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Effects of ethyl formate on fruit quality and target pest mortality for harvested strawberries

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Abstract

Strawberry fruit, western flower thrips and two-spotted spider mites were exposed to a range of ethyl formate (EF) concentrations from 0.8 to 2.4%. Ethyl formate treatments included both single and multiple applications of EF, the multiple applications having a venting period between each application. Additionally, target pests were exposed to EF and elevated levels of CO_2 from 5 to 95 kPa, balance air to total 101 kPa. Exposure to EF resulted in significant mortality for western flower thrips; however, complete control was achieved only in the treatments with the highest cumulative exposures, multiple applications of 0.8% or 2.4% EF. Two-spotted spider mites were less susceptible to EF with the most effective treatment, 2.4% EF, resulting in 66% mortality. Low levels of CO_2 (5 kPa or 10 kPa) combined with 1.3% EF significantly increased two-spotted spider mite mortality, however, levels of CO_2 $\geq$20 kPa significantly decreased mite mortality compared to treatments with EF in air. There was no significant difference in mortality for western flower thrips exposed to 0.8% EF in the presence of CO_2 at 5, 10, 20, and 40 kPa when compared to 0.8% EF in air. Treatment with 0.3% EF with $\geq$40 kPa CO_2 resulted in significantly decreased western flower thrips mortality compared with that of 0.3% EF in air. However, for 0.8% EF, western flower thrips mortality only declined in an atmosphere of $\geq$80 kPa CO_2. There was no significant difference in strawberry condition between treated and untreated fruit, however increased levels of acetaldehyde, ethanol, ethyl acetate and EF were detected in fruit exposed to EF. In two separate experiments, strawberry fruit showed calyx damage in fruit exposed to concentrations of 0.8% or 1.6% EF, respectively.

Keywords: Carbon dioxide; Disinfestation; *Fragaria* × *ananassa* Duch; *Frankliniella occidentalis* (Pergande); Fruit quality; Postharvest; *Tetranychus urticae* Koch; Two-spotted spider mite; Western flower thrips

1. Introduction

The current treatment for postharvest strawberry disinfestation of pests such as western flower thrips [*Frankliniella occidentalis* (Pergande)] and two-spotted spider mite (*Tetranychus urticae* Koch) is methyl bromide fumigation. An alternative treatment is desirable due to the reduced availability and increased cost of methyl bromide as a result of its phase out in 2005 for all uses except quarantine treatments. Low molecular weight volatile compounds such as ethyl formate (EF) are produced by many fruit and vegetables and are important flavor and aroma components. They also have been shown to have insecticidal and fungicidal properties (Nursten, 1970).

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These low molecular weight volatile compounds can potentially degrade to biogenic levels in the tissues of treated commodities before the product reaches the market. This is an advantage over conventional chemicals, which can persist as residues in food products.

Ethyl formate has been used for disinfestation of pests in stored dried fruit since 1927 (Simmons and Gertler, 1945), and also has been used in grains, stored drybeans, tobacco, and other stored products (Merck Index, 1983). More recently, EF has been tested for use in some fresh commodities. For example, packaged head lettuce infested with green peach aphid was exposed to 0.5–1.5% EF at 15 °C under vacuum for up to 2 h. Although aphid mortality was high (93–98%), complete control was not achieved, while damage to lettuce was extensive at higher concentrations and longer exposure times (Stewart and Aharoni, 1983; Stewart and Mon, 1984). Additionally, Western flower thrips on harvested strawberries were completely controlled by 0.5% EF fumigation under vacuum for 1 h at 21 °C with no noticeable effects on berry quality, including fruit flavor (Aharoni et al., 1980). Ethyl formate completely controlled California red scale on harvested grapefruit at concentrations of 1.5% and 3.0% for 3 h at 21 °C with no detectable off-flavors or phytotoxicity (Aharoni et al., 1987). However, 2.0% EF (2 h exposure at 21 °C) did not control Fuller rose beetle on harvested lemons (Soderstrom et al., 1991).

This study evaluated the use of EF alone and in combination with various concentrations of carbon dioxide as a postharvest fumigant for control of the two most common pests on California strawberries, western flower thrips and two-spotted spider mites. Initial treatments indicated that increased concentrations of CO_2 in the presence of EF could increase pest mortality. The effects of EF and CO_2 treatments on strawberry condition and quality were also investigated.

2. Materials and methods

2.1. Ethyl formate concentration in the presence of strawberry fruit

Strawberry fruit (*Fragaria* × *ananassa* Duch. cv. 'Diamante') were obtained from a local distributor and sorted for calyx and overall berry condition. Strawberry fruit (550 g, 15% load factor by weight) were placed in a 3.8 L jar and exposed to 0.8, 1.6, 2.4% or 3.2% EF for 120 min. Airspace samples were drawn from the treatment jars at 20 min intervals to monitor reductions in EF concentration resulting from absorbtion of the material by the fruit. An empty jar was injected with 1.6% EF as a control. Headspace samples were also drawn during treatment to monitor ethylene, CO_2 and O_2 concentrations at 0, 60, and 120 min from jars containing strawberry fruit both with and without EF.

2.2. Target pests

Western flower thrips were reared in the laboratory on fresh green beans at a constant temperature of 24 °C (12L:12D). For exposure to EF, a section of green bean with 100–200 s instar thrips was placed in a 59 ml plastic portion cup (Solo Cup Company, Urbana, IL) with a vented lid. Two-spotted spider mites were reared in the laboratory on cotton seedlings at a constant temperature of 24 °C (24L:0D). For exposure to EF, mite-infested cotton cotyledons containing 50–200 mites of mixed stages were placed in portion cups with vented lids. Portion cups containing pests were placed inside treatment jars (two cups per jar), with or without strawberry fruit present, and treated as described below.

2.2.1. Ethyl formate treatment of fruit and pests in air

Strawberry fruit, two-spotted spider mites and western flower thrips were exposed to EF at 24 °C in 3.8 l glass jars (3 replications; 20 berries per treatment; 550 g) sealed with rubber stoppers. Reagent grade, 2 °C liquid EF (Fisher Scientific, Pittsburgh, PA, purity 99.5%) was injected through a rubber septum covering an inlet port in the rubber stopper onto filter paper affixed to the underside of the stopper. A separate outlet port was used to sample the airspace in the treatment jars for EF concentration using a 3 mm diameter plastic tube in the outlet port protruding approximately half-way into the jar. Airspace samples were obtained during tests using a gas-tight, 1 ml syringe. Ethyl formate concentration was measured using a gas chromatograph (GC-9AM; Shimadzu Scientific Instruments, Columbia, MD) fitted with a 60/80 carbopack column with 5% carbowax (Supelco, Bellefonte, PA), flame ionization detector at 250 °C,

injection port temperature of 250 °C (N_2 as carrier gas) and oven temperature of 85 °C.

For initial treatments, EF concentrations and exposure times were as follows; 0.8% EF for 60, 90 min or 120 min, 1.6% EF for 60 min, and 2.4% EF for 60 min. For treatments with multiple EF applications, berries, thrips and mites were fumigated at 60 min intervals at 24 °C with 0.8% EF at two (0.8%–0.8%) or three (0.8%–0.8%–0.8%) repeat applications, each separated by a 60 min venting period at 24 °C.

2.2.2. Ethyl formate treatment of fruit and pests in CO_2

To test the effects of elevated levels of CO_2 in the presence of EF on target pests, jars containing pests were purged with 5, 10, 20, 40, 80 kPa or 95 kPa CO_2, balance air to total 101 kPa, until equilibration was achieved, after which the jars were sealed. Treatment jars were then injected with 0.3% or 0.8% EF for western flower thrips, or 1.3% EF for two-spotted spider mites. In a separate test, EF was applied at 1.6, 1.8% or 2.0% each with 10 kPa CO_2 for 60 min. Following EF treatment, fruit and pests were held in ambient air (24 °C) for 60 min after which strawberry fruit were placed at 0 °C (RH 60%) for 24 h, then evaluated for quality. Target pests were held at 24 °C for 48 h, then evaluated for mortality.

2.3. Fruit quality

Post-treatment fruit quality evaluations included firmness, internal and external color, soluble solids, volatile content, calyx damage, and berry damage. Fruit firmness and color were measured on opposite sides of each fruit. Firmness was measured using the penetration force of a 3 mm diameter cylindrical metal tip mounted to a penetrometer (Ametek, Largo, FL). Color was measured with a Minolta Chromameter (model CR-300; Ramsey, NJ) in CIE L*a*b* mode under CIE Standard Illluminant C. Internal color was measured by slicing the berry longitudinally and taking one reading per side adjacent to the central cavity. Calyx and berry damage were evaluated subjectively and scored as one (none), two (slight, <20% affected), three (moderate, 20–60% affected) or four (severe, >60% affected). Calyx damage presented as vascular tissue darkening, discoloration, and drying. Berry damage included bruising, decay, and loss of fruit integrity (weakened and easily ruptured skin surface).

2.3.1. Strawberry fruit soluble solids and volatiles

Five composite samples of four fruit each (each sample representing a replication) were juiced together. Soluble solids content of the juice was measured using a refractometer (Abbe model 10450; American Optical, Buffalo, NY). Fermentative volatile content was determined as described by Ke et al. (1991). Five milliliters of juice were placed in a glass tube (16 mm × 125 mm), sealed with a rubber septum and stored at −22 °C. For subsequent analysis, samples were thawed in a 24 °C water bath for 5 min, then held for 1 h at 65 °C. A 1 ml headspace sample was analyzed for acetaldehyde, methanol, ethanol, ethyl acetate, and ethyl formate by gas chromatography. Volatile compounds were quantified by comparison to known standards.

Data were analyzed by JMP Statistical Discovery (SAS Institute, 1989) using analysis of variance. A Tukey–Kramer significant difference test (HSD) was used for mean separation of fruit quality and pest mortality data.

3. Results

3.1. Fumigant concentration

The EF concentration in treatment jars decreased rapidly in the presence of strawberry fruit (15% by weight). EF concentrations in the jars equilibrated after approximately 100 min to less than 30% of the initial concentration (Fig. 1). Concentrations of CO_2 in the untreated control jars were recorded as 0.4, 0.7, and 1.4% at 0, 60, and 120 min, respectively. Concentrations of CO_2 in the EF treatment jars were 0.3, 1.1, and 2.0%, respectively. Levels of O_2 were maintained above 18.3% for the duration of the treatment in both the control and EF treatments. Ethylene was not detected in any of the samples.

3.2. Target pest mortality

3.2.1. Mortality with ethyl formate in air

All EF treatments resulted in significant western flower thrips mortality; however, complete mor-

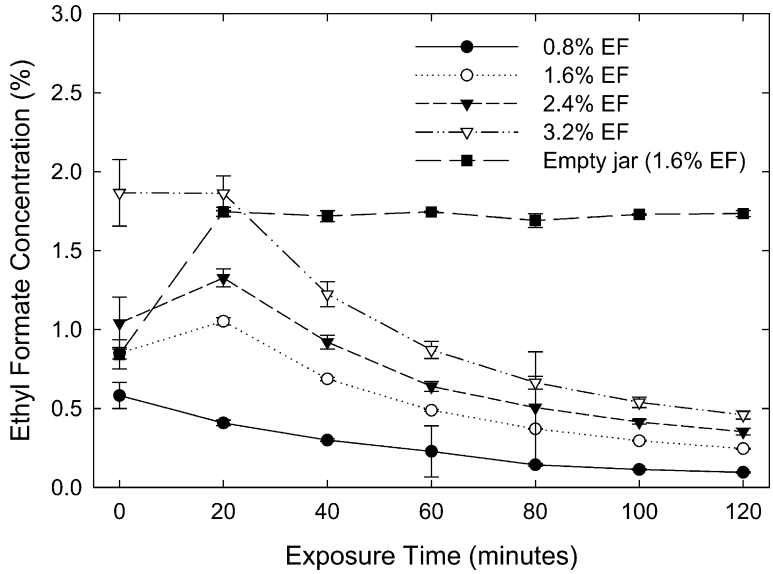

Fig. 1. Ethyl formate concentration (%) over time during exposure of strawberry fruit to 0.8, 1.6, 2.4% or 3.2% EF for 2 h at 24 °C (load factor 15% by weight). An empty jar was injected with 1.6% EF as a control.

tality was achieved only in the treatments with the highest cumulative exposures: 0.8%–0.8%, 0.8%–0.8%–0.8%, and 2.4% (Table 1). Two-spotted spider mites were less susceptible to EF. The treatments with the two highest cumulative exposures, 0.8%–0.8%–0.8% and 2.4%, resulted in 57 and 66% mortality, respectively (Table 1).

