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Efficacy of Using In-carton Fumigation with the Quarantine Treatment Against Codling Moth on Apples Intended for Export to Japan

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Summary. **The two-component quarantine treatment was shown to be effective against at least 7,000 codling moth (*Cydia pomonella*) fifth instar larvae infesting 'Fuji' apples (*Malus ×domestica*) in each required confirmation test involving two sizes of cartons. After cold storage for 55 days at 36 °F (2.2 °C), infested fruit were placed in vented cartons, either 20-lb [7 × 12 × 12.5 inches (17.8 × 30.5 × 31.8 cm)], or 40-lb [12 × 12.5 × 20.5 inches (30.5 × 31.8 × 52.1 cm)], then fumigated with 0.056 oz/ft^3 (56 g·m^{-3}) of methyl bromide for 2 hours at 50 °F (10.0 °C). After each treatment, either no survivors were present or no moribund larvae survived beyond the first week of post evaluation of the larvae.**

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Apples produced in the Pacific northwestern United States must be treated for the codling moth, before the fruit are exported to Japan [Ministry of Agriculture, Forestry and Fisheries-Japan (MAFF-Japan), 1950]. The current approved method is a two-component quarantine treatment which consists of a 55-d cold treatment at 36 °F or below, followed by a 2-h methyl bromide fumigation of 0.056 oz/ft^3 at 50 °F (Hansen et al., 2000; Moffitt et al., 1988). However, in the original confirmation test, apples were treated in wooden bins, which contained nonsorted fruit from the field or nongraded fruit sorted to size only. Because the high quality fruit destined to Japan are identified only after going through the packing line, the industry is now required to fumigate fruit other than those intended for the Japanese market. Thus, it would be economically beneficial to the industry if excess fruit were not fumigated and only fruit intended for export to Japan were fumigated after sorting in shipping cartons. In our study, we evaluated treatment efficacy of the approved method on fruit packed in specially built vented cartons during fumigation. The carton sizes were the standard 40-lb container and the 20-lb gift-pack box.

Materials and methods

Artificial infestation of apples with codling moth larvae. Organically grown 'Fuji' Washington Extra Fancy size [2.5 inches (6.35 cm) diameter] were obtained from commercial fruit packing and marketing concerns in Washington state and shipped to the USDA–ARS Laboratory in Wapato, Wash., before testing. The fruit were from the 2000 apple season and were stored in controlled atmosphere conditions since harvest.

Codling moth larvae were obtained from the colony reared at the Wapato laboratory, where they were maintained on a soy-wheat germ-starch artificial diet at about 81°F (27.2°C), 40-58% relative humidity (RH), with a 16:8-h light:dark photoperiod (Toba and Howell 1991).

Previous tests have shown that the fifth instar is the larval stage of the codling moth most tolerant to methyl bromide (Hansen et al., 2000). Therefore, four late fourth to early fifth instar larvae were removed from artificial diet and placed near the stem end of each apple. Late fifth instars were excluded because they would form cocoons for pupation instead of feeding. The infested apples were held overnight (12-18 h) at about 75 °F (23.9 °C), 60% to 70% RH, with a 16:8-h light:dark photoperiod, enclosed in fiberboard trays lined with polyethylene liners and a lid made of standard fiberglass window screen.

For each replicate, 3,312 fifth instar larvae were used in the treatment and 512 fifth instar larvae were used as untreated controls. To insure that only larval mortality was recorded, the control insects were evaluated on the same day that the infested apples began the cold treatment. The number of larvae required for treatment was based on the proportion of larvae that survived in the untreated controls (estimated total number of larvae to be treated = initial number used in the infestation period · [number live of controls/initial number of controls]). This is the same procedure used in the original confirmation test (Hansen et al., 2000).

