A Two-component Quarantine Treatment for Postharvest Control of Codling Moth on Apple Cultivars Intended for Export to Japan and Korea

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SUMMARY. Confirmatory tests were performed on a two-component quarantine treatment against the codling moth (*Cydia pomonella* L.) (Lepidoptera: Tortricidae) for seven apple [*Malus sylvestris* (L.) var. *domestica* (Borkh.) Mansf.] cultivars ('Delicious,' 'Golden Delicious,' 'Braeburn,' 'Fuji,' 'Gala,' 'Jonagold,' and 'Granny Smith') intended for export to Japan and Korea. Treatment consists of a 55-day cold storage at 40 °F (2.2 °C) or below, followed by a 2-hour methyl bromide fumigation (0.056 oz/ft³ or 56 g·m⁻³) at 50 °F (10 °C). No eggs or larvae survived this treatment. Comparison tests were conducted on all cultivars to demonstrate no difference in insect responses between a previously accepted cultivar and proposed cultivars. Concentration-mortality responses were determined for each of the components and no statistical differences were found in the regression slopes of pest mortality with controlling variable (either cold exposure or fumigation) among all cultivars. Descriptive mathematical models, developed for the effects of cold storage on egg mortality and for methyl bromide fumigation on larvae mortality, were sigmoid curve equations.

Apples are an important export crop for growers of the Pacific Northwest. Most countries accept these fruit based on the systems approach including intensive inspections at the packing house for arthropods of quarantine concern (Jang and Moffitt, 1994; Moffitt, 1989) and, in some cases, a mandatory cold storage period. Only Japan and the Republic of Korea require a specific quarantine treatment for codling moth (*Cydia pomonella*) (Lepidoptera: Tortricidae) [Ministry of Agriculture, Forestry and Fisheries-Japan (MAFF-Japan), 1950; Ministry of Agriculture and Forestry-Republic of Korea, 1982].

Until recently, Japan required a specified detailed procedure to be followed for a quarantine treatment for codling moth to be accepted (Waddell et al., 1990). First, life stages of quarantine concern were identified. Second, the most resistant of these stages were determined. Third, a large-scale test, involving as many as 30,000 individual insects in fruit, must show that the treatment was effective by having no survivors. Next, confirmatory tests

of efficacy were conducted under the observation of inspectors. Approval of subsequent cultivars required comparative concentration-response tests with a previously accepted cultivar to show no significant difference in treatment LC₅₀s (lethal concentrations of fumigant at 50% mortality) due to cultivar (Robertson and Preisler, 1992). Finally, a confirmatory test of efficacy was done, again under MAFF-Japan observation, using one of the proposed cultivars from the comparative concentration-response tests. The type of data and how they are analyzed were specified by MAFF-Japan.

Apples may be infested by eggs and larvae of the codling moth. Eggs are less susceptible to methyl bromide fumigation than the larvae (Gaunce et al., 1980; Tebbets et al., 1986). Eggs have three embryonic stages: white, red-ring, and blackhead stages (Richardson et al., 1982); the red-ring stage is the most resistant to low temperatures (Moffitt and Burditt, 1989a). Among the larvae, fifth instars are the least susceptible to cold (Toba and Moffitt, 1991; Yokoyama and Miller, 1989) and to methyl bromide fumigation (Moffitt et al., 1988).

Postharvest control of codling moth was initially attempted by cold treatment. Newcomer (1936) found that codling moth eggs and larvae on bartlett pears (*Pyrus communis* L.) died in less than 30 d at 30 to 39 °F (-1.1 to -0.6 °C). Moffitt and Albano (1972) destroyed all codling moth eggs and nondiapausing larvae in less than a month after cold storage at 39 ± 0.5 °F (-0.6 ± 0.3 °C) of infested immature apples. Moffitt and Burditt (1989b) produced complete mortality of codling moth eggs (red-ring stage) on 'Delicious' and 'Golden Delicious' apples within 42 d at 32.2 to 35.8 °F (0.1 to 2.1 °C).

Methyl bromide fumigation has been examined to control codling moth on commercial fruit in the United States including sweet cherries (*Prunus avium* L.) (Anthon et al., 1975, 1977; Moffitt et al., 1992) and nectarines [*Prunus persica* L. (Batsch) Nectarine Group] (Yokoyama et al., 1987a, 1988, 1990), and on New Zealand sweet cherries and nectarines (Waddell et al., 1990). Gaunce et al. (1980) determined the concentration-response of codling moth life stages in immature apples to methyl bromide fumigation at 73.4 ± 1.8 °F (23 ± 1 °C)

and recommended 0.032 oz/ft³ (32 g·m⁻³) for 2 h as an operational treatment. Using cages containing rearing diet, Tebbets et al. (1986) examined the concentration–response of codling moth mortality from methyl bromide fumigation at a greater range of temperatures. Codling moth eggs are more resistant to methyl bromide fumigation and the concentration required for quarantine control can produce unacceptable phytotoxic effects on apples (Moffitt and Burditt, 1989b).

