Alternative Fumigants to Methyl Bromide for Killing Pupae and Preventing Emergence of Apple Maggot Fly (Diptera: Tephritidae)[1]

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Abstract Effects of methyl bromide, ECO_2FUME (phosphine gas + CO_2), Vapam (sodium methyldithiocarbamate), chloropicrin, Telone II (1, 3 dichloropropene), and chloropicrin + Telone II on killing the pupae and preventing adult emergence of apple maggot fly, *Rhagoletis pomonella* (Walsh), was determined. In an experiment performed inside glass flasks, pupal mortality caused by all fumigants 7 d after a 4-h exposure was significantly higher than in the control, but effects of the five fumigants on pupal mortality 7 d post exposure did not differ consistently even though chloropicrin caused the highest absolute mortality. In contrast, fumigant effects on adult emergence were consistent and clearer in that chloropicrin, Telone II, and chloropicrin + Telone II prevented all emergence of adult flies over 111 - 112 d whereas methyl bromide, ECO_2FUME, and Vapam reduced it by only 41 - 73% compared with controls. The differences in pupal mortality and adult emergence patterns suggest that the fumigants acted slowly. This is the first study that shows chloropicrin, Telone II, and the two in combination could be alternative fumigants to methyl bromide for killing pupae and preventing emergence of *R. pomonella*.

Key words *Rhagoletis pomonella*, methyl bromide, ECO_2FUME, Vapam, chloropicrin, Telone II

Much emphasis has been placed on finding alternatives to methyl bromide for controlling agricultural pests (Miller 2001) since the *Montreal Protocol on Substances That Deplete the Ozone Layer* international treaty was adopted in Montreal in 1987 to phase out ozone-depleting substances. Methyl bromide has been used to treat fruit for tephritid fruit fly larvae for many years (e.g., Jones and Schuh 1953, Sandford 1962, Roth and Richardson 1970) and continues to this day (Hallman and Thomas 2011). Methyl bromide also has been tested against pupae of apple maggot fly, *Rhagoletis pomonella* (Walsh) (Diptera: Tephritidae), but results indicate that pupae are able to survive 8-h exposures to this fumigant (Roth and Richardson 1970).

Rhagoletis pomonella is a quarantine pest of apple [*Malus domestica* (Borkh.) Borkh] in the western U.S. that is abundant in western Washington state (Yee and Goughnour 2008). There the fly attacks apples in backyards and in small orchards. Fly pupae are nonmobile and occur in the soil from fall to summer, and consequently fumigation of soil is an option for managing the fly. Fumigants also could be used as

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treatments for rooted apple trees exported to Canada, which are regulated for *R. pomonella* (Canadian Food Inspection Agency 2010), as well as for potted, ornamental plants in nurseries. Soils in pots with these plants could potentially harbor pupae. For example, the fly also attacks small ornamental fruits such as cotoneasters (*Cotoneaster* spp.) (Yee and Goughnour 2008). Hypothetically, larvae that develop in cotoneaster fruit could drop into the soil below, pupate, be shipped across state lines or country borders, and eventually produce adults that could attack apples.

Non ozone-depleting fumigants used against stored products pests, nematodes, and various soil-borne organisms include phosphine (Liu 2008), sodium methyldithiocarbamate (Johnson et al. 1979), chloropicrin (South et al. 1997), and 1, 3 dichloropropene (Qiao et al. 2011). Despite having been available for many years and having been shown to be effective against many pest organisms, none of these fumigants has been tested against pupae of *R. pomonella*. Therefore, the objectives of this study were to determine possible alternative fumigants to methyl bromide for killing pupae and preventing emergence of *R. pomonella*.

Materials and Methods

Insect source. Apple maggot pupae originated from naturally-infested apples and ornamental hawthorn fruit (*Crataegus monogyna* Jacq.) collected in Clark and Skamania counties in southwestern Washington state in August and September 2010. Apples and hawthorns were brought to a laboratory at the Washington State University and Extension Unit in Vancouver and placed in tubs for rearing of larvae. Larvae that emerged from fruit pupated in the tubs and were collected periodically over a 2-month period. Pupae were then stored in 0.473- l containers with moist soil (a 1:1:1 volume mix of sand:peat moss:vermiculite) and chilled at 3 - 4°C for 6 months (November 2010 to May 2011) before exposure to fumigants. Pupae were removed from chilling and held at 7 - 16°C for 7 d before exposure to fumigants. A total of 1400 pupae was used for the experiment.

