EFFICACY OF PLANT VOLATILES FOR POST-HARVEST CONTROL OF WESTERN FLOWER THRIPS IN FRESH PRODUCE

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ABSTRACT

Insect infestation of fresh produce exported from New Zealand is an ongoing issue for exporters. Seventeen low-hazard volatile compounds were screened, for their potential as fumigants for controlling adults, nymphs and eggs of western flower thrips (WFT), *Frankliniella occidentalis* (Pergande). WFT infested chrysanthemum flowers were exposed to volatiles in glass jars with circulation fans. Higher mortality was achieved for adults than for nymphs/eggs. Mortality after the 6 h exposure was greater than after the 2 h exposure. Compounds that had good efficacy against WFT included 2-propyn-1-ol, diallyl sulphide (the main compound of garlic oils), 2-methyl-(E)-butenal and ethyl acetate. Of these, 2-propyn-1-ol caused significant phytotoxicity at the concentrations tested. The other three were tolerated by the plant material and are worth further investigation.
Keywords: broccoli, chrysanthemum, *Frankliniella occidentalis*, fumigants, phytotoxicity, plant volatiles, western flower thrips.

INTRODUCTION

Fresh produce exported from New Zealand has to comply with the quarantine regulations of the importing country. Detection of quarantine pests can lead to compulsory fumigation treatment with methyl bromide or destruction of produce. This project is part of an ongoing effort to provide NZ exporters with preshipment treatments that will assist in meeting NZ export clearance obligations and reduce pest arrivals in the importing country. Effective low-hazard fumigants are required to replace current ozone depleting and highly toxic fumigants.

Western flower thrips (*Frankliniella occidentalis* Pergande, WFT) lives primarily in flowers and is now a significant pest of greenhouse crops around the world (Tommasini & Maini 1995). It was one of the three most commonly found species in thrips-infested consignments imported into the Netherlands between 1987 and 1993 (Vierbergen 1995). It is also found on outdoor crops such as onion, apples, sweet pepper, peas and cucumber. Adults lay eggs into plant tissue and the average incubation period for WFT eggs at 20ºC is 6.4 days (Lublinkhof & Foster 1977).

Previous work on onion thrips showed that adults and nymphs were killed with ethyl formate (Vapormate™) but eggs laid in leek plant tissue were not killed (van Epenhuijsen et al. 2007). Simpson et al. (2007) demonstrated that ethyl formate was also effective against WFT. However, ethyl formate has been shown to be phytotoxic to leek leaves, while another low hazard compound, ethyl acetate, was both effective against thrips and not as phytotoxic (van Epenhuijsen et al. 2007).

A large number of low-hazard compounds have potential as fumigants for fresh produce, such as alcohols (Burditt et al. 1963; Weber et al. 1987; Dentener et al. 1998; Hammond et al. 2000; Jamieson et al. 2003), aldehydes (Aharoni et al. 1980; Hammond et al. 2000; Simpson et al. 2003; Lee et al. 2004), essential oils (Kostyukovsky et al. 2002; Park & Shin 2005), esters, ethers and ketones (Burditt et al. 1963).

A range of these materials was selected for testing against WFT to identify compounds with promise for development as a low-hazard fumigant for preshipment treatment of fresh produce. As well as efficacy against WFT phytotoxic damage to fresh produce was assessed, using chrysanthemum flowers and broccoli florets as model tissues.

MATERIALS AND METHODS

Fumigant compounds

Seventeen chemicals and mixtures were tested for efficacy against WFT (Table 1).

TABLE 1: Compounds used in toxicity and phytotoxicity trial.

Trt no.	Label name	Rate used (g/m^3)	Chemical formula	Chemical group	Supplier
1	Untreated control	-	-	-	-
2	Ethyl alcohol/ ethanol+5% glacial acetic acid[1]	81.95	C_2H_5OH	Alcohol	Merck KgaA, Germany +BDH Chemicals, UK
3	2-propyn-1-ol	14.05	C_3H_4O	Alcohol	Merck Ltd, NZ
4	Propionaldehyde	72.13	C_3H_6O	Aldehyde	Merck Ltd, NZ
5	Propylene oxide	106	CH_3CHCH_2O	Epoxide	BDH Chemicals, UK
6	Ethyl formate	25.4	$C_3H_6O_2$	Ester	Merck-Schuchardt, Germany
7	Acetone	101.4	$(CH_3)_2CO$	Ketone	Merck KgaA, Germany
8	Trans-2-pentenal	4.8	C_5H_8O	Aldehyde	Sigma-Aldrich Pty Ltd Australia
9	Ethyl acetate	154.9	$C_4H_8O_2$	Ester	BDH Chemicals, UK
10	Diethyl ether	130.2	$C_2H_5OC_2H_5$	Ether	Merck KgaA, Germany
11	Allyl acetate	6.1	$C_5H_8O_2$	Ester	Merck Ltd, NZ
12	Tert-butyl-methylether	154.9	$C_5H_{12}O$	Ether	Merck Ltd, NZ
13	P-anisaldehyde	238.2	$C_8H_8O_2$	Aldehyde	BDH Chemicals, UK
14	Trans-anethole	259.9	$C_{10}H_{12}O$	Aromatic unsaturated ether	Merck Ltd, NZ
15	Diallyl sulphide	256.4	$C_6H_{10}S$	Organosulphur compound	Merck-Schuchardt, Germany
16	Manuka oil[2]	548	NA	Essential oil	Tairawhiti Pharmaceuticals, NZ
17	Acetaldehyde	80.2	CH_3CHO	Aldehyde	Sigma Chemical Co, USA
18	2-methyl-(*E*)-butenal	41.5	C_5H_8O	Aldehyde	Sigma Aldrich Chemie GmbH, Germany

