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ECO₂FUME[®] FOR POSTHARVEST DISINFESTATION OF HORTICULTURE PRODUCE

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ABSTRACT

ECO₂FUME[®] is a cylinder gas formulation of phosphine that was developed for the grain industry for use in SIROFLO[®] systems. ECO₂FUME can be applied rapidly and can readily be used to top up PH₃ concentrations during a fumigation, a feature used in SIROFLO. It avoids problems with unexpended residues of PH₃-generating compounds, which can occur when using solid PH₃-generating formulations. These attributes give ECO₂FUME the potential for treatment of produce requiring shorter duration fumigations than those used for grain. Research on the development of alternatives to methyl bromide for disinfestation of cut flowers for export, led to the registration of ECO₂FUME for this purpose in Australia in March 1999. Fumigation requirements are an exposure time of 15 h with an initial PH₃ concentration of 1 g m⁻³ and a minimum temperature of 15°C. Further work is in progress on produce with much longer shelf life than flowers and for which longer exposure times of up to 48 h have been used. The potential for use of ECO₂FUME for disinfestation of oranges infested with larvae of the Queensland fruit fly *Bactrocera tryoni*, has been demonstrated. It has been shown to be effective in controlling eggs, larvae and pupae of the lightbrown apple moth *Epiphyas postvittana*, on pears, and larvae of the codling moth *Cydia pomonella*, in apples without injuring the produce.

INTRODUCTION

ECO₂FUME[®] - 2% phosphine with carbon dioxide (CO₂) as a carrier gas - is a cylinder gas formulation of phosphine (PH₃) that was developed for the grain industry for use in SIROFLO[®] systems (Winks and Russell, 1994). Solid formulations of aluminium and magnesium phosphide have been used for many years to generate PH₃ for fumigation of grain. These formulations gradually release PH₃ to provide the desired toxic concentration. ECO₂FUME has the advantage that it can be applied rapidly to provide a desired concentration of phosphine. Also it can readily be used to top up PH₃ concentrations during a fumigation, a feature used in SIROFLO. Another advantage is that ECO₂FUME

avoids problems with unexpended residues of PH_3 generating compounds, which can occur when using solid PH_3 generating formulations. These attributes give ECO_2FUME the potential for treatment of produce for which shorter duration fumigations than for grain are required.

The implementation of decisions of the Montreal Protocol to reduce the use of methyl bromide (MB), because it is an ozone depleting chemical has resulted in international interest in developing alternative treatments (Anon., 1998). MB has been used extensively for soil fumigation and for pre-shipment and quarantine purposes. Its effectiveness and rapid action often makes it the fumigant of choice.

This paper describes efforts to find alternatives to MB for postharvest treatment of a range of cut flowers and fruits, and the selection of ECO_2FUME as a promising fumigant formulation. The initial study was on flowers partly because whereas MB is effective against important pests of flowers, it also reduces the vase life of many flowers. The flowers were mainly Australian wildflowers, as these comprise about 90% of Australian flower exports. The fruits were citrus, (for which MB is the only fumigant registered in Australia), apples and pears.

METHODS

Flowers

Laboratory studies were carried out in which a range of cut flowers, mostly Australian wildflowers, was screened against the fumigants carbon disulphide, carbonyl sulphide, cyanogen, ethyl formate (Eranol), hydrogen cyanide, metham sodium and PH_3 , with MB being used for comparison. Fumigant concentrations used were based, where possible, on established quarantine dosages. They ranged from 0.1 mg L^{-1} for PH_3 to 32 mg L^{-1} for MB and exposure times ranged from 0.5 h for hydrogen cyanide to 16 h for PH_3 .