Experimental design. Six fumigants were evaluated in the experiment (Table 1). Rates used were label rates. The rate of methyl bromide used (Table 1) was equivalent to that listed for fumigating plants (nonfood product) (0.048 kg/m^3) (Great Lakes Chem.Corp. 2005). The rate for ECO$_2$FUME was equivalent to the high rate listed for raw agricultural commodities, processed foods, animal feed, and nonfood commodities (Cytec Industries, Inc. 2005). The rates used for Vapam (AMVAC Chem. Corp. 2010), chloropricrin (Trical 2006), Telone II (Dow AgroSciences 2009), and chloropicrin + Telone II were equivalent to high rates listed for fumigating soils. The percentages of chloropicrin and Telone II in the chloropricrin + Telone II treatment (Table 1) were the same as in the Telone C17 product manufactured by DowAgroSciences (2010).

On 11 May, 20 pupae were placed on the bottom of each of 35 one-l Erlenmeyer flasks (21 cm tall and 12 cm wide at the bottom), with 5 replicates for the control and each of the 6 fumigant treatments. Pupae were exposed inside flasks without soil. Controls were not exposed to any fumigant. Methyl bromide was a liquid in the compressed state, and was removed from the cylinder using a syringe and injected into a 15.2 × 15.2 cm Kynar gas sampling bag with a silicone septum valve for receiving gases (Cole-Parmer, Vernon Hills, IL). The methyl bromide volatilized immediately inside the bag. The desired amount of methyl bromide gas (Table 1) was removed from the bag using a syringe and injected into the flask with pupae through a hole in

Table 1. Fumigants tested in 2011 experiment against *R. pomonella* pupae

Fumigant (physical state when delivered)	Manufacturer or Source	EPA Reg. No.	Rate/liter
Methyl Bromide (Meth-O-Gas® 100) (liquid)	Great Lakes Chem. Corp., West Lafayette, IN	5785 - 11	12.1 ml[a]
ECO$_2$FUME (2% phosphine gas + CO_2) (gas)	Cytec Industries Inc., West Paterson, NJ	68387 - 7	39 ml[b]
Vapam (42.0% sodium methyldithiocarbamate) (liquid)	AMVAC Chem. Corp., Los Angeles, CA	5481 - 468	230 µL[b]
Chloropicrin (99% chloropicrin = trichloronitromethane, TriClor Fumigant) (liquid)	Trical, Inc., Hollister, CA	58266 - 2-11220	92 µL[b]
Telone II (1, 3 dichloropropene, 97.5%) (liquid)	DowAgroSciences, Indianapolis, IN	62719 - 32	170 µL[b]
Chloropicrin (16.5%) + Telone II (81.2%) (liquid)	Trical, Inc., DowAgroSciences	58266 - 2-11220, 62719 - 32	170 µL[b]

[a] Equivalent to 0.048 kg/m^3 on label for nonfood products. [b] Maximum label rate.

a rubber stopper on top of the flask. The hole was then plugged. Parafilm® (Pechinney Plastic Packaging, Chicago, IL) was wrapped around the stopper and flask to ensure a tight seal. ECO$_2$FUME was directly removed from the cylinder as a gas through a regulator connected to a tube and syringe. The syringe was then injected into a gas sampling bag as with methyl bromide. Vapam, chloropricrin, Telone II, and chloropicrin + Telone II were liquids and were removed directly from the cylinders using syringes and placed in 15-ml Nalgene vials and tightly sealed. The desired amount for each fumigant was then removed from a vial using a syringe and then injected into the flask holding the pupae through the hole in the stopper. The hole was plugged and the Parafilm was wrapped around the stopper. The Telone II and Vapam did not volatilize immediately and the liquid when injected into the flask ran down to the bottom of the flask and contacted the pupae. Pupae were exposed to fumigants for 4 h in the shade. Ambient temperatures between 1100 and 1500 h when pupae were exposed to fumigants ranged from 13.8 - 14.2°C (Hobo data logger, Onset Computer Corp., Cape Cod, MA). On 12 May, the same number of replicates and fumigant application methods as on 11 May were used. However, the 20 pupae in each flask were placed inside a small bag made of light nylon tulle fabric with 1.5 mm^2 openings (Walmart, Bentonville, AR), tied, and suspended with a string 5 cm above the bottom of the flask so that the pupae could not contact the liquid. Ambient temperatures during exposure between 1000 and 1400 h ranged from 14.9 - 16.1°C.