[1]Mixture made up.
[2]Ketones removed.

Application method

Treatments were carried out in 1.6 litre glass jars (phytotoxicity testing) and 4.3 litre glass jars (WFT toxicity testing) with metal lids and were done in a fumehood at 20°C. Each treatment was replicated four times throughout the trial.

The chemicals were applied using a dispensing pipette onto a piece of cotton wool taped below a 13 mm access hole in the lid. Immediately after application the hole was sealed with a rubber bung and kept closed for the duration of the trial. The cotton wool was changed for every replicate if the boiling point of the test chemical was over 100°C (treatments 3, 11, 13, 14, 15 and 18).

Computer fans (diameter 40 mm (Manhattan 12V)) suspended halfway down inside the jars were used to circulate the volatile. The wire cable was pulled through a rubber bung that closed a second hole in the lid. Electrical bullet terminals were used to connect and disconnect the parallel-wired fans.

Plant materials

Chrysanthemum plants (*Chrysanthemum morifolium* 'Mum'; Knights Nursery, Auckland) were ordered in advance at different stages and grown-on to produce open flowers. Flower stalks were placed in moist cotton wool and wrapped in aluminium foil to keep them fresh during the treatment period. Broccoli heads, freshly cut at Riverside Gardens (Ashhurst), were divided into florets of approximately 30 g.

The pieces of plant material were suspended inside the jars, in a mesh-wire cage (diameter 65 mm, length 150 mm) that could just slide through the jar opening. After treatment, the lids of the fumigation chambers were taken off, and the plant material was taken out of the jar and aerated in the fume hood for 2 min.

Phytotoxicity testing

Compounds in Table 1 were initially applied to WFT infested flowers and broccoli at a rate of 1 mol/m^3 for 2 h. Where no phytotoxicity was observed from a treatment, this was followed by a second test at 1.5 mol/m^3, and subsequently by a third test at 1.75 mol/m^3. Any phytotoxicity was noted.

Phytotoxicity was tested using chrysanthemum stalks approximately 70 mm long with an open flower and leaves attached, and broccoli florets. After treatment the cotton wool and aluminium foil were removed from the chrysanthemum flowers, which were then placed in 40 ml beakers with water at 20°C. The pieces of broccoli were laid out on plastic weigh trays and covered with plastic sheets. Flowers and broccoli were assessed for reduced shelf life. Any signs of burning of the leaves and petals were noted over a period of 4 weeks for the flowers and 2 weeks for the broccoli.

Toxicity to WFT

Western flower thrips were reared at Crop & Food Research, Palmerston North, on chrysanthemum flowers at 21±1.5°C, 40-55% RH, 16:8 h light:dark. Chrysanthemum plants with open flowers were placed in the WFT rearing unit between heavily infested plants for 4 days to enable thrips to move on to the chrysanthemum plants. Flowers were removed when adults were found and first browning symptoms on petals occurred. In addition, for the 6 h treatment, between four and seven adult thrips were collected and placed on the flowers to be treated. Flowers used in these tests were approximately 30 mm long, fully open and without buds.

Resources available for this trial did not permit more detailed exploration of application rates for each compound tested, therefore effective rates from previous ethyl formate and ethyl acetate trials were converted to mols/m^3 and equivalent amounts were used for the other compounds. Rates for the treatments in the final WFT toxicity experiment were chosen once the effect of the phytotoxicity was known (Table 1). WFT infested flowers were treated for either 2 h or 6 h as described above. Four jars (replicates) were treated for each treatment.

After aeration of the flowers, live and dead thrips were counted. For the 2 h treatment thrips were dislodged from the flowers by knocking the flower twice, 10 times on the collection tray. For the 6 h treatments, the flowers were flicked three times to dislodge thrips onto the collection tray, then the flower was taken apart and all dead and live thrips found in 60 s were counted. The plant material was checked 5 days after treatment to enable the eggs laid in the plant tissue to hatch.

