The potential of ethyl formate + carbon dioxide to control a range of horticultural pests

M.J. Griffin, L.E. Jamieson, A. Chhagan, N.E.M. Page-Weir, J. Poulton, V.A. Davis, F. Zulhendri and P.G. Connolly

The New Zealand Institute for Plant & Food Research Limited (Plant & Food Research), Private Bag 92169, Auckland
Corresponding author: Melissa.Griffin@plantandfood.co.nz

Abstract Ethyl formate (EF) is a 'generally recognised as safe' (GRAS) compound that can be applied as a fumigant to disinfest fresh produce. This paper reports initial research to determine the dose responses of various pests to ethyl formate+carbon dioxide treatment during 1-, 2-, 3- and 4-h treatments at ambient temperatures between 18 and 23°C. Thrips (mixtures of *Thrips tabaci* and *Frankliniella occidentalis*) on lupin flowers, obscure mealybugs (*Pseudococcus viburni*) on potatoes, and greedy scale (*Hemiberlesia rapax*) on potatoes were more susceptible than either lightbrown apple moth (*Epiphyas postvittana)* eggs on plastic and fifth instar larvae on apples, or codling moth (*Cydia pomonella)* second/third instars and fifth instars on apples. Further efficacy studies are warranted to investigate the commercial viability of EF+CO_2 treatment of fresh produce against other pests, effects on fruit quality, and commercial application logistics.

Keywords ethyl formate, generally recognised as safe, GRAS, codling moth, lightbrown apple moth, mealybugs, scale, thrips.

INTRODUCTION

New Zealand and international horticultural export industries are moving away from using methyl bromide for postharvest disinfestation because of international restrictions on its use (Montreal Protocol), damage to produce and toxicity to workers. A review of Generally Recognised as Safe (GRAS) postharvest disinfestation technologies identified ethyl formate (EF) as having insecticidal properties against a number of pests in short-treatment durations (L.E. Jamieson, unpublished data). EF is a naturally occurring plant volatile that breaks down into formic acid and ethanol in the presence of water (Desmarchelier 1999). EF is registered for use in New Zealand as VAPORMATE™: a non-flammable formulation containing 16.7% by weight EF and 83.3% by weight liquid carbon dioxide. The addition of carbon dioxide has varying effects on the efficacy of ethyl formate depending on the pest species and life stage (Simpson et al. 2007). Addition of 10% CO_2 increased efficacy of ethyl formate against prepupae and adults of western flower thrips, crawlers and adults of grape mealybug and 2-day-old eggs of omnivorous leafroller (Simpson et al. 2007). In contrast, the efficacy of ethyl formate with CO_2 for other species and life stages was similar to that of ethyl formate with air or in some instances lower. However, Haritos et al. (2006) reported benefits of added CO_2. Thus,

EF mixed with 20% CO_2 increased mortality of adults and free living stages of three grain beetles (rice weevil, *Sitophilus oryzae;* red flour beetle, *Tribolium castaneum*; and lesser grain borer, *Rhyzopertha dominica*) compared with treatment using EF alone.

EF has been shown to be effective in the fumigation of a number of products and their associated pests (De Lima 2006; Simpson et al. 2007; van Epenhuijsen 2007; Damcevski et al. 2010; Finkelman et al. 2010). This paper summarises initial trials to determine the relative susceptibilities of a variety of horticultural pests to ethyl formate combined with CO_2 at ambient temperature.

MATERIALS AND METHODS

Insect supply and set up

Codling moth (CM; *Cydia pomonella* L.) larvae were provided from laboratory cultures reared at Plant & Food Research (PFR) as described by Jamieson et al. (2013). Three to five codling moth second/third instar larvae were placed in a small fine mesh cage (approximate diameter 2.8-3.5 cm and 1 cm high) and sealed over the calyx of an apple with Blu-Tack™. Five larvae-infested apples were placed on a cardboard fibre apple tray inside a mesh bag and the insects left to burrow in and establish at 20°C for 2-4 days before treatment. Each replicate was made up of 10 larvae-infested apples. Fifth instar codling moth larvae, reared in conditions to induce diapause (12:12 h light:dark (L:D)), were placed in tissue paper to cocoon for 2-4 days. Tissue paper containing cocooning larvae was cut out and 50 such larvae per replicate were placed around apples for treatment.