Combining fumigation and cold storage can result in an effective treatment with little phytotoxic damage. Seo et al. (1971) showed that a combination treatment of methyl bromide fumigation and cold storage was efficacious against the melon fly (*Bactrocera cucurbitae* Coquillett), the oriental fruit fly (*Bactrocera dorsalis* Hendel), and the Mediterranean fruit fly (*Ceratitidis capitata* Wiedemann) (Diptera: Tephritidae) in papayas (*Carica papaya* L.) and avocados (*Persea americana* Mill.). For the codling moth, Moffitt (1971) used 0.032 oz/ft³ (32 g·m⁻³) of methyl bromide for 2 h at 23.9 to 25.6 °C followed by 60 d of cold storage at 30.9 °F (–0.6 °C) to eliminate larvae in ‘Delicious’ and ‘Golden Delicious’ apples. Morgan et al. (1974) destroyed all codling moth larvae in five apple cultivars with 0.032 oz/ft³ (32 g·m⁻³) of methyl bromide for 2 h at about 63 °F (17 °C) followed by 31 to 35 d of cold storage at 31.1 °F (–0.5 °C).

A proposed treatment for apples exported to Japan would thus involve both cold storage and fumigation. Cold treatment would control codling moth eggs, whereas the cold stressed larvae would be more susceptible to the fumigant than to the fumigant alone. Current commercial practices for apples

in Washington include lowering the fruit temperature as quickly as possible and holding the fruit in cold storage until marketed. A cold storage treatment followed by fumigation would be preferable because it allows for improved commercial options while the fruit are in cold storage and only those fruit selected for the Japanese and Korean markets need to be fumigated. Furthermore, fruit damage from methyl bromide fumigation increases with increasing temperatures during fumigation (Drake et al., 1988, Drake and Moffitt, 1998). Treating cold fruit would avoid the problems associated with fumigating warm fruit.

The proposed treatment consisted of two components, cold treatment at 36 °F (2.2 °C) or below for a minimum of 55 d followed by fumigation with methyl bromide at 0.056 oz/ft³ (56 g·m⁻³) for 2 h at 50 °F (10 °C) or above. MAFF–Japan required that the confirmatory tests for ‘Delicious’ and ‘Golden Delicious’ consist of at least 10,000 each of codling moth eggs and larvae. This number was subsequently reduced to 7,000 for the other apple cultivars.

Since the completion of this study, the United States has challenged Japan, in the court of the World Trade Organization (WTO), on the necessity to test each additional cultivar. The WTO has decided that additional cultivar testing is not required once a commodity (e.g., cherry, apple, etc.) has been accepted. Thus, this report presents the results of the two-component tests of efficacy on apple cultivars and the final concentration–response comparisons with codling moth larvae in five subsequent cultivars. The phytotoxic analyses associated with these cultivars have been reported by Drake et al. (1988) and Drake and Moffitt (1998).

Materials and methods

CULTIVARS AND SCHEDULES. MAFF–Japan required that the cultivar testing be conducted in a specific sequence (Table 1). The first confirmatory tests in 1993 used commercially mature, Fancy, size 138 ‘Delicious’ apples from Washington for both treatment components. In the 1997 confirmatory tests, the cold storage component was conducted using organic, unwaxed, mature Fancy or Extra Fancy size 113 ‘Fuji’ apples from Washington (Union Gap, Wash.). In the cold storage–methyl bromide fumigation tests, organically grown unwaxed immature ‘Fuji’ apples from California were used; samples were sent to the Washington State Department of Agriculture to analyze for residues of organophosphate, organochlorine, and carbamate insecticides. Filler apples, fruit used to maintain the required load factor during fumigation, were organically grown ‘Fuji’ apples from California in the first test and from commercial sources in Washington for the remaining tests.

The comparison tests on cold storage were conducted in 1997 on mature Fancy or Extra Fancy size 113 fruit of ‘Braeburn,’ ‘Fuji,’ ‘Gala,’ ‘Jonagold,’ ‘Granny Smith,’ and ‘Golden Delicious.’ Comparison tests on methyl bromide fumigation were done with unwaxed organic apples of the same cultivars except ‘Delicious’ was substituted for ‘Golden Delicious.’ All fruit were mature Fancy or Extra Fancy size 113 from commercial orchards in Washington.