Treatment facilities. As required by the two-component quarantine treatment, codling moth larvae in fruit were exposed to low temperatures prior to fumigation. All cold treatments were conducted in a controlled environment chamber 7.2 ft long × 6.2 ft wide × 8.9 ft high (2.19 × 1.89 × 2.71

m) with a volume of 397 ft^3 (11.2 m^3), and equipped with a combination cooling-heating system to maintain the specified temperature within a 1.8 °F (1.0 °C) range. Temperatures during treatment were monitored every 6 h during the 55-d storage by using a personal computer with commercial thermistors (Techni-Systems; Chelan, Wash.).

Before fumigation, infested apples were placed in two types of specially built vented corrugated apple cartons, a 20-lb carton (7 inches high × 12 inches wide × 12.5 inches long) and a 40-lb carton (12 inches high × 12.5 inches wide × 20.5 inches long). The corrugated liner board was constructed with no waxes or additives. The corrugation glue was an unmodified cornstarch adhesive, Clinton 106 (Archer Daniels Midland, Clinton, Iowa). The cartons were assembled with A-385 Freezer Pack hot melt glue (North West Adhesives, Vancouver, Wash.).

Each carton had four vertical parallel vents on each of the two lateral sides. The vents were 4.8 inches high and 0.8 inch long (12.19 × 2.03 cm). Each vent created an opening into the carton interior of 3.84 inch^2 (24.776 cm^2). The carton vents were covered by polypropylene screen material XN 5340, with a mesh size of 0.3 inches high × 0.5 inches long (0.76 × 1.27 cm).

Fumigations were conducted inside the same fumigation chamber used for the original 'Delicious' and the five cultivar varieties (Moffitt et al., 1988; Hansen et al., 2000). The fumigation chamber, 4.4 ft wide × 8.1 ft high × 4.5 ft long (1.34 × 2.47 × 1.37 m), was equipped with a combination heating-cooling system to maintain a specified temperature within a 1.8 °F range. An air circulation system moved air in the chamber continuously throughout the exposure period. Before the fumigation confirmation tests, the chamber was evaluated for airtightness by pressurizing to 25 mm (1.0 inch) in the detection arm of a kerosene manometer (USDA, 1998). The resulting pressure decay, a drop from the 25 mm [0.029 lb/inch^2 (0.20 kPa) pressure] to 2.5 mm [0.003 lb/inch^2 (0.02 kPa) pressure] in the detection arm of the manometer, was ≥60 s and was within an acceptable range. Methyl bromide retention was measured for 48 h with an initial dosage of about 0.010 oz/ft^3 (10 g·m^{-3}) at 50 °F and with no more than 30% gas loss for the final reading. Temperatures during fumigation were monitored using a personal computer with commercial thermistors (Techni-Systems; Chelan, Wash.). Five temperature probes were used, one each in the upper and lower parts of the chamber and three in the load. Thermistors were calibrated within 0.2 °F (0.11 °C) using an ASTM (American Society for Testing and Materials, Conchohocken, Pa.) certified thermometer. Temperatures were recorded at 0, 10, 30, 60, and 120 min of exposure.

Brass bulk-head connectors were installed in the three cartons that were sampled during measurement of the methyl bromide fumigation. Sampling lines constructed of Tygon tubing lead through to the outside of the chamber. Concentrations of methyl bromide were determined by using gas chromatography with an electron capture gas chromatograph (HP-5890 series II; Hewlett-Packard, Avondale, Pa.) with the column containing Poropak Q 50/80 packing (Alltech Assoc., Inc.; Deerfield, Ill.); samples were not diluted. Four gas sampling probes were used, one in the central part of the chamber and three in the stacked cartons. Samples were taken at 10, 30, 60, and 120 min of exposure for each fumigation. Concentration-time (CT) products of the headspace (expressed as gram-hours per cubic meter) and percentage of sorption were calculated (Monroe, 1969).

The cartons were stacked on a pallet inside the chamber. The stacking pattern consisted of seven cartons per layer on a 3.3 ft long × 4.0 ft wide (1.01

Table 1 . Average and SE of air and fruit temperatures for cold storage of fifth instar codling moth-infested apples during the 55-d cold treatment, grouped by carton size of posttreatment packaging.