Lightbrown apple moth (LBAM; *Epiphyas postvittana* (Walker)) larvae and eggs were provided from laboratory cultures reared at PFR as described in Jamieson et al. (2013). Fifth instar LBAM larvae were placed into large vented pots with tissue paper. One pot represented one replicate, with 30-50 individual LBAM larvae depending on availability. LBAM eggs were 4-6 days old and stored at 20°C, and assessed before treatment for clearing and eye spot formation, which is an indication that the egg is fertile. Each replicate had a minimum of 100 eggs that were assessed as having obvious embryo development before treatment.

Obscure mealybugs (*Pseudococcus viburni* (Signoret)) were reared at PFR in Auckland on sprouting red skinned potatoes at 20°C, 16:8 h L:D. Mealybug-infested potatoes were collected from the colony and placed into vented pots with mesh lids and bases. Each pot represented one replicate, with a minimum of 30 individuals of each life stage.

Greedy scale (*Hemiberlesia rapax* (Cormstock)) were reared on red skinned potatoes and butternut squash at PFR in Auckland at 20-23°C, 16:8 h L:D. Insect-infested potatoes from the colony were placed into vented pots, identical to those used for mealybugs. Each pot represented one replicate, with a minimum of 100 individuals of each life stage.

Lupin flowers (*Lupinus arboreus*), naturally infested with a mixture of thrips species (onion thrips *Thrips tabaci* (Lindeman) and western flower thrips *Frankliniella occidentalis* (Pergande)), were collected from the coastal area surrounding Piha, Auckland, and placed into vented vials. One vial represented one replicate.

For each pest 3–4 replicates were set up and treated.

Treatment procedure

Ethyl formate+CO_2 was applied at ambient temperature (18–23°C), using a 250-litre chamber fitted with a liquid ring vacuum pump (Model TRMB 32-75, Travini Pumps, USA), a compound gauge (WIKA EN-8371, 316 L) and a heated water bath to volatilise EF. A 110-mm spark-proof fan inside the chamber ensured even distribution of the applied gases. Containers of insects were loaded into the chamber and the door was sealed.

The treatment procedure started by decreasing the pressure in the chamber to -70 kPa; at this low pressure the EF could be injected and drawn into the chamber. EF was injected into the chamber via the water bath heat exchange system (held at 90°C) to volatilise the EF liquid.

The chamber was held at low pressure for 15 min before the pressure was increased to ambient by letting in air and the calculated amount of CO_2 that represented the ratio of EF:CO_2 in a VAPORMATE™ formulation (Table 1).

The concentration of EF in the chamber was measured, where possible, every 10 min by taking a 1-ml sample and injecting into a Philips® PYE UNICAM PU4500 Chromatograph. The mean, maximum and minimum values are shown in Table 1. At the end of the treatment time the chamber and fruit were vented for 30 min. For the first 10 min the air inlet at the back was opened and the pressure decreased slightly so the chamber could be flushed with air, at the same time keeping the pressure close to atmospheric pressure so that air could enter the chamber. The door was then opened and left for 20 min.

After treatment the insects were kept at 20°C before assessment. CM larvae, LBAM larvae, obscure mealybug and greedy scale were assessed 2-4 days after treatment. Thrips were assessed 1 day after treatment and the LBAM eggs were assessed 10 days after treatment. All mobile pests were assessed as live (movement) or dead (no movement) when prodded. Sessile life stages of greedy scale were assessed according to morphological characteristics described by Jamieson et al. (2013). LBAM eggs were classified as live if hatched and dead, if fertile and unhatched. Infertile eggs were not included in analyses.

Statistical analysis

For mortality response figures (Figures 1–12), the loess smoothing function (Chambers & Hastie 1992) was used in R (R Development Core Team 2012) to draw a smooth line through the mean mortality points for each pest after exposure to different EF (+CO_2) at each EF concentration/time. An angular transformation (arcsin(sqrt(p))) was applied to the percentage (p) to stabilise the variance (i.e. so that the error bar was appropriate over the entire range of 1-100%). Standard errors for each treatment were calculated at every treatment concentration. The root mean square of these SEMs gave a mean SEM for each life stage exposed to each treatment.