Table 1
Percent mortality of second instar western flower thrips and two-spotted spider mites (mixed stages) following single and multiple exposures to ethyl formate at 24 °C in the presence of strawberry fruit (load factor by weight 15%)

Ethyl formate concentration (%)	Time exposed (minutes)	Western flower thrips	Two-spotted spider mites
0	–	7.2 a[a]	4.5 a
0.8	60	99.7 b	18.3 a,b
0.8	90	99.6 b	22.3 ab
0.8	120	99.4 b	26.5 b
0.8–0.8[b]	60–60	100.0 b	45.6 c
0.8–0.8–0.8	60–60–60	100.0 b	56.5 c,d
1.6	60	99.9 b	52.3 c,d
2.4	60	100.0 b	66.3 d

[a] Means in a column followed by the same letter are not significantly different at the 5% level.

[b] Series of numbers refers to multiple injections; 0.8%–0.8% refers to exposure to 0.8% EF for 1 h, jar opened for 1 h to ambient air, then exposure to 0.8% EF for a second hour.

3.2.2. *Mortality with ethyl formate combined with elevated CO_2*

Mortality of western flower thrips exposed to 0.3% EF with CO_2 concentrations of 5, 10, and 20% was similar to thrips exposed to 0.3% EF in air (0.03 kPa CO_2) (Table 2). There was no difference in mortality

Table 2
Percent mortality of second instar western flower thrips and two-spotted spider mites (mixed stages) exposed to ethyl formate (EF) for 60 min at 24 °C in varying concentrations of CO_2 without fruit present

Carbon dioxide (%)	Western flower thrips		Two-spotted spider mite
	0.3% EF	0.8% EF	1.3% EF
Untreated control[a]	12.5 a[b]	6.8 a	3.3 a
0.03	79.4 b	96.6 d	57.8 c
5	83.7 b	97.7 d	73.8 d
10	89.0 b	97.6 d	76.7 d
20	78.2 b,c	96.4 c,d	30.6 b
40	60.4 c,d	91.0 b,c,d	23.1 b
80	49.4 d,e	82.9 b,c	18.3 b
95	37.0 e	79.0 b	18.6 b

[a] Untreated control thrips and mites were not exposed to EF, however respective mortality data is shown in the appropriate columns as a comparison to EF and carbon dioxide treatments.

[b] Means in a column followed by the same letter are not significantly different at the 5% level.

for western flower thrips exposed to 0.8% EF in the presence of CO_2 at 5, 10, 20, and 40 kPa when compared to 0.8% EF in air. Treatment with 0.3% EF with ≥40 kPa CO_2 resulted in decreased western flower thrips mortality compared with that of 0.3% EF in air, 5 kPa or 10 kPa CO_2. However, for 0.8% EF, western flower thrips mortality did not decline until the CO_2 concentration reached ≥80 kPa CO_2. Two-spotted spider mites exposed to 1.3% EF showed a sharp increase in mortality with the inclusion of 5 kPa and 10 kPa CO_2 compared with mites treated with 1.3% EF in air (Table 2). Mortality then rapidly declined at 20 kPa CO_2 to below that of 1.3% EF in air, and continued to decrease as concentrations of CO_2 increased, although there was no significant difference in mortality between 20 and 95 kPa CO_2.

In follow-up treatments with 1.6, 1.8% or 2.0% EF in air or in 10 kPa CO_2, there were no significant differences in western flower thrips mortality between treatments, or between atmospheres (Table 3). However, two-spotted spider mite mortality increased with increasing EF concentration and was significantly higher in treatments including 10 kPa CO_2 as compared to those with EF in ambient air.