Large-scale fumigations with the PH_3 formulation ECO_2FUME were carried out in 27 m^3 modified steel shipping containers. The containers were fitted with fans to recirculate gases within the chambers and to ventilate them on completion of fumigations. Thermostatically controlled heaters (setting usually 20°C) were used to heat air within the chambers to ensure that the temperature of the flowers was kept at 15°C or above. The fans helped to distribute heat. In many of the fumigations a 5-10 sec burst of Pestigas[®] was released into the chamber, to agitate insect pests, 10 min before introduction of ECO_2FUME . To monitor gas input, the mass of each gas introduced into the chamber was measured by weighing the gas bottles before and after each fumigation. Gas concentrations were monitored using sampling lines and gas detector tubes (Dräger[®]). Flowers in buckets of water were placed on trolleys that were wheeled into the chambers for fumigation. Flowers and foliage, either cut and in containers of water or whole potted plants, infested with insects were exposed in cages. Cage lids were removed just before fumigation commenced (Muhunthan *et al.*, 1997, Williams and Muhunthan, 1998).

The insects exposed were mainly species that had caused rejections of consignments of export flowers after the flowers had been treated with insecticide dips or aerosol treatments; for example larvae of the moth *Strepsicrates ejectana* (Walker), a pest of *Thryptomene* spp. which form leaf and webbing shelters on *Thryptomene*. The insects *Oryzaephilus surinamensis* (L.), *Tribolium castaneum* (Herbst), and were also screened. The flowers and foliage tested were those that

are regularly exported together with some considered to have promise for the future. The flowers included proteas, banksias, waxflowers, flannel flower, smoke bush, waratahs, Acacias, *Thryptomene*, *Phyllica* and *Leptospermum* cultivars.

Citrus

Insect fumigations: Citrus infested with larvae of Queensland fruit fly (Qfly) *Bactrocera tryoni* (Froggatt) is unacceptable in the marketplace. Disinfestation treatments must be applied to most fruit produced in Queensland and northern New South Wales to ensure that it is free of Qfly. Fruit must also be treated when, periodic outbreaks of Qfly occur in southern New South Wales, South Australia and Victoria.

Export quality navel oranges were infested with Qfly eggs. The rind of each orange was pierced with a spiked block, which made 20 holes to make it easy for flies to oviposit. Navel oranges were placed on top of fly mesh cages of adult fruit flies, which laid through the mesh into the oranges. Several series of fumigations were carried out and for each series 20 cartons of infested oranges and two cartons of uninfested "control" oranges were used. The oranges were held in a quarantine insectary at $26 \pm 2^\circ\text{C}$ and 40%-70% r.h. to provide favourable conditions for the development of Qflies and prevent any escapes. The "control" oranges were held in a refrigerator at $\sim 11^\circ\text{C}$.

Cartons of infested and control oranges were fumigated with ECO_2FUME in a 900 L chamber fitted with a heater and a gas recirculation and exhaust fan. PH_3 concentrations were determined using gas detector tubes (Dräger) at the beginning of a fumigation half way through (generally) and at the end. Sometimes additional ECO_2FUME was added half way through a fumigation to boost the PH_3 concentration.

The first fumigations were based on the schedule developed for wildflowers (Muhunthan *et al.*, 1997). Initial PH_3 concentrations of 0.94 g m^{-3} to 1.36 g m^{-3} , exposure times of 16 h to 24 h and temperatures of 20°C to 23°C were used. In the final series of fumigations initial phosphine concentrations of 1.67 g m^{-3} were used with an exposure time of 48 h and temperatures of 23°C to 25°C (Williams *et al.* 2000).

Assessing effectiveness of fumigations: After fumigation, the oranges were placed in plastic boxes (580 x 380 x 165 mm), each box having a perforated base, which fitted on top of a box of similar dimensions with a solid base, covered with fine grade vermiculite. Any mature larvae that developed, left the fruit, passed through the perforations and pupated in the vermiculite. Each pair of boxes was enclosed in a Terylene voile bag to prevent escapes. About 10 days later the vermiculite was sieved for puparia. If larvae were found, sieving was repeated a few days later. The number of puparia produced was compared with the number produced from groups of infested unfumigated oranges. Four groups of ten infested oranges were sampled from the cartons and each group was placed in a plastic box and puparia were collected, using the same method as for the fumigated oranges. A few puparia from both fumigated and unfumigated oranges were kept to see if they would complete development to the adult stage, before being killed. Effectiveness of the fumigations was assessed by estimating percentage mortality using the number of puparia produced compared with the estimated number of larvae exposed. The estimate of larval numbers was based on the average number of pupae per orange produced from the batches of

unfumigated infested oranges. Lower 95% confidence limits for mortality were found using exact calculations based on the binomial distribution. This is the method of calculation used by Couey and Chew (1986) to relate the number of insects exposed to mortality and probit values.