Carton size[z]	Test	Temp °F (°C) Air Avg	Air SE	Fruit Avg	Fruit SE
20-lb	1	36.7 (2.6)	0.2 (0.1)	37.6 (3.1)	0.7 (0.4)
	2	36.7 (2.6)	0.2 (0.1)	36.7 (2.6)	1.1 (0.6)
	3	26.9 (2.7)	0.2 (0.1)	36.9 (2.7)	0.4 (0.2)
40-lb	1	36.7 (2.6)	0.2 (0.1)	36.1 (2.3)	0.2 (0.1)
	2	36.7 (2.6)	0.2 (0.1)	36.9 (2.7)	0.4 (0.2)
	3	36.7 (2.6)	0.2 (0.1)	37.0 (2.8)	0.4 (0.2)

[z]20-lb carton = 7 x× 12 × 12.5 inches (17.8 × 30.5 × 31.8 cm); 40-lb carton = 12 × 12.5 × 20.5 (30.5 × 31.8 × 52.1 cm).

Table 2. Fruit and air temperatures, percent sorption, and concentration-time (CT) values inside the chamber and within the carton during fumigation with 0.056 oz/ft^3 (56 g·m^{-3}) of methyl bromide for 2 h at 50 °F (10 °C) in efficacy tests of codling moth-infested 'Fuji' apples in two different sizes of vented cartons.

Carton size[z]	Test	Temp °F (°C) Fruit[y] Avg	Fruit SE	Air[x] Avg	Air SE	Fumigation In chamber Sorption (%)	In chamber CT[w] (g·h^{-1}·m^{-3})	In carton Sorption (%)	In carton CT (g·h^{-1}·m^{-3})
20-lb	1	50.2(10.1)	0.4 (0.2)	51.1 (10.6)	0.7 (0.4)	28.2	86.2	25.5	85.7
	2	50.7 (10.4)	0.4 (0.2)	48.0 (8.9)	0.9 (0.5)	14.9	83.2	17.4	85.7
	3	49.5 (9.7)	0.4 (0.2)	48.9 (9.4)	0.9 (0.5)	42.2	94.8	36.4	94.7
40-lb	1	50.2 (10.1)	0.0 (0.0)	51.1 (10.6)	0.5 (0.3)	14.2	88.9	11.1	89.0
	2	49.3 (9.6)	0.0 (0.0)	50.9 (10.5)	0.9 (0.5)	30.7	85.9	20.5	83.1
	3	48.7 (9.3)	0.5 (0.3)	50.4 (10.2)	0.9 (0.5)	34.9	80.6	34.4	81.1

[z]20-lb carton = 7 × 12 × 12.5 inches (17.8 × 30.5 × 31.8 cm); 40-lb carton = 12 × 12.5 × 20.5 inches (30.5 × 31.8 × 52.1 cm).
[y]Based on two temperature probes taking measurements at the start, and 10, 30, 60, and 120 min into fumigation.
[x]Based on three temperature probes taking measurements at the start, and 10, 30, 60, and 120 min into fumigation.
[w]CT (concentration-time) values determined using the method of Monroe (1969), where 1.0 g·h^{-1}·m^{-3} = 0.001 oz-h/ft^3.

Table 3. Number of surviving fifth instar codling moth larvae in untreated controls and estimated number of larvae treated for cold storage-fumigation[z] in efficacy tests with infested 'Fuji' apples in difference sizes of vented cartons.