ARTIFICIAL INFESTATION WITH CODLING MOTH EGGS. Codling moth eggs were obtained from a colony reared at the USDA–ARS Yakima Research Laboratory since 1960. The colony was maintained on a soy–wheat germ–

Table 1. History of confirmatory and comparison tests for the two-component treatment against the codling moth in apple cultivars intended for export to Japan.

Year	Test	Component	Target life stage	Cultivar	
				Proposed	Standard
1993	Confirmatory	Cold storage	Egg	Delicious	None
		Cold storage + fumigation	V instar ^z	Delicious	None
1997	Comparison	Cold storage	Egg	Five cultivars ^y	Golden Delicious
		Fumigation	V instar	Five cultivars	Delicious
1997	Confirmatory	Cold storage	Egg	Fuji ^w	None
		Cold storage + fumigation	V instar	Fuji	None

^zFifth instar larval stage used.

^yThe five cultivars were ‘Braeburn,’ ‘Fuji,’ ‘Gala,’ ‘Jonagold,’ and ‘Granny Smith’.

^w‘Fuji’ represented ‘Braeburn,’ ‘Fuji,’ ‘Gala,’ ‘Jonagold,’ and ‘Granny Smith’ in the 1997 confirmatory test.

starch, artificial diet at about 81 °F (27 °C), 40% to 58% relative humidity (RH), with a 16/8 h light/dark photoperiod (Toba and Howell, 1991). Eggs were obtained by allowing codling moth adults to oviposit for 48 h on apples of a single cultivar in a fiberboard tray. After the adults were removed, apples with eggs were held for 48 h longer at about 75 °F (24 °C), 60% to 80% RH, with a 16/8 h light/dark photoperiod to allow them to develop to the red ring stage (48 to 96 h old).

ARTIFICIAL INFESTATION WITH CODLING MOTH LARVAE. Codling moth larvae were also obtained from the same culture reared at the USDA-ARS Yakima Research Laboratory. Previous tests had shown that the fifth instar codling moth is the larval stage of those found in fruit most tolerant to methyl bromide (H.R. Moffitt, unpublished data). Therefore, late fourth and early fifth instars were removed from artificial diet and placed directly on the fruit of each cultivar so that larvae can enter into the fruit and initiate feeding. Late fifth instars were excluded because instead of feeding they formed cocoons for pupation. Infestation rates were three larvae per apple for the comparison tests and four larvae per apple for the confirmatory tests. Before treatment, the infested apples were held overnight (12 to 18 h) at about 75 °F, 60% to 70% RH in fiberboard trays lined with polyethylene, which allowed larvae to enter the apples and start feeding.

COLD STORAGE FACILITY. Infested apples were stored in a controlled environment chamber (7.2 ft [2.2 m] long, 6.2 ft [1.9 m] wide, 8.9 ft [2.7 m] high, 400 ft³ [11.3 m³] volume) equipped with a combination cooling-heating system to maintain 36 °F within a 1.8 °F (1 °C) range. Fruit pulp and room temperatures were recorded using a personal computer with thermistors (Techni-Systems; Chelan, Wash.) and univariate statistics calculated using Quattro Pro v.7 (Corel, 1996).

CONFIRMATORY TESTS—COLD STORAGE. Natural mortality of the codling moth eggs (the controls) were determined by securing apples with red ring stage eggs and allowing the eggs to develop for 7 d in a rearing room at about 75 °F, 60% to 80% RH with a 16/8 h light/dark photoperiod. The number of control eggs examined were

4,043 for 'Delicious' apples and 800 for 'Fuji' apples. Eggs were examined for egg hatch (survival) using a dissecting microscope. To determine efficacy, red-ring eggs (46,091 eggs for 'Delicious' apples and 7,235 for 'Fuji' apples) were held in cold storage at 36 °F. After 8 weeks of cold treatment, these eggs were removed and allowed to develop in the rearing room under the same conditions as the untreated controls. After 7 d of development, the eggs were examined in the same manner as the untreated controls. Rate of survival of the treated eggs was corrected for natural mortality (unhatched eggs) by the equation % survival treated eggs = [no. of hatched treated eggs]/[(total no. treated eggs)(no. of hatched control eggs/total control eggs)] × 100%, as required by MAFF-Japan.