Concentration mortality data for each replicate were fitted using generalised linear model (Chambers & Hastie 1992) with the complementary log-log (clog-log) link (Preisler & Robertson 1989), with concentration as the explanatory variable, to derive estimated lethal concentration (g/m^3) to achieve 99% mortality (LC_{99}). These estimates were calculated as the concentration to achieve a mortality of $c + (1 - c) \times m$, where c was the control mortality and m the estimated proportion mortality. For each life stage, a geometric mean LC and its associated standard error (SEM) were estimated, from which a 95% confidence interval (CI) was calculated. Non-overlap of the 95% CIs is approximately equal to a test for difference at P=0.01.

Table 1 The calculated amount of CO_2 for each concentration of ethyl formate (EF) to mimic an application of VAPORMATE™ (VM: EF + CO_2) and the actual amounts of EF measured.

Concentration (g/m^3)		Calculated (%)		Measured EF (%)		
EF	VM	EF	CO_2	Mean	Maximum	Minimum
5	30	0.16	1.29	0.05	0.07	0.02
10	60	0.32	2.57	0.10	0.21	0.04
15	90	0.48	3.86	0.15	0.31	0.08
20	120	0.64	5.14	0.19	0.45	0.04
30	180	0.96	7.72	0.47	0.80	0.21
40	240	1.28	10.29	0.57	0.86	0.29
50	300	1.60	12.86	0.99	1.47	0.73
60	360	1.92	15.43	1.11	1.14	0.93

RESULTS

The predicted lethal concentrations for 99% mortality (LC_{99}) for all species and life stages tested are shown in Table 2. The most tolerant species was codling moth second/third instars, requiring a predicted 63.5 g EF/m^3+CO_2 in a 4-h treatment to achieve 99% mortality. The number of codling moth recovered from apples ranged between 75 and 210 per treatment (33–93% recovered), indicating that cannibalism was a potential issue. Cannibalism may have also contributed to the high mortality rates in the untreated control (31.2–55.6%). Increasing EF concentration resulted in an increase in mean mortality of second/third instar codling moth larvae (Figure 1). At 5 g EF/m^3+CO_2, mean mortality ranged from 47.8 to 75%. However, at 60 g EF/m^3+CO_2, mean mortality ranged from 96.1 to 100%. Increasing treatment time did not have a significant effect on the mortality of second/third instar codling moth larvae, the majority of which were located inside apples.

Increasing both EF concentration and treatment time from 1 to 3 h resulted in increased mean mortality of fifth instar diapausing CM larvae (Figure 2). At 5 g EF/m^3+CO_2, mean mortality was between 0 and 1.42%. However, at 60 g EF/m^3+CO_2, mean mortality increased to 66.8% for a 1-h exposure and 100% for 3- and 4-h treatment times. Treatment for 2 h gave variable results. Estimated lethal concentrations for a 2- to 4-h treatment were 33.4-51.4 g EF/m^3 (Table 2).

Increasing both EF concentration and treatment time increased mortality of fifth instar LBAM larvae (Figure 3). At 5 g EF/m^3+CO_2, mean mortalities ranged from 0 to 3.1%. However, at 60 g EF/m^3+CO_2, mean mortalities ranged from 90.2% for a 1-h exposure to 99% for a 3- to 4-h exposure. Mean estimated 99% lethal concentrations were 23.5-37.4 g EF/m^3 for a 3- or 4-h exposure (Table 2).

Mean mortality of LBAM eggs (percentage of fertile unhatched) within untreated controls was 27.2 – 31.1%. LBAM eggs were tolerant to EF treatment, requiring a 3- to 4-h exposure to 50-60 g EF/m^3 to achieve >99% mortality (Figure 4). Estimated 99% lethal concentrations for a 3- to 4-h treatment were 57-62.4 g EF/m^3 (Table 2).