Fruit quality

Twenty uninfested oranges from each consignment were included in each fumigation and subsequently examined for appearance in comparison with control batches. Five of the treated oranges from each fumigation were cut open and assessed for rind condition, juiciness and taste after a day airing, and again, up to 4 weeks later.

Pome fruit

Preliminary disinfestation trials were conducted to gauge the effectiveness of ECO₂FUME fumigations on the codling moth, *Cydia pomonella* (L.) and the lightbrown apple moth (LBAM), *Epiphyas postvittana* (Walker) and to see if such fumigations had any adverse effects on pears.

Insect Fumigations: The insect stages used were 5th instar codling moth and 4th, 5th and 6th instar and pupae of LBAM. The insects were fumigated over 48 hours using an initial concentration of approximately 1.4 g m⁻³ PH₃. Phosphine concentrations were determined using Dräger gas detector tubes.

First instar codling moth larvae were allowed to infest apple thinnings and held at 25°C until the 5th instar was reached. The infested thinnings were fumigated in a 900 L chamber. After fumigation, the larvae were removed from the apples and assessed for mortality.

LBAM were treated in 3 replicate fumigation chambers constructed from 200 L drums. Each drum was fitted with a pressure release valve, input and output hoses and the lid was sealed in place with silicone rubber and clamps. The LBAM were fumigated in cardboard fruit boxes, one per chamber. Three groups of 70 4th instar larvae were placed in plastic boxes (120 x 180 x 80 mm) containing a pear and several strips of corrugated cardboard to provide shelters. This process was repeated for the other instars and for 6th instar larvae that were allowed to pupate. Each fruit box was loaded with approximately 50 pears amongst which was placed a box of each of the LBAM stages to be treated. The box was then placed in a fumigation drum and ECO₂FUME was introduced. After treatment the larvae and pupae were removed and assessed for mortality.

Fruit quality: Packham's Triumph pears were picked during commercial harvest. Three replicates of 100 fruit each (50 treated and 50 control) were treated with ECO₂FUME.

Fruit were exposed to 48-hour fumigations in the chamber used in the codling moth experiments. The concentration of PH₃ was determined initially (aimed at ~ 1.4 g m⁻³); after 24 h, and the gas was topped up at this time, if necessary, to give a concentration of ~ 0.7 g m⁻³; and at 48 h. As only one chamber was available, the 3 replicates were treated sequentially, with the pears stored at ambient temperature while awaiting treatment.

During the fumigation the control fruit were held in air at 20°C. After the fumigation both treated and control fruit were stored at -1°C for 5 weeks to

induce ripening. They were then assessed for firmness, skin colour, total soluble solids (TSS), internal and external browning or any other injury symptoms.

RESULTS

Flowers

The laboratory tests with seven fumigants indicated that PH_3 was the least phytotoxic of the fumigants tested and that it killed many of the insects exposed (Weller *et al.*, 1996, Williams, 1996).

The large scale fumigations demonstrated that to kill some of the problem pests, exposure times of 15-16 h were required with initial PH_3 concentrations of about 1 g m^{-3} . Also it was found possible to reduce the PH_3 concentration if Pestigas[□] was applied before $\text{ECO}_2\text{FUME}^{\square}$. This is demonstrated by results obtained with larvae and pupae of *S. ejectana* (Table 1).