CONFIRMATORY TESTS—COLD STORAGE WITH METHYL BROMIDE FUMIGATION. For each test of 'Delicious' and 'Fuji' cultivars, the treated fruit were infested with at least 3,200 fifth instar larvae and untreated controls had at least 760 larvae. From fruit dissection, control larvae were evaluated for survival on the same day that the treated infested apples began the cold treatment. Using established protocols, the number of larvae treated was estimated using the number of live larvae found in the untreated controls for that cultivar [estimated total number of treated larvae = initial number of larvae on fruit! (number live of controls/initial number of controls)].

The next day, infested fruit contained in perforated polyethylene picking lugs [23 inch (59 cm) long × 15 inch (39 cm) wide × 7 inch (18 cm) high] were exposed to the same low temperature regime, in the same environmental chamber, as was done for the eggs in the cold storage tests at 36 °F. Air and fruit pulp temperatures were monitored as described before.

Methyl bromide fumigations were conducted inside a chamber [4.4 ft (1.35 m) wide, 8.1 ft (2.48 m) high, and 4.5 ft (1.38 m) deep, total volume = 160.4 ft³ (4.61 m³)] with an external air circulation system. The fumigation chamber was equipped with a combination heating-cooling system to maintain a specified temperature within a 1.8 °F (1 °C) range. An air circulation system, including the addition of two fans on the top bin, circulated air in the chamber continuously throughout the

exposure period. For each fumigation, a 50% load factor (load volume: chamber volume) was achieved by using wooden bulk bins and filler apples as needed. The dimensions of the three bulk bins were as follows: two were 4.0 ft (1.22 m) long, 4.0 ft wide, and 2.2 ft (0.66 m) high outside diameter (OD) [fruit volume = 32.0 ft³ (0.906 m³), wood volume = 3.1 ft³ (0.088 m³)]; one was 4.0 ft long, 4.0 ft wide, and 1.3 ft (0.39 m) high OD [fruit volume = 19.0 ft³ (0.538 m³), wood volume = 2.2 ft³ (0.062 m³)].

After 55 d of cold storage at 36 °F, infested apples were enclosed in nylon organandy cloth bags inside the same lugs used during the cold treatment. These lugs were equally distributed within the middle bin of each test. The apples were maintained for about 36 h at the planned fumigation temperature of 50 °F to allow time for the larvae to enter the fruit. Infested apples were then fumigated with methyl bromide at 0.056 oz./ft³ (56 g·m⁻³) for 2 h at 50 °F with a minimum 2-h aeration period after treatment. All fumigations were done at normal atmosphere pressure with a 50% load factor. Temperatures during fumigation were monitored using a personal computer with thermistors (Techni-Systems; Chelan, Wash.). Concentrations of methyl bromide were determined by gas chromatography with 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.

After fumigation, the treated infested apples were held at about 75 °F, 70% to 80% RH, with a 16/8 h light/dark photoperiod for 4 d until the survival evaluation period. During evaluation, the treated apples were dissected for live, moribund, and dead larvae. Moribund larvae were held with immature apples and daily observations were made to determine mortality. No statistical tests were done because no larvae survived the treatments.

COMPARISON TEST—COLD STORAGE. Natural mortality of codling moth eggs (controls) by each cultivar was determined by securing apples containing about 800 eggs of the red-ring stage and allowing the eggs to develop for at least 10 d at about 75 °F, 60% to 80% RH, with a 16/8 h light/dark photoperiod. Eggs were examined using a

dissecting microscope for egg hatch (survival).

Time-mortality effects were examined by placing egg-infested apples in cold storage at 36 °F. A sufficient number of apples of each cultivar containing about 800 eggs was removed each week for 6 weeks and allowed to develop under the same conditions as the untreated controls. After at least 10 d of development, the eggs were examined for survival in the same manner as for the untreated controls. The test was concluded when there was 0.00% survival in 2 consecutive weeks, the procedure approved by MAFF-Japan.

Univariate statistics of survival data were done with PROC MEANS (SAS Institute, 1982a, 1982b). Concentration-mortality data were pooled and TableCurve (Jandel Scientific, San Rafael, Calif.) was used to screen 90 potential mathematical models for the best fit of the mortality-concentration response.

COMPARISON TESTS—METHYL BROMIDE FUMIGATION. The concentration-response of the fumigation component was done without the preceding cold storage because otherwise insufficient numbers of larva would be available to show relationships. The effect of different concentrations of methyl bromide on codling moth survival were examined for 'Braeburn,' 'Fuji,' 'Gala,' 'Jonagold,' and 'Granny Smith' cultivars each compared with 'Delicious.' Fumigations were in 7.5-gal (28.3-L) fiberglass chambers (model 55300 vacuum desiccators; Labