formate in two flasks (44.33 mg/kg) and 2 μl of ethyl formate in other two flasks (88.66 mg/kg). Just like fortified standards, sample in duplicate were prepared by using treated apples without adding ethyl formate after thawing it to room temperature. The samples and standards were kept at room temperature for 3 h and then head space samples were taken and analyzed with GC-FID.

**Commercial-Scale Trials.**

**Commercial-Scale Cool Room Fumigation Trials.** The fumigation trials were conducted at Newton Brothers’ Orchard (Manager, Harvey Giblett), Manjimup, Western Australia (−34.2418403, 116.1455942). Ten electric frying pans (Sunbeam Classic Banquet Frypan, model FP5910) at medium heat were used for vaporization of liquid ethyl formate. The pans, placed on the top of fruit stacks, were used for vaporizing ethyl formate. Fumigation was conducted at headspace air temperature of 10 °C and relative humidity (RH) 75–80% of cool room, with intergranular air temperature and RH of the bulk, 2 m below the fruit, to be between 8–9°C and 80–85%, respectively. The circulation fans on the wall of the cool room of 900-cm³ dimension with 90% volume occupied by apples were run to disperse vapor throughout the storage area during vaporization. A Logitech WiLife outdoor camera with master system USB connected to a computer was used for monitoring vaporization of ethyl formate from electric frying pans during fumigation. Dosages of 50–55 g/m³ ethyl formate were applied for all five large-scale fumigation trials.

**Commercial-Scale Bioassay.**

*G. platensis* adults were collected from blue gum plantations at Manjimup. When preparing the fumigation bioassays, five *G. platensis* adults were transferred to plastic vials (100 ml) with screen lids and perforated bottoms. The vials were placed in different locations throughout the cool room including some being suspended down the sides of stacks on the end of string lines at headspace air temperature of 10 °C and RH 75–80% of cool room, with intergranular air temperature and RH of the bulk, 2 m below the fruit, to be between 8–9°C and 80–85%, respectively. The treated and untreated insect numbers used were 500–1,200 and 200–300 adults, respectively, for each trial. All the bioassay results were compared with controls in triplicate, which were kept in adjacent cool room having same temperature and RH as the treated cool room and with the same number of weevils. After 24-h fumigation, the cool room was opened and aired for 2–4 h and then all vials were retrieved. To assess insect mortality, vials were transferred to a laboratory at 25°C to check for recovery. End point was delayed for 2 d to account for possible recovery of insects.

**Measuring Temperature and RH of Cool Rooms.** During the fumigation, headspace air temperature and RH were automatically recorded in the cool room with a HOBO data logger unit (Model H08-004-02, Onset Computer Corporation, MA, www.onsetcomp.com). To measure fruit temperature, the HOBO probe sensor was inserted 2 m below the fruit at the top of bin stacks in the center of the cool room. To measure the headspace air temperature and RH, the HOBO was hung 0.5 m above the fruit at the top of the bin stacks. The recorded data were read with BoxCar Version 3.6+ for Windows (Onset Computer Corporation) software. The HOBO units had previously been calibrated in the laboratory against each other and an alcohol-filled glass thermometer as well as a range of glycerol and water solutions for RH.

**Gas Sampling and Monitoring.** To monitor the concentration of ethyl formate in cool rooms during the exposure and ventilation periods, 8–10 nylon gas sampling lines (3 mm i.d.) were installed at various positions inside the cool room before the application of ethyl formate. Gas samples were drawn from the storage through these lines using an electric pump to a position 5 m from the storage, which was outside the fumigation area. The gas samples were stored in Tedlar sample bags (1 liter) until analysis (usually within 1 h) using the gas chromatographic conditions previously described.

**Results and Discussion**

**Laboratory Bioassay of Ethyl Formate.** All bioassay results were compared with untreated controls, which were kept under the same temperature between 22–24°C and 55% RH, with the same number of weevils. For bioassays without apples, adult mortality was observed after 24-h exposure. Complete control (100% mortality) was achieved at 30, 40, and 80 mg/liter of ethyl formate at 22–24°C. However, mortality of 0, 13, 72, and 81% were observed at 5, 15, 20, and 25 mg/liter of ethyl formate as shown in Fig. 1. End point mortality readings taken at 2 and 4 d after the end of fumigation did not show any revival of weevils. Bioassay with fruits showed that due to some ethyl formate being absorbed by the fruit at a loading of 90–95% apples, complete control was achieved at 40 mg/liter of ethyl formate at 22–24°C for 24 h as shown in Fig. 2. The concentration of the formulation declined rapidly as shown in Fig. 3 within the first 10 h, particularly within the first four hours 75–85% of ethyl formate was absorbed by fruit. These are typical results, and the loss of fumigant from the gas phase followed the expected pattern with an initial rapid sorption giving way to a long-term trend about 10 h after dosing. This result is consistent with previous trials of ethyl formate on wheat, barley, oats, and peas (Desmarchelier et al. 1998, Ren et al. 2003, Ren and Mahon 2006).