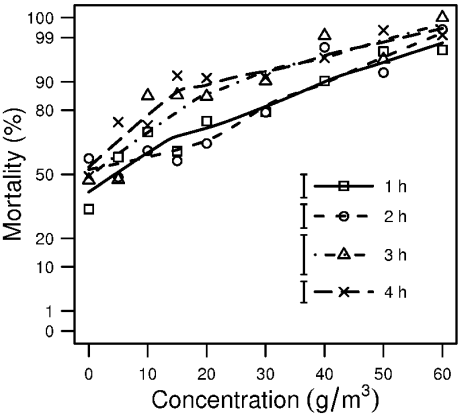

Figure 1 Percentage mortality of second and third instar codling moth larvae at 0–60 g ethyl formate (EF)/m^3 + CO_2 for 1, 2, 3 and 4 h. n = 75–210 per data point.

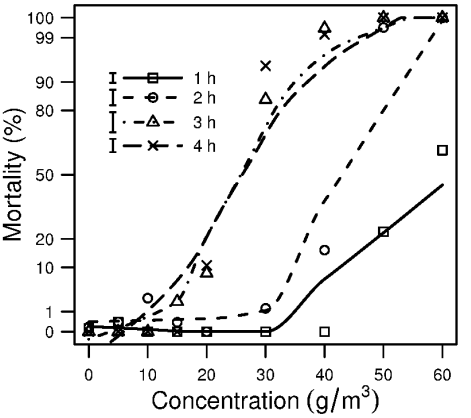

Figure 2 Percentage mortality of fifth instar codling moth larvae at 0–60 g ethyl formate (EF)/m^3 + CO_2 for 1, 2, 3 and 4 h. n =130–167 per data point.

Increasing EF concentration increased the mortality of obscure mealybug (Figures 5–7). Crawlers were the most tolerant life stage assessed after treatment (Figure 5) and this probably resulted from treated eggs hatching. Mean estimated 99% lethal concentrations for obscure mealybug crawlers were 27.5-35.1 g/m^3 for 1-, 3- or 4-h exposures (Table 2).

Table 2 The predicted concentrations (g/m^3) of ethyl formate (EF) with CO_2 to cause 99% mortality (LC_{99}) of various pests exposed to 0–60 g/m^3 EF for 1, 2, 3 and 4 h (LC_{99} (95% CI)). Where LC_{99} could not be calculated because 100% mortality was achieved at lower concentration, the concentration where 100% was achieved is presented. Pests are second and third instar codling moth (CM: 2/3*) and fifth instar codling moth programmed for diapause (CM: D5*); lightbrown apple moth fifth instar larvae (LBAM: 5*) and lightbrown apple moth eggs (LBAM: eggs); obscure mealybug crawlers, nymphs and adults (OMB: crawlers, OMB: nymphs and OMB: adults, respectively); first, second and third instar greedy scale (Scale: 1*, Scale: 2*, Scale: 3*, respectively). n = total number of insects treated in the treatment time.

	Treatment time (h)			
Pest: life stage	1	2	3	4
CM: 2/3*	72.3 (58.5-89.2) n = 1079	65.7 (53.2-81.1) n = 927	59.7 (48.3-73.7) n = 872	63.5 (51.4-78.4) n = 938
CM: D5*	Mortality <90%[1] n = 1617	51.4 (46.2-57.1) n = 1443	34.2 (30.7-38) n = 1483	33.4 (30.1-37.2) n = 1518
LBAM: 5*	62.6 (31.6-123.9) n = 1121	- n =1022	37.4 (18.9-74.1) n = 1008	23.5 (11.9-46.6) n = 1305
LBAM: eggs	Mortality <90%[1] n = 4275	Mortality <90%[1] n = 3717	57 (44.4-73.3) n = 3790	62.4 (48.6-80.2) n = 3530
OMB: crawler	35.1 (25.8-47.8)[2] n = 1608	- n = 1434	27.5 (21.3-35.3) n = 3365	30.7(19.8-47.5)[3] n =3901
OMB: nymph	24.2 (18.8-31.2) n =3654	24.6 (19.1-31.7) n =3904	9.3 (6.9-12.7)[2] n =3493	10 (100%)[4] n =2956
OMB: adult	23 (17.9-29.6) n =1585	21.7 (16.9-27.9) n =2083	10 (100%)[4] n =1383	10 (100%)[4] n =1457
Scale: 1*	19.9 (14-28.2) n =2649	15.3 (10.8-21.7) n =2511	5 (100%)[4] n =2435	15 (100%)[4] n =2328
Scale: 2*	19.2 (13.5-27.3) n =2044	13.6 (9.6-19.4) n =2460	5 (100%)[4] n =2449	10 (100%)[4] n =2316
Scale: 3*	18.8 (13.2-26.7) n =4156	16.3 (11.5-23.2) n =3953	16.6 (11.7-23.6) n =3699	10.4 (5.7-19.2)[3] n =3306
Thrips: adult	10.5 (6.2-17.8)[2] n = 1501	10 (100%)[4] n =609	15 (100%)[4] n =905	10 (100%)[4] n =286
Thrips: larvae	36.6 (23.8-56.4) n =3044	33.5 (21.8-51.7) n =1742	26.4 (17.1-40.6) n =1932	Inconsistent data[5] n =270