For both 11 and 12 May tests, stoppers on the flasks after the 4-h exposures were removed and fumigants released into the air outdoors. After fumigants were released, the pupae were stored inside an unheated shed (7.5 - 31.4°C, mean 21°C) with exposure to natural daylight inside clear, sealed 0.473-l containers with 57 g of 15% moist 1:1:1 peat moss:sand:vermiculite soil for 111 - 112 d (until 31 August 2011).

Efficacy of the fumigant treatments on both dates was determined by (1) numbers and percentages of pupae that were dead at 7 d postexposure to fumigants and (2) numbers and percentages of adults that emerged over the 111 - 112 d postexposure. Mortality was recorded at 7 d postexposure by dissecting 5 of the 20 pupae from each replicate and examining their condition. Live pupae were creamy yellow; dead pupae were dried or a brown semiliquid. Adult emergence was determined by allowing the other 15 pupae per replicate to develop. The numbers of flies that emerged were counted every 3 - 7 d over the 111 - 112 d.

Data analysis. Numbers of dead pupae and of adults that emerged were analyzed using a Kruskal-Wallis test because data were not normal even after square-root transformation, followed by Fisher's least significant difference (LSD) test. The LSD method is appropriate for rank data according to Conover (1980). Ranks were analyzed and presented, but means ± SE are also presented for easier interpretation. Percentage pupae dead and percentage adult emergence were analyzed using tests of more than 2 proportions, followed by a Tukey-type multiple comparison (Zar 1999). Data from 11 and 12 May were analyzed separately to take into account slight differences in methods and temperatures between days. Mean days of adult emergence among treatments were analyzed with one-way analysis of variance.

Results

Pupal mortality caused by all fumigants 7 d after a 4-h exposure was significantly higher than in the control, but effects of the 5 fumigants on pupal mortality did not differ consistently (Table 2). Chloropicrin, however, caused the highest absolute mortality and performed better than Vapam and Telone II as measured by numbers of dead pupae in both 11 and 12 May tests.

In contrast to the pupal mortality results, adult emergence results (Table 3) were consistent and clearer in that chloropicrin, Telone II, and chloropicrin + Telone II prevented all emergence of adult flies over 111 - 112 d; whereas, methyl bromide, ECO_2FUME, and Vapam reduced it by only 41 - 73% compared with controls. Mean emergence day after fumigant exposure for the control and for methyl bromide, ECO_2FUME, and Vapam treatments did not differ (combining 11 and 12 May results, $n = 10$, $P > 0.05$) (control, 56.8 ± 1.3 d; methyl bromide, 56.6 ± 1.1 d; ECO_2FUME, 53.6 ± 1.0 d; Vapam, 55.9 ± 1.2 d; $F = 1.62$; df = 3, 36; $P = 0.2029$). Flies from all treatments emerged at 42 - 74 d and none emerged during the remainder of the 111 - 112 d monitoring period. Near the end of the monitoring period, all puparia seen in the chloropicrin, Telone II, and chloropicrin + Telone II treatments were brown and shriveled, an indication pupae were dead. Live puparia are off-white in color.

Discussion

The differences in pupal mortality and adult emergence of *R. pomonella* suggest that the fumigants acted slowly and that use of pupal mortality, even at 7 d postexposure, as a measure of fumigant efficacy can be misleading. Chloropricin killed more

Table 2. Numbers ± SE and percentages of *R. pomonella* pupae that were dead 7 d after a 4-h exposure to fumigants inside flasks

	11 May 2011		12 May 2011	
Fumigant	Mean no. dead ± SE (mean rank)	Total % dead (n = 25)	Mean No. dead ± SE (mean rank)	Total % dead (n = 25)
Control	0.2 ± 0.2 (3d)	4c	1.4 ± 0.7 (5.8c)	28c
Methyl Bromide	4.4 ± 0.2 (23.3ab)	88ab	4.2 ± 0.4 (21.4ab)	84ab
ECO_2FUME	4.2 ± 0.2 (20.9abc)	84ab	4.0 ± 0.3(19.2b)	80ab
Vapam	3.0 ± 0.5 (12.5c)	60b	4.0 ± 0.3 (19.2b)	80ab
Chloropicrin	4.8 ± 0.2 (28.1a)	96a	5.0 ± 0 (30.0a)	100a
Telone II	3.8 ± 0.2 (16.7bc)	76ab	3.0 ± 0 (9.0c)	60bc
Chloropicrin + Telone II	4.2 ± 0.4 (21.5a)	84ab	4.2 ± 0.4 (21.4ab)	84ab

5 replicates of the control and treatment on each date, 5 pupae per replicate. Ranks inside parentheses or percentages within columns followed by the same letter are not significantly different (P > 0.05). Ranks (Kruskal-Wallis test, followed by LSD test): critical T = 12.58; 11 May: T = 21.329; critical LSD = 8.489; 12 May: T = 20.664; critical LSD = 8.762. Percentages (Test of more than 2 proportions, followed by Tukey-type multiple comparisons): critical chi-square$_{0.05,\,6}$ = 12.592; 11 May: chi-square = 70.432; 12 May: chi-square = 42.054; critical LSD for both: 4.170.

pupae after 7 d than did Telone II, suggesting chloropicrin was more efficacious in killing pupae, but these 2 treatments and chloropicrin + Telone II were similar in that they prevented all adult emergences. This suggests pupae exposed to Telone II and to chloropicrin + Telone II took longer to die than those exposed to chloropicrin alone. It also suggests that combining chloropicrin and Telone II slightly reduces the toxicity of chloropicrin.

Results indicate methyl bromide, ECO_2FUME, and Vapam at label rates cannot kill 100% of *R. pomonella* pupae after a 4-h exposure, at least under our test conditions of 13.8 - 16.1°C. Methyl bromide killed 84 and 88% of pupae within 7 d, however, which is inconsistent with work showing that methyl bromide did not kill pupae >5 d after formation of the puparium at 32 mg per liter for 24 h and 48 mg for 8 h at 21.1 - 23.3°C (Roth and Richardson 1970). Differences between these and our results could be due to variations in susceptibility of fly populations or age of pupae when exposed. Complete kill of pupae of western cherry fruit fly, *Rhagoletis indifferens* Curran, exposed to methyl bromide was difficult and occurred only after 24 h of exposure to the fumigant (Jones and Schuh 1953).

Chloropicrin, Telone II, and chloropicrin + Telone II at label rates killed high numbers of *R. pomonella* pupae within 7 d and were apparently more toxic to the pupae than methyl bromide, ECO_2FUME, and Vapam under our test conditions. Chloropicrin and Telone II also have been shown to be effective against fungi and nematodes (South et al. 1997, Qiao et al. 2011), indicating they act on a broad range of soil-inhabiting organisms. Chloropicrin costs $3.60/pound and Telone II costs $1.78/pound (Trident Agricultural Products, Woodland, WA), but the 2 are comparable in application cost because

Table 3. Numbers ± SE and percentages of adult *R. pomonella* flies that emerged over 111 - 112 d after pupae were exposed to fumigants for 4 h inside flasks

Fumigant	11 May 2011 Mean No. ± SE (mean rank)	11 May 2011 % Emerged (n = 75)	12 May 2011 Mean No. ± SE (mean rank)	12 May 2011 % Emerged (n = 75)
Control	13.2 ± 0.5 (33.0a)	88.0a	12.8 ± 0.4 (33.0a)	85.3a
Methyl Bromide	5.4 ± 1.0 (22.0b)	36.0b	3.4 ± 0.2 (18.2c)	22.7c
ECO_2FUME	6.2 ± 1.0 (23.6b)	41.3b	7.6 ± 0.6 (26.2b)	50.7b
Vapam	6.2 ± 1.0 (23.4b)	41.3b	6.6 ± 0.9 (24.6b)	44.0bc
Chloropicrin	0 (8.0c)	0c	0 (8.0d)	0d
Telone II	0 (8.0c)	0c	0 (8.0d)	0d
Chloropicrin + Telone II	0 (8.0c)	0c	0 (8.0d)	0d

5 replicates of the control and treatment on each date, 15 pupae per replicate. Ranks inside parentheses or percentages within columns followed by the same letter are not significantly different (P > 0.05). Ranks (Kruskal-Wallis test, followed by LSD test): critical T = 12.58; 11 May: T = 31.164; critical LSD = 4.0348; 12 May: T = 32.935; critical LSD = 2.473. Percentages (Test of more than 2 proportions, followed by Tukey-type multiple comparisons): critical chi-square$_{0.05,\ 6}$ = 12.592; 11 May: chi-square = 229.094; 12 May: chi-square = 234.488; critical LSD for both: 4.170.

the label rate for Telone per volume is higher. However, chloropicrin and Telone II need to be tested against *R. pomonella* pupae in soil before they can be considered candidates for field use against this pest. Any toxic effects of these materials on specific nursery plants that could host *R. pomonella* also need to be determined if these plants are to be fumigated against the pest. Nevertheless, this is the first study that shows chloropicrin, Telone II, and the 2 in combination could be alternative fumigants to methyl bromide for killing pupae and preventing emergence of *R. pomonella*.

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