3.3. Fruit quality

There were no significant differences between treated and untreated strawberry fruit for firmness, color, berry damage, or soluble solids (Table 4). There was no difference in calyx damage between fruit exposed to 0.8% EF and control fruit for any of the exposure times tested, including multiple EF applications; however, exposure to 1.6 and 2.4% EF for 60 min resulted in slight to moderate and severe calyx damage, respectively (Table 4). In the follow-up experiment, the was no increase in calyx damage in fruit exposed to 1.6, 1.8% or 2.0% EF as compared with the control fruit (Table 3), and inclusion of CO_2 with EF had no effect on calyx damage.

Table 3
Percent mortality of second instar western flower thrips and two-spotted spider mites (mixed stages) and calyx damage for strawberry fruit following exposure to ethyl formate at 24 °C for 60 min (load factor by weight 15%)

Ethyl formate concentration (%)	Atmosphere	Western flower thrips	Two-spotted spider mites	Strawberry calyx damage[a]
0	–	15.2 a	14.6 a	1.8 a
1.6	Air	99.9 b	63.9 b	2.3 a
1.6	CO_2	100.0 b	73.1 c	2.4 a
1.8	Air	100.0 b	59.7 b	2.4 a
1.8	CO_2	100.0 b	80.1 d	2.6 a
2.0	Air	100.0 b	73.0 c	2.6 a
2.0	CO_2	99.7 b	86.8 e	2.6 a

Means in a column followed by the same letter are not significantly different at the 5% level.

[a] Damage score: one (none), two (slight), three (moderate), four (severe).

Table 4
Mean effects of single and multiple exposures to ethyl format at 24 °C on volatile content and strawberry fruit quality. Fruit evaluation after treatment and 24 h at 0 °C

Ethyl formate concentration (%)	Time exposed (minutes)	Fruit quality						Volatiles (μmol mol^{-1})			
		Calyx damage[a]	Berry damage	Firmness (*N*)	Soluble solids (%)	External color (*H*)	Internal color (*H*)	Acet-aldehyde	Ethanol	Ethyl acetate	Ethyl formate
0	–	1.4 a,b[b]	1.1	3.5	7.1	39.7	61.0	0.6 a	7.7 a	2.6 a	0.5 a
0.8	60	1.1 a	1.1	3.5	7.0	39.7	63.8	1.0 a,b	13.7 a	3.3 a	0.9 a,b
0.8	90	1.5 b	1.2	3.6	7.2	39.6	62.9	1.6 a,c	39.9 a,c	8.9 a,b	1.0 a,c
0.8	120	1.5 b	1.3	3.3	6.9	39.8	63.8	1.7 a,b,c	26.4 a,b	5.9 a,b	1.5 c
0.8–0.8[c]	60–60	1.3 a,b	1.1	3.4	7.0	40.4	62.2	2.1 c	59.4 a,c	13.2 b	1.6 c
0.8–0.8–0.8	60–60–60	1.4 a,b	1.1	3.6	7.1	40.6	63.7	2.5 d,c	99.3 c,d	27.5 c	1.5 b,c
1.6	60	2.3 c	1.3	3.5	7.2	41.2	60.8	2.7 d,c	83.3 b,c,d	16.8 b	1.5 b,c
2.4	60	4.0 d	1.2	3.6	7.3	39.0	66.5	3.3 d	122.4 d	31.3 c	1.4 b,c
Significance (*P*)		<0.5	ns	ns	ns	ns	ns	<0.5	<0.5	<0.5	<0.5

[a] Damage score: one (none), two (slight), three (moderate), four (severe).

[b] Means in a column followed by the same letter are not significantly different at the 5% level.

[c] Series of numbers refers to multiple injections; 0.8%–0.8% refers to exposure to 0.8% EF for 1 h, jar opened for 1 h, then repeated exposure to 0.8% EF for a second hour.