[1]LC_{99} was not calculated because mortality did not exceed 90%.
[2]Mean calculated using only two replicates because mortality did not exceed 90% in other replicate.
[3]Only one replicate, because mortality did not exceed 90% in other replicates.
[4]LC_{99} was not calculated because mortality reached 100% at lowest concentration.
[5]LC_{99} was not calculated because mortality increase with concentration increase was not consistent across all replicates.

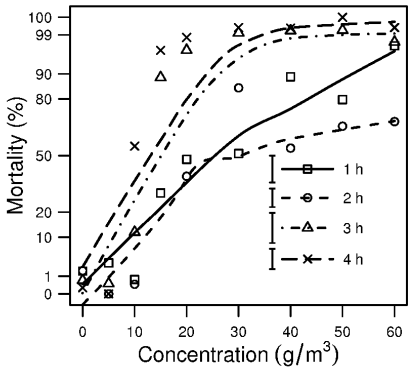

Figure 3 Percentage mortality of fifth instar lightbrown apple moth at 0–60 g ethyl formate (EF)/m^3 + CO_2 for 1, 2, 3 and 4 h. n = 75–159 per data point.

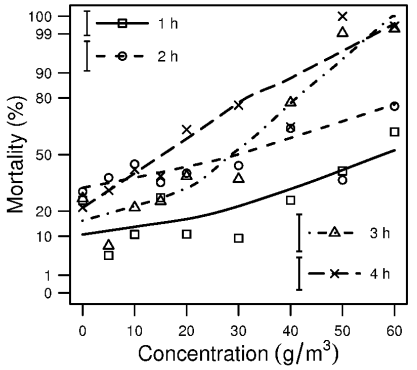

Figure 4 Percentage mortality of lightbrown apple moth eggs at 0–60 g ethyl formate (EF)/m^3 + CO_2 for 1, 2, 3 and 4 h. n = 170–538 per data point.

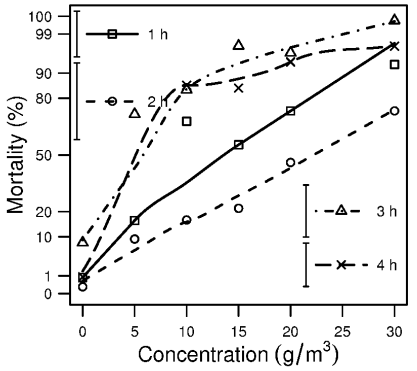

Figure 5 Percentage mortality of obscure mealybug crawlers at 0–30 g ethyl formate (EF)/m^3 + CO_2 for 1, 2, 3 and 4 h. n = 46–970 per data point.

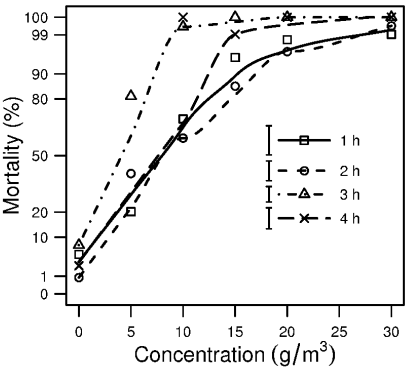

Figure 6 Percentage mortality of obscure mealybug nymphs at 0–30 g ethyl formate (EF)/m^3 + CO_2 for 1, 2, 3 and 4 h. n = 323–1085 per data point.