**Phytotoxicity Studies of Ethyl Formate.** In comparison with untreated apples, no morphological changes in terms of color, texture, and firmness were observed even after 1, 2, and 3 wk of treatment. No brown spots or any damage were observed in treated apples as compared to control when cut from the center vertically after fumigation. The data were consistent with the previous experiments on strawberries where...
little or no calyx damage and no berry damage were observed when exposed to 0.8% ethyl formate at 24°C for 2 h (Simpson et al. 2004). Table grape exposure to ethyl formate at concentration up to 0.5% for 1 or 2 h were found to be well tolerated with the exception of increased rachis browning; however, after 2-d storage the rachis of untreated fruit was similarly browned (Simpson et al. 2007). In the case of onion no effect on skin color, firmness, or incidence of rots and phytotoxicity were observed up to 324 g/m$^3$ of Vapormate (equivalent to 54 g/m$^3$ of ethyl formate) for 2 h (Van Epenhuijsen et al. 2007). Vacuum fumigation of

Fig. 1. Mortality data for *G. platensis* adults after 24-h exposure between 22–24°C to ethyl formate at different concentrations without apples.

Fig. 2. Ethyl formate Concentration × time products (mg h/liter) with mortality (%) of *G. platensis* adults in fumigation chambers containing apples.
packaged lettuce with 0.5% ethyl formate for 2 h did not result in loss of commodity quality (Stewart and Aharoni 1983, Stewart and Mon 1984).

**Residue Studies of Ethyl Formate.** Residue studies show that after one day aeration, ethyl formate residues in apple had declined to background levels (0.05–0.2 mg/kg) shown in Table 1. During the holding period, ethyl formate declined in fruit without the need for fan-forced aeration. After one day, residues were above those in the untreated control sample, but below the level of 1 mg/kg which is the current Australian maximum residue level (MRL) for ethyl formate in dried fruit. These results are consistent with previous commercial-scale trials with ethyl formate on wheat, barley, oats, and peas (Desmarchelier et al. 1998, Ren et al. 2003, Ren and Mahon 2006).

**Commercial-Scale Ethyl Formate Application Method.** The electric frying pans used to vaporize ethyl formate were a simple yet highly efficient method for conducting the trials. It was hard to confirm complete vaporization of ethyl formate inside the cool rooms without use of an outdoor camera. With the use of camera, for instance we know, 50 liters of ethyl formate can be vaporized and delivered to a 900-m³ cool room in less than 15 min. While this method was suitable for assessment of ethyl formate to disinfest apples of the weevils, it could be a worker safety issue because large amount of liquid ethyl formate have to be carried by hand to the top of fruit stacks. Therefore, new outdoor highly efficient ethyl formate application system needs to be developed for commercial fumigation.

**Large-Scale Bioassay of Ethyl Formate Against G. platensis Adults.** The total of more than 6,000 and 3,000 adult weevils were included in fumigated areas and as untreated controls, respectively, during five commercial-scale trials. Total mortality was achieved in all the treated plastic vials compared to no mortality in the untreated controls. No revival of weevils was observed even after two days culture at 25°C in the laboratory. This number of test insects with no survivors could be considered an acceptable result to substantiate the use of ethyl formate as a commercial phytosanitary quarantine treatment for export fruit. HOBO data logger unit shows the temperature in cold rooms were about 10°C and RH 75–80% at the time of fumigation.

In conclusion, following laboratory study and commercial trials on total 2,000 tons of Pink Lady apples in Manjimup, ethyl formate has shown a great potential for preshipment treatment of export apples to control for G. platensis adults. Application is being made for

![Fig. 3. Ethyl formate concentration (mg/liter) over time in head space of fumigation chamber containing apples at 40 and 80 mg/liter for 24-, 48-, and 96-h exposure.](image)

Table 1. Ethyl formate residues (mg/kg) in treated apples at 40 and 80 mg/liter and untreated control samples in the laboratory at different exposure durations after no aeration and aeration for one day.

<table>
<thead>
<tr>
<th>Dosage (mg/liter)</th>
<th>Exposure time (d)</th>
<th>After exposure no aeration (mg/kg)</th>
<th>After one day of aeration (mg/kg)</th>
</tr>
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<tbody>
<tr>
<td>40</td>
<td>1</td>
<td>10.4</td>
<td>0.09</td>
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<td></td>
<td>2</td>
<td>12.5</td>
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<td></td>
<td>4</td>
<td>13.2</td>
<td>0.10</td>
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<tr>
<td>80</td>
<td>1</td>
<td>18.5</td>
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<td></td>
<td>2</td>
<td>16.2</td>
<td>0.11</td>
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<tr>
<td></td>
<td>4</td>
<td>16.3</td>
<td>0.08</td>
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<tr>
<td>Untreated control</td>
<td>1</td>
<td>0.05</td>
<td>0.08</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0.10</td>
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<td>0.08</td>
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registration of the fumigant for ongoing commercial use. As next stage work, we are developing onsite non-flammable ethyl formate application system.

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