Nymphs, eggs and adults were recorded separately. Mortality rates and 95% confidence intervals were estimated using generalised linear models with binomial distributions, fitted using GenStat (Version 9 for Windows, 2006, VSN International Ltd, Hemel Hempstead, UK).

RESULTS

Phytotoxicity

Several of the treatments caused severe damage to chrysanthemum flowers, which was particularly noticeable at higher rates. Symptoms of damage included leaf damage from minor burning with 2-propyn-1-ol (treatment 3) to significant burning with acetaldehyde (trt 17), and damage to flowers from drooping petals with allyl acetate (trt 11) to collapse of the whole flower with trans-2-pentenal (trt 8).

Symptoms of phytotoxicity were clearer on chrysanthemum flowers than on broccoli florets. Different broccoli heads and different broccoli tissue types showed different symptoms. In addition, there was a lack of clear distinction between chemical damage and the normal processes of tissue senescence. An off-odour was noticeable in some treatments, e.g. trans-2-pentenal (trt 8), allyl acetate (trt 11) and acetaldehyde (trt 17). In treatments 8 (trans-2-pentenal) and 11 (allyl acetate) the broccoli was exuding a transparent liquid. The broccoli treated with trans-anethole (trt 14) remained green long after the other treatments had gone yellow, but it also gave off a smell reminiscent of liquorice.

Toxicity to WFT

The mean percentage mortality of WFT adults and nymphs/eggs is shown in Figure 1. In general, adults tended to be more susceptible to treatments with volatiles than nymphs/eggs. Also, a 6 h exposure to volatiles tended to be more efficacious than a 2 h exposure, in particular against nymphs/eggs. There were large 95% confidence limits associated with mean mortalities due to low numbers of insects treated in some flowers.

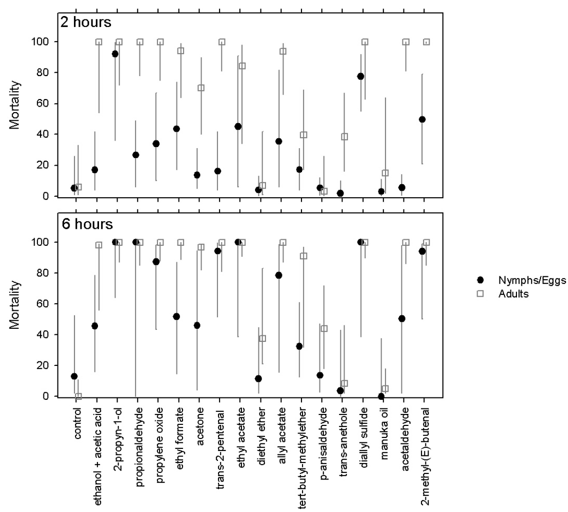

FIGURE 1: Mean mortality (%) of Western flower thrips after a 2 or 6 h exposure to volatiles (bars are 95% confidence intervals).

After a 2 h exposure, mortality levels of WFT treated with 2-propyn-1-o1 and diallyl sulphide were >76% (Table 2). At the longer 6 h exposure, all compounds resulted in high mean mortalities (>78%), except for treatments 10 (diethyl ether), 12 (tert-butyl-methyl ether), 13 (P-anisaldehyde), 14 (trans-anethole), 16 (manuka oil) and the untreated control (Table 3). Four treatments (2-propyn-1-ol, diallyl sulphide, 2-methyl-(E)-butenal and ethyl acetate) resulted in 100% mortality and a further four treatments had over 90% mortality.

TABLE 2: Mean mortality (%, with 95% confidence interval) of total thrips (adults, nymphs and eggs) after 2 h exposure to volatiles. The total number of WFT treated is also indicated.

Treatment no./name		% mortality	(95% CI)	No. WFT treated	
				Adults	Nymphs/Eggs
3	2-propyn-1-ol	98	(42, >99)	11	43
15	diallyl sulphide	76	(51, 90)	9	80
18	2-methyl-(E)-butenal	68	(38, 88)	17	42
6	ethyl formate	58	(28, 83)	14	37
5	propylene oxide	49	(20, 79)	13	44
11	allyl acetate	49	(19, 80)	16	27
8	trans-2-pentenal	44	(21, 70)	17	60
9	ethyl acetate	41	(8, 85)	5	13
4	propionaldehyde	38	(17, 66)	15	58
2	ethyl alcohol/ 5% glacial acetic acid	30	(11, 58)	6	54
7	acetone	25	(12, 44)	21	116
12	tert-butyl-methylether	16	(6, 35)	14	109
17	acetaldehyde	15	(5, 38)	16	70
1	untreated control	6	(1, 29)	21	72
14	trans-anethole	5	(1, 22)	14	79
10	diethyl ether	5	(1, 22)	12	97
13	p-anisaldehyde	5	(1, 19)	15	117
16	manuka oil	4	(1, 18)	8	88

TABLE 3: Mean mortality (%, with 95% confidence interval) of total thrips (adults, nymphs and eggs) after a 6 h exposure to volatiles. The total number of WFT treated is also indicated.

Treatment no./name		% Mortality	(95% CI)	No. WFT treated	
				Adults	Nymphs/Eggs
3	2-propyn-1-ol	100	(87, 100)	27	15
4	propionaldehyde	100	(71, 100)	16	1
9	ethyl acetate	100	(84, 100)	25	7
15	diallyl sulphide	100	(87, 100)	33	7
18	2-methyl-(E)-butenal	98	(82, >99)	37	17
5	propylene oxide	97	(84, >99	31	14
11	allyl acetate	95	(76, 99)	34	6
8	trans-2-pentenal	93	(79, 98)	29	21
17	acetaldehyde	86	(55, 97)	17	5
7	acetone	83	(66, 93)	37	16
2	ethyl alcohol/ 5% glacial acetic acid	78	(53, 91)	19	14
6	ethyl formate	86	(62, 96)	28	11
12	tert-butyl-methylether	59	(39, 77)	27	26
13	p-anisaldehyde	29	(14, 51)	22	20
10	diethyl ether	18	(7, 38)	22	22
14	trans-anethole	6	(2, 20)	29	30
1	untreated control	6	(2, 19)	31	30
16	manuka oil	4	(<1, 15)	40	14

DISCUSSION

This study provides indicative information about the efficacy to WFT of volatiles from 'low-risk' compounds. The approach was to test a number of these compounds but limit the range of treatment concentrations and durations due to restricted numbers of insects available. Greater mortality was achieved for adults of WFT than for nymphs/eggs, and higher mortality was achieved in the 6 h treatments than in the 2 h treatments. Burditt et al. (1963) tested 108 compounds against eggs and larvae of Oriental fruit fly and Mediterranean fruit fly and found that materials toxic to one species were generally toxic to the other. They used 2 h exposure periods. Hammond et al. (2000), testing a range of volatile aldehydes against *Myzus persicae* and *Aphis fabae*, found propanal, (E)-2-pentenal, and 2-methyl-(E)-butenal to be most promising but they also caused phytotoxic effects. These substances were effective against WFT in this trial.

Hammond et al. (2000) also reported that these aldehydes were effective against mealybugs, thrips and whitefly. Some compounds, notably the essential oils, are reported to be highly effective (Park & Shin 2005), but need a fumigation duration of more than 24 h. In the present trial manuka oil only gave low WFT mortalities. The goal was to investigate compounds that were effective in short fumigation times, to fit with the logistics of fresh produce handling, so manuka oil may not be appropriate in this situation.

Compounds that resulted in high mortalities against WFT included 2-propyn-1-ol, diallyl sulphide (the main compound of garlic oils), 2-methyl-(E)-butenal and ethyl acetate. Of these, 2-propyn-1-ol caused significant phytotoxicity at the concentrations tested. The other three did not cause noticeable damage to the plant material. These substances warrant further investigation on a wider range of fresh produce.

A range of factors has been found to influence the level of phytotoxicity of volatile compounds. Otto & Daines (1969) state that humidity levels within the treatment jars could influence the amount and type of tissue damage through increased stomatal opening. Absorption of the treatment compounds will vary but could be significant. For example, ethyl formate as a gas is highly absorbed by fresh produce, especially at raised humidities (Hilton & Banks 1997; B. Page, pers. comm.). Apples treated with ethanol vapour are reported by Jamieson et al. (2003) to have increased levels of ethanol, metabolised to acetaldehyde and ethyl acetate in their tissues. Phytotoxicity of plant volatiles to flowers is reported by Kostyukovsky et al. (2002) to depend on the species and the variety of the flowers. They state that keeping treated flowers at low temperature prevented phytotoxicity effects with some flowers. Symptoms of phytotoxicity were fairly clear on chrysanthemum flowers but assessment of damage to broccoli florets was more complex.

Different broccoli heads and different tissue types showed different symptoms, together with some lack of clear distinction between chemical damage and the normal process of tissue senescence. Observations indicated that some materials caused significant phytotoxic damage at the rates tested. Compounds that were particularly damaging included trans-2-pentenal, acetaldehyde, allyl acetate, 2-propyn-1-ol and trans-anethole.

The most insecticidal of these compounds was 2 propyn-1-ol. There appeared to be no consistent relationship between phytotoxicity and insecticidal activity.

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