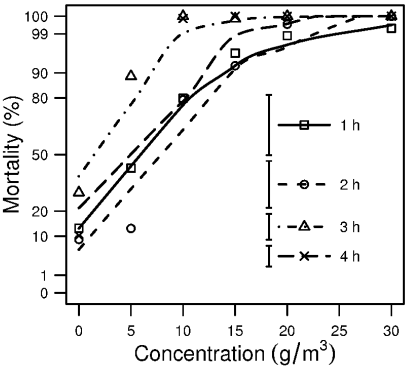

Figure 7 Percentage mortality of obscure mealybug adults at 0–30 g ethyl formate (EF)/m^3 + CO_2 for 1, 2, 3 and 4 h. n = 56–459 per data point.

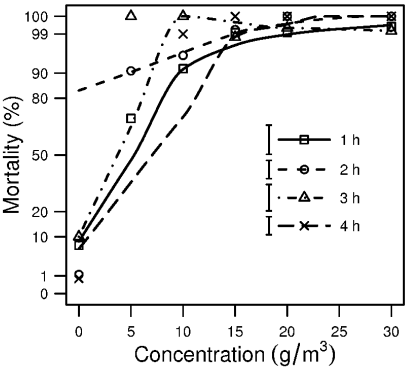

Figure 8 Percentage mortality of first instar greedy scale at 0–30 g ethyl formate (EF)/m^3 + CO_2, for 1, 2, 3 and 4 h. n = 300–821 per data point.

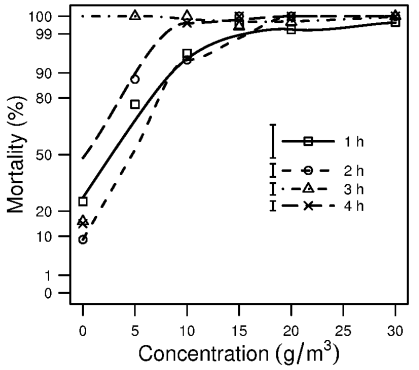

Figure 9 Percentage mortality of second instar greedy scale at 0–30 g ethyl formate (EF)/m^3 + CO_2 for 1, 2, 3 and 4 h. n = 301–817 per data point.

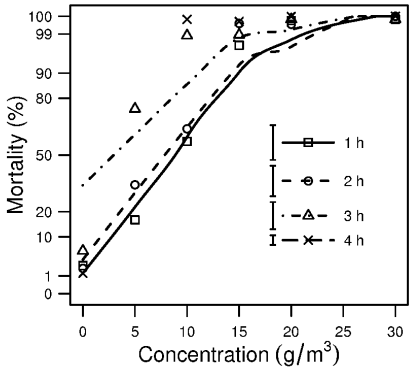

Figure 10 Percentage mortality of third instar greedy scale at 0–30 g ethyl formate (EF)/m^3 + CO_2 for 1, 2, 3 and 4 h. n = 393–1375 per data point.

The tolerance of the different life stages of greedy scale insects to EF were similar for a 1-h exposure (Table 2, Figures 8–10), with an estimated concentration of 18.8-19.9 g EF/m^3 required to kill 99%. For the longer treatments, 100% mortality was reached at the lower doses for first and second instar scale, while third instar greedy scale tended to be more tolerant, requiring an estimated 10.4-16.6 g EF/m^3 for 99% mortality.

Thrips larvae of the mixed species (onion thrips and western flower thrips) were more tolerant than thrips adults (Table 2, Figures 11 & 12). An estimated 26.4-36.6 g EF/m^3 would be required to kill 99% of thrips larvae in 1-3 h.