TABLE 1
Effects of 15 - 16 hour fumigations with Pestigas[□] and $\text{ECO}_2\text{FUME}^{\square}$ on *Strepsicrates ejectana*

Temperature (°C)	Pestigas (g)	ECO_2FUME (kg)	PH_3 conc. (g m^{-3})		<i>Strepsicrates ejectana</i>			
			Initial	Final	Larvae		Pupae	
					Live	Dead	Live	Dead
20.5 (19.6-22.8)	120	3.12	1.4	1.0	0	18	0	24
20.0	0	0	0	0	18	0	24	0
21.0 (16.3-23.1)	160	2.02	1.0	0.6	0	45	0	12
20.0	0	0	0	0	45	0	12	0
21.9 (19.5-26.7)	120	2.04	1.0	0.7	0	103	0	1
20.0	0	0	0	0	103	0	1	0
18.1 (17.5-18.9)	120	1.12	0.27	0.15	0	75	0	47
20.0	0	0	0	0	75	0	47	0
18.2 (16.1-22.2)	140	1.4	0.32	0.19	0	96	-	
20.0	0	0	0	0	96	0	-	

Citrus

Results of the final series of fumigations of navel oranges are given in Table 2.

Pome fruit

Insect fumigations: During the codling moth fumigation the concentration of PH_3 dropped from 1.5 g m^{-3} to 0.8 g m^{-3} after 24 h and to 0.4 g m^{-3} after 48 h. Sixty larvae were recovered from the apple thinnings, all of which were dead.

In the LBAM fumigations the concentration of phosphine decreased from 1.4 g m^{-3} to 0.8, 0.6 and 1.0 g m^{-3} respectively for each replicate. All stages of LBAM were dead when assessed.

TABLE 2
Fumigations of Washington navel oranges infested with Queensland fruit fly larvae exposed to ECO₂FUME for 48 hours at 23°C to 25°C

Temp. (°C)	Phosphine concentration (g m ⁻³)			Estimated No. of fly larvae	No. of pupae	Mortality (%)	95% lower confidence limit
	Initial	Middle	Final				
23	1.67	0.29-0.58	0.10	19,552	0	100	99.985
25	1.67	0.21-0.70	0.14	16,256	1	99.994	99.971
25	1.67	0.35-0.70	0.14	12,960	0	100	99.977
23-25	1.67	0.58-0.70	0.10-0.14	48,768	1	99.998	99.990

Fruit Quality: Phosphine recorded during the three replicate fumigations were:

- Rep .1: 1.5 g m⁻³ to 0.5 g m⁻³ after 24 h, topped up to 0.7 g m⁻³ final concentration 0.3 g m⁻³.
- Rep .2: 1.7 g m⁻³ to 0.7 g m⁻³ after 24 h, no top up required, final concentration 0.4 g m⁻³.
- Rep .3: 1.7 g m⁻³ to 0.2 g m⁻³ after 24 h, topped up to 0.6 g m⁻³ final concentration 0.2 g m⁻³.

Fumigated pears ripened slightly less rapidly than the control pears. Treated fruit were significantly more green and displayed non-significant trends towards lower TSS and higher firmness. There was no occurrence of internal or any other injury. Informal taste tests indicated no off flavours.

CONCLUSIONS

Results of the flower fumigation research enabled BOC Gases to obtain an extension of registration of ECO₂FUME to cover fumigation of cut flowers for export, which became effective in March 1999 (ECO₂FUME is now a registered product of CYTEC Industries Inc.). The registered application conditions are a dosage of 700 ppm PH₃ (approximately 1g m⁻³) for 15 h at a minimum temperature of 15°C. Under these conditions there was minimal damage to flowers and foliage and all test arthropods were killed with the exception that some eggs of certain arthropods, eg. those of two-spotted mites, survived in some fumigations. Consequently the registration is for adult and larval stages, not eggs. It is noted that use in conjunction with Pestigas can reduce the PH₃ concentration required. The fumigation schedule is being used commercially for flowers exported to Japan and the USA.

The citrus study shows ECO₂FUME has potential for use as a replacement for MB for disinfestation of citrus from Qfly. Further work needs to be done to develop means of utilising the fumigation technique by the citrus industry. It is anticipated that, as with grain fumigations, there will be no problems with residues, but this needs to be demonstrated.

The preliminary study with pome fruit also further demonstrated the potential for use of ECO₂FUME for postharvest disinfestation of fruit. ECO₂FUME may also be suitable for disinfestation of vegetables. Leesch (1984) investigated alternatives to MB for fumigation of iceberg lettuce and found that PH₃ was a good alternative, but had the disadvantage of needing to be generated from solid formulations. This problem would be overcome by using ECO₂FUME.

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