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ERADICATION OF EUCALYPTUS WEEVILS IN APPLES BY ETHYL FORMATE

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ABSTRACT

Export of Pink Lady™ apples from Australia have been significantly affected by infestations of adult eucalyptus weevil (eucalyptus snout beetle or gum tree weevil). These weevils do not damage apple trees or fruit, but rest at the petiole portion of apples when selecting overwintering sites. As a result apples infested with live eucalyptus weevils leads to rejection for export. Usage of methyl bromide as post harvest treatment is restricted under the Montreal Protocol. Therefore, it has become important to develop an alternative safe fumigant as an eradication method for eucalyptus weevil on apple.
Laboratory experiments were conducted to evaluate ethyl formate, which is a naturally occurring volatile chemical present in many plant commodities, as fumigant for eradication of the eucalyptus weevil on apples. Laboratory and cool storage trials show that ethyl formate is highly toxic to the eucalyptus weevil and low phytotoxic to the fruit. Complete control can be achieved at 30 g m^{-3} of ethyl formate at 25ºC for 24 hours exposure with and without apple. In comparison with untreated apples, the colour and texture have no change 1, 2 and 3 weeks after treatment. Four field trials were conducted in cool storages (the capacity ranged from 250-900 tonnes) in Western Australia. The ethyl formate was applied at dosage of 50-55 g m^{-3} and low temperature (4-8ºC) for 24 hours exposure. All eucalyptus weevils were killed and after 1 day aeration, residue of ethyl formate declined to natural levels (0.05-0.2 mg kg^{-1}). Phytotoxicity studies showed no effect on morphology or taste of apples.

Key words: apple, ethyl formate, fumigant, fumigation, eucalyptus weevil

INTRODUCTION

Australia conducts a small but important export trade in Pink Lady™ apples, but recent exports have been significantly affected by infestation of adult eucalyptus weevil. The insect was accidentally introduced to WA where it inhabits blue gum (Eucalyptus globulus) plantations, but in autumn, some adult weevils seek shelter in apple orchards during harsher weather. The weevils do not damage apple trees or fruit, but rest at the stalk when selecting overwintering sites. When subjected to quarantine inspection in Australia prior to overseas export, such fruit would be rejected, especially for the lucrative British and European markets. Until successful management programs for the weevil can be developed, the issue of bulk picking, packing and transporting is uncertain and even shipment in cartons is at risk.

Due to restrictions governing use of methyl bromide as mandated by the Montreal Protocol, use of naturally occurring plant volatiles as potential fumigants for post-harvest treatment of insect pests was considered a priority for investigation. One such compound is ethyl formate which has long history as a fumigant for stored products (Cotton and Roark, 1928) and for dried fruit in particular (Simmons and Fisher, 1945; Banks and Hilton, 1996). For the past few years, ethyl formate has been re-evaluated as an alternative fumigant for grain stored in unsealed farm bins (Annis, 2002; Ren et al., 2003; Ren and Mahon, 2006). It is registered as a fumigant for dried fruit in Australia and has a history of safe use as a 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 (Desmarchelier, 1999). 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 colourless liquid with a low boiling point (54.1^{o}C) and has a pleasant aromatic odour. Its flammable limit is 85 g m-3. The US Food and Drug Administration (FDA, 1984) reviewed its use as a flavouring agent and characterised it as safe.

Experiments have been conducted using ethyl formate as a post-harvest fumigant for some pests of table grapes (Simpson et al., 2007) and thrips in onion (Van Epenhuijsen et al., 2007). Here we report the effectiveness of various concentrations of ethyl formate in controlling eucalyptus weevil both at laboratory and commercial scale cool storages.

MATERIALS AND METHODS

Fruit and insect samples

For both laboratory study and field trials, Cripps Pink apples (also known as Pink Lady™) were supplied by Newton Brothers' Orchard (Western Australia, WA). The fruit samples were stored at 5ºC in a cool room. Adult eucalyptus weevils collected from blue gum plantations in Manjimup (WA) were used for bioassay.

Reagents and apparatus

Ethyl formate used for laboratory study was supplied by Sigma Aldrich, reagent grade, 97% purity. For commercial scale fumigation, food grade ethyl formate, supplied by Bronson & Jacobs Pty Ltd., Australia.

One litre Erlenmeyer flasks (Bibby Sterilin, Staffordshire, Cat. No. FE 1 L/3) were used for preparation of standards; 250 mL Erlenmeyer flasks (Crown Scientific, Code FE1L3) equipped with cone/screw-thread adapter (Crown Scientific, Code ST 5313) with 7/16" blue septa (Grace Davison Discovery Sciences, catalog: 6518) were used for fumigation; 120 mL glass bottles (Plasdene Glass Pak, Perth) were used to monitor weevils after fumigation; and 4 litre glass jars (Plasdene Glass Pak, Perth) with screw tight lids were used for phytotoxicity and residue studies; 4 L glass jars were used for the fumigation of the apple samples plus insects.