Carton size[x]	Test	Untreated control			Treatment		
		Infested (no.)	Survivor (no.)	Survivor (%)	Infested (no.)	Treated[y] (no.)	Survivor (no.)
20-lb	1	552	442	80.1	3,312	2,652	0
	2	552	459	83.2	3,312	2,754	0
	3	552	415	75.2	3,312	2,490	0
	Total	1,656	1,316		9,936	7,899	0
40-lb	1	552	453	82.1	3,312	2,718	0
	2	552	421	76.3	3,312	2,526	0
	3	552	347	62.9	3,312	2,082	0
	Total	1,656	1,221		9,936	7,326	0

[z]Methyl bromide fumigation with 0.056 oz/ft^3 (56 g·m^{-3}) for 2 h at 50 °F (10 °C).
[y]Number of treated larvae estimated by the percent survival of the untreated controls: Treated number = (infested number) (% control survival).
[x]20-lb carton = 7 × 12 × 12.5 inches (17.8 × 30.5 × 31.8 cm); 40-lb carton = 12 × 12.5 × 20.5 inches (30.5 × 31.8 × 52.1 cm).

× 1.22 m) wooden pallet for both the 20-lb and the 40-lb cartons. The 20-lb cartons were stacked ten layers high on the pallet in the chamber for a total of 70 cartons and the 40-lb cartons were stacked six layers high for a total of 42 cartons.

TREATMENT AND EVALUATION. After 55 d at low temperature, the cartons for each replication were removed from cold storage, stacked on a pallet inside the chamber and conditioned at the planned fumigation temperature of 50 °F for about 36 h. Temperature data were entered into a Quattro Pro version 7 spreadsheet (Corel Corp., Ottawa, Ont., Canada) and univariate statistics calculated by using the internal function formulas. Then, apples infested with fifth instar larvae were fumigated with methyl bromide at 0.056 oz/ft^3 for 2 h at 50 °F with a minimum 2-h aeration period. All fumigations were conducted at normal atmospheric pressure with a load factor of about 50%. Paired *t* tests were conducted with SAS (SAS Institute, Cary, N.C.) by using PROC MEANS for the differences between the variables and selecting the T and PRT options.

After fumigation the treated apples were held at about 75 °F, 70% to 80% RH, with a 16:8-h light:dark photoperiod for 3 to 4 d until the evaluation period where the treated apples were dissected for larvae. Moribund larvae were held with immature apples and weekly observations were made to determine delayed mortality, as was done in the previous confirmation tests (Moffitt et al., 1988; Hansen et al., 2000). The test for successful efficacy was no survivors with a minimum of 7,000 treated larvae in fruit for each carton size. Any survivor would negate the fumigation using the vented 20-lb and 40-lb cartons.

Results and discussion

At least 7,000 larvae were needed to demonstrate treatment efficacy for each carton size. Because the average recovery rates of infested larvae were 79.5% for the 20-lb carton test and 73.8% for the 40-lb carton test, 9,936 larvae over three replicates were used to infest apples for each test.

In the 55-d cold storage, infested fruit for both carton tests had internal average temperatures of 36.8 °F (2.67 °C) (Table 1). Considering the duration of the cold storage, including the standard defrost periods, this was very close to the target 36 °F. The low standard errors indicate that the temperatures were constant during storage times.

Three fumigation replicates were needed for each of the two cartons with the temperatures consistent with the treatment requirement (Table 2). CT values from inside the cartons and in the chamber were similar for each of the replicates (Table 2), indicating that methyl bromide entered the cartons unobstructed. Paired *t* tests indicated no significant difference between inside the chamber and within the carton for percent sorption ($t = 1.67$, $P > 0.05$) and CT values ($t = 0.07$, $P > 0.05$).

The evaluations after fumigation resulted in all larvae dead within the first week after evaluation (Table 3). At the time of evaluation, most larvae were black, suggesting death from the 55-d cold storage at 36 °F before fumigation. Thus, the two-component treatment against codling moth larvae is efficacious for fruit in vented cartons.

In-carton fumigation may have application for other fruit. Yokoyama et al. (1990, 1994) demonstrated quarantine efficacy in codling moth-infested nectarines contained in sealed unvented shipping containers. Hinsch et al. (1992) reported no significant damage to the fumigated nectarines in these containers. In our cartons, vents allowed rapid evacuation of the fumigant during aeration.

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