3.3.1. Volatile compounds

Strawberry acetaldehyde (Aa), ethanol, and ethyl acetate concentrations were greater with increased exposure to EF, either from multiple exposures or by exposure to higher concentrations of EF (1.6% or 2.4%) (Table 4). The highest concentration of Aa was detected in fruit treated with 2.4% EF, with an Aa concentration more than five-fold higher than that of the control fruit. The highest ethanol and ethyl acetate concentrations were detected in fruit treated with 2.4% EF, which had ethanol and ethyl acetate concentrations 16 and 12-fold higher than that of the control fruit, respectively. Ethyl formate concentrations were approximately three-fold higher in treated fruit compared with control fruit, with the exception of fruit exposed to 0.8% EF for 90 min or less (Table 4).

4. Discussion

Our results indicate that low concentrations of EF do not impact strawberry fruit quality for the parameters measured, even when multiple applications are used. These findings are similar to those of Aharoni et al. (1980) who detected no calyx damage, organoleptic quality loss, or off odors in strawberries exposed to 0.5% or 1.0% EF under vacuum. In our study, increases in volatiles were detected in treated fruit compared with untreated control fruit, although it is uncertain as to whether these changes would be perceived by human sensory evaluations. Formal taste tests were not conducted because EF is not a registered material at this time.

The rapid absorption of EF by the strawberry fruit concurs with the findings of researchers experimenting with other commodities. Stewart and Aharoni (1983) reported even higher absorption rates in packaged head lettuce compared with that of our strawberry fruit. Our results did not indicate a significant increase in western flower thrips or two-spotted spider mite mortality with increasing exposure time to EF, presumably due to the depletion of the fumigant.

The small amount of CO_2 that accumulated in the treatment jars during the test would not have a significant impact on mortality. In fact, control mortality was very low with only 7.2% mortality for WFT and 4.5% mortality for two-spotted spider mites. In addition, a similar accumulation of CO_2 would be expected in commercial applications using the same load factor.

Ethyl formate effectively controlled western flower thrips at concentrations that did not affect strawberry fruit quality. Attempts to control two-spotted spider mite were unsuccessful, even with high concentrations and multiple applications of EF. While multiple application of EF increased mite mortality, mortality remained well below 100%. Combined treatments with EF and 10 kPa CO_2 increased mite mortality significantly, but did not result in complete control.

Reports in the literature indicate that elevated CO_2 atmospheres can increase, decrease, or have no impact on insect mortality when combined with various fumigants (Bond and Buckland, 1978; Soderstrom et al., 1991; Simpson et al., 2003). Pest mortality in the presence of increasing CO_2 concentrations with acetaldehyde (Simpson et al., 2003) or methyl bromide (Jones, 1938) followed a similar pattern to our mortality data with combined EF and CO_2 treatments. Studies on the effects of CO_2 on certain insect species indicate that 10–15 kPa CO_2 has an excitatory effect on the insects' respiratory system (McGovran, 1932). When insects are exposed to concentrations of CO_2 in excess of 20 kPa, an initial increase in respiration is rapidly followed by suppressed respiratory function (Kitchel and Hoskins, 1935). Although the exact mode of action of increased CO_2 alone or in the presence of fumigants on insects is unknown, increased concentrations of CO_2 have been associated with spiracular opening and elevated water loss in insects under low humidity (Mellanby, 1934).

Our study indicates that, for EF, the concentration of CO_2 is critical and response varies according to the target pest and EF concentration used. For Western flower thrips, EF combined with $\leq$20 kPa CO_2 did not provide increased mortality compared to EF treatments in air; however, mortality of two-spotted spider mites increased in the presence of 5 kPa or 10 kPa CO_2 with EF. The effect of CO_2 was reversed at higher concentrations, and $\geq$20 kPa or $\geq$40 kPa CO_2 resulted in lower mite or thrips mortality, respectively, compared to treatments with EF in air.

This is an important consideration for future work with combinations of CO_2 and fumigants, as the optimum proportions of these gases may be species specific for optimal performance of any given fumigant. Our results indicate that mortality of at least two types

of pest species can be significantly decreased if CO_2 levels in the presence of the fumigant are too high.

Ethyl formate is currently in the process of being formulated with CO_2 for commercial use in Australia and New Zealand. Our results indicate that CO_2 used in commercial preparations could reduce or increase the mortality of certain pests, depending on the concentrations used and the targeted pest. Further studies are warranted for targeted pests to insure that optimal levels of CO_2 are used in combination with EF.

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