A 100 µL syringe (SGE, Melbourne, Cat. no. 005250) and 5 uL syringe (SGE, Melbourne, Australia; Cat. no. 001000 5F) were used for injection of gas samples into the gas chromatographs (GC) and transfer of liquid ethyl formate to make gas standards; 50 mL air tight syringes (SGE, Melbourne; Cat. no. 008900) were used to withdraw air from empty flasks to make the standard.

Analysis of ethyl formate

Ethyl formate was determined using DPS portable GC companion 600 equipped with a flame ionisation detector (FID) after isothermal separation on a 30 m × 0.53 mm (i.d.) 3 um, metallic column, Restek 800-356-1688 phase MXTr-S, (Catalogue no. 70285, serial no. 702152) at oven temperature 90°C, detector temperature 150°C and carrier flow helium regulator 55 KPa and air regulator 100 Kpa.

All the samples and standards were injected in duplicate. The concentrations of ethyl formate were calculated on the basis of peak areas as compare to gas standards.

Laboratory bioassays

Fumigation was carried out in 250 mL Erlenmeyer flasks without apples at 5, 10, 15, 20, 25, 30, 40 and 80 g m^{-3} of ethyl formate with 25 adult weevils in each were taken. For bioassays with apples, seven 4 litre glass jars were loaded 90-95% full with apples and 100 adult weevils in each. The jars were sealed with airtight lids equipped with septa as an injection port and a cone-shaped filter paper. Three jars were treated with 40 g m^{-3}, three with 80 g m^{-3} of ethyl formate and one served as control.

The concentration of ethyl formate was measured by gas chromatography (GC-FID) at intervals over the exposure period of 24 hours. After 24 hours fumigation, flasks or jars were opened to check mortality and the insects were transferred to new 120 mL bottles containing fresh blue gum leaves at 25ºC to check for their recovery.

Laboratory phytotoxicity and residual studies

For these studies eight apples were placed in each of seven 4 litre glass jars. As mentioned above, jars were treated with 40 and 80 g m^{-3} of ethyl formate and one as control. After 24, 48 and 96 hours fumigation, one jar each of 40 and 80 g m^{-3} were opened and the apples were checked for morphological and physiological changes compared with unfumigated fruit. For morphological changes fruits were looked visually for any spots, skin damage, texture, change in colour compare to control. This was done in both whole and apple cut from the petiole.

For analysis of ethyl formate residues, one apple each from 24, 48 and 96 hours 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.

Commercial scale cool room fumigation trials

Application and bioassay

Two different methods for application of ethyl formate were tested at Newton Brothers' Orchard Western Australia. In the first electric frying pans and in second a new inhouse made unit was used for vaporization. Dosages of 50-55 g m^{-3} were applied for 24 h for all large scale trials. For bioassays plastic vials with weevils having screen lids were placed in different locations throughout the cool room, the treated and unexposed insect numbers used were 800-1200 and 200-300 adults respectively for each trial.

Gas sampling and monitoring

For analysis of ethyl formate gas samples were drawn from the storage through nylon lines using an electric pump. The gas samples were stored in Tedlar® sample bags (1 L) until analysis using the gas-chromatographic conditions previously described.

RESULTS AND DISCUSSION

Laboratory bioassay of ethyl formate

All bioassay results were compared with untreated controls kept under the same conditions, with the same number of weevils. For bioassays without apples 100% adult mortality was achieved at 30 g m^{-3} and above of ethyl formate at 22-24ºC. However, mortality of 81, 72, 13 and 0% were observed at 25, 20, 15 and 5 g m^{-3} of ethyl formate (Figure 1). End point mortality readings taken at two and four days did not show any revival of weevils. In case of