DISCUSSION

The most susceptible pests in this trial to EF+CO_2 were the mixed thrips species, greedy scale and obscure mealybug. These were generally controlled (99% mortality) by a 10.5-36.6 g EF/m^3+CO_2 treatment for 1-2 h. The most tolerant pests were codling moth (particularly internal second/third instar larvae) and LBAM (particularly eggs). These tolerant species/life stages required a 3- to 4-h exposure to 59.7-63.5 g EF/m^3+CO_2. This pattern of lepidopteran species being tolerant to EF treatment and thrips, mealybug and scale insects being susceptible has also been reported by De Lima (2009, 2010) and Jamieson et al. (2013). De Lima (2009, 2010)

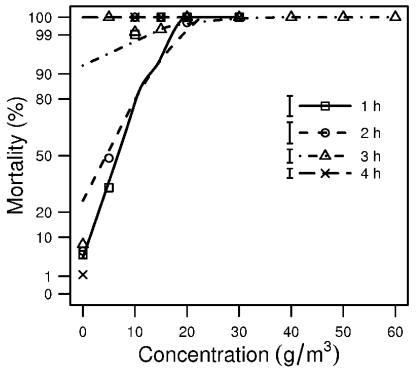

Figure 11 Percentage mortality of adult thrips at 0–60 g ethyl formate (EF)/m^3 + CO_2 for 1, 2, 3 and 4 h. n = 40–340 per data point.

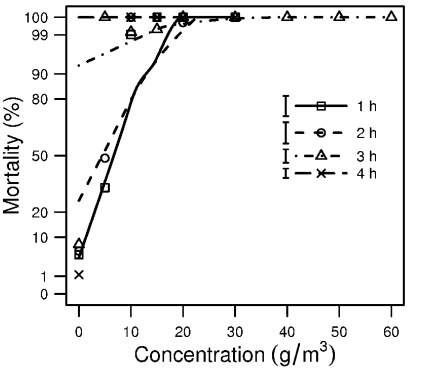

Figure 12 Percentage mortality of thrips larvae at 0–60 g ethyl formate (EF)/m^3 + CO_2 for 1, 2, 3 and 4 h. n = 3–746 per data point.

evaluated EF for controlling a range of pests on citrus and grapes in Australia and recommended a 1-2 h exposure to 20-24 g EF/m^3 for susceptible species (i.e. longtailed mealybug, two-spotted mite, western flower thrips, plague thrips and citrus mealybug) or 30-40 g EF/m^3 for tolerant species (i.e. lightbrown apple moth, Fuller's rose weevil and red-back spider) at 20-25°C. These recommendations were less severe than what the present study has found and it is speculated that this is because the present study was carried out with pests in the presence of fruit, which enabled EF to be hydrolyzed into formic acid and ethanol (Desmarchelier 1999). Codling moth second/third instar larvae that bored into the fruit were harder to kill, probably because less of the EF reached the insect. LBAM eggs were also more tolerant and other studies have shown that eggs are often the most tolerant life stage to EF treatment (Simpson et al. 2007; van Epenhuijsen 2007; De Lima 2009; De Lima 2010).

EF + CO_2 is being used as a safe, packhouse-friendly fumigation treatment as part of a systems approach to managing quarantine pests on bananas and pineapples in the Philippines (H. Krishna, KC Associates Ltd, personal communication). It is also being evaluated to control Californian red scale and western flower thrips on citrus in the USA (F. Pupin, University of California, unpublished data).

Results from this study show that EF+CO_2 is a promising postharvest disinfestation treatment that has the potential to control a range of pests on New Zealand fresh produce exports. Further research is required to test a wider range of pests and life stages that create market access challenges for fresh fruit and vegetable exports. In addition, it will be important to measure the quality of fresh produce after EF treatment. Few reports exist that describe the tolerance of fresh produce to EF. Aharoni et al. (1980) and Simpson et al. (2004) reported few or no effects on strawberry quality after fumigation by 15.5–24.7 g/m^3 EF for 1 h at 21-24°C. There have been reports of no adverse effect on quality of citrus or grapes after exposure to 37.8-40 g EF/m^3 (De Lima 2010; F. Pupin, University of California, unpublished data). Grapes tolerated 54 g EF/m^3 for 4 h at temperatures between 5 and 20°C (De Lima 2009) and apricots tolerated 30-153 g EF/m^3 (Chhagan et al. 2013). Furthermore, few reports exist that describe the sorption rates of EF by fruits and packaging, which has an impact on the amount of EF available for pest kill. Therefore, further studies of the efficacy against other pests and life stages, fruit quality and commercial application logistics are required to investigate the viability of EF+CO_2 as a commercial treatment.

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