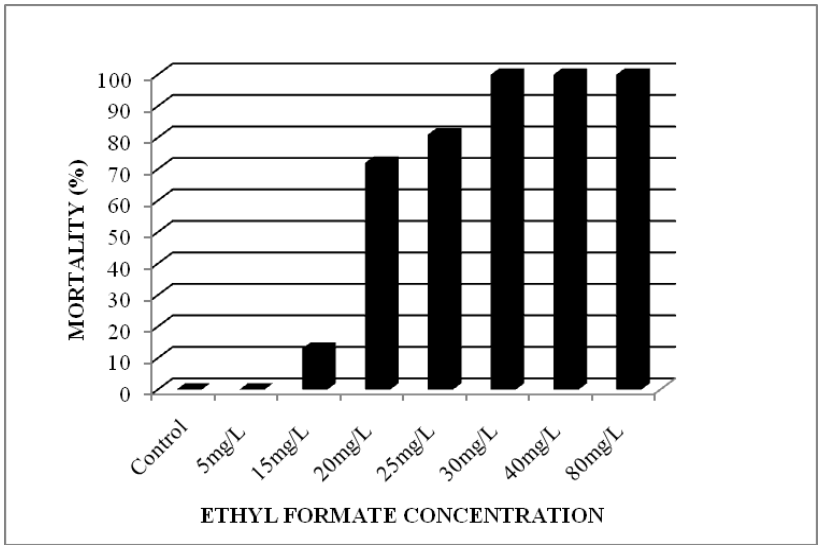

Fig. 1- Mortality of eucalyptus weevils at different levels of ethyl formate 22-24ºC for 24 hours exposure

bioassay with apples as some ethyl formate being absorbed by the fruit 100% control was achieved at 40 g m^{-3} of ethyl formate at 22-24ºC for 24 hours. The loss of fumigant in the chamber during fumigation showed that about 50% of applied ethyl formate was absorbed (Figure 2). The concentration of the formulation declined rapidly within the first 4 hours. This result is consistent with previous trials of ethyl formate on wheat, barley, oats and peas (Desmarchelier et al., 1998; Ren and Mahon, 2003, 2006).

Phytotoxicity and residue studies of ethyl formate for apples

Residue studies showed that after one day aeration ethyl formate residue in apple have declined to background levels (0.05-0.2 mg kg^{-1}) as compare to 0 day aeration. (Table1). These results are consistent with previous commercial-scale trials with ethyl formate on wheat, barley, oats and peas (Desmarchelier et al., 1998; Ren and Mahon, 2003, 2006).

In comparison with untreated apples, the colour and texture of fruit subjected to fumigation with ethyl formate showed no change and had no effect on morphology even after 1, 2 and 3 weeks of treatment.

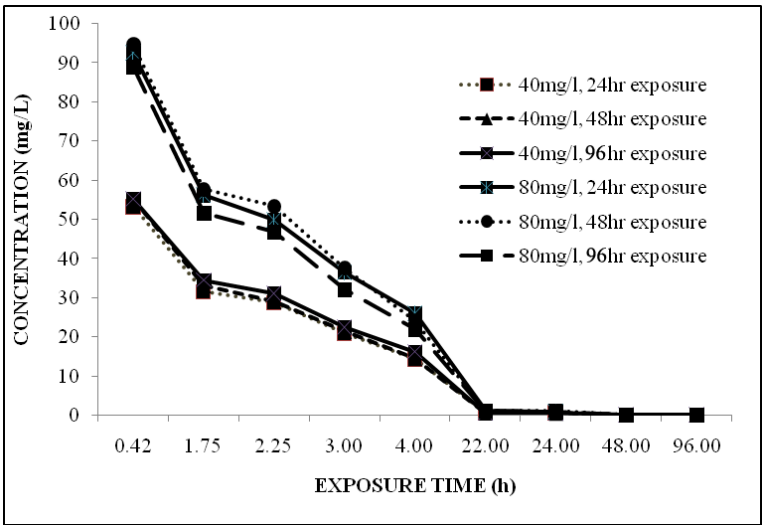

Fig. 2- Sorption of ethyl formate by apples with time for two dosage rates and three exposure time with ethyl formate after removal from fumigation chamber

Commercial scale bioassay of ethyl formate against eucalyptus weevils

Ethyl formate application methods

The new ethyl formate nitrogen purging unit, developed in house, was highly efficient in vaporising and delivering ethyl formate into the commercial-sized cool storage areas. For example, 50 litres of ethyl formate can be vaporised and delivered to a 900 m^3 cool room in less than 45 minutes. The unit works reliably and has no OH&S issue. This technology has great potential to offer application of ethyl formate for pre-shipment treatment of other insect pests of fruit and vegetables.

Table 1. Ethyl formate residues in treated apples at 40 and 80 g m^{-3} at different exposure durations as compared to untreated control samples

Dosage (g m^{-3})	Exposure time (days)	After exposure (no aeration)	One day of aeration
40	1	10.4	<0.05
	2	12.5	<0.05
	4	13.2	<0.05
80	1	18.5	<0.05
	2	16.2	<0.05
	4	16.3	<0.05
Untreated control	1	<0.05*	<0.05
	2	<0.05	<0.05
	4	<0.05	<0.05

*. <0.05 is GC detection level

Bioassays

Total mortality was achieved in all the treated plastic vials compared to no mortality in the untreated controls. 5000-6000 number of dead test insects with no survivors could be considered an acceptable result for the purpose of substantiating this use of ethyl formate as a commercial phytosanitary treatment for required quarantine inspections for export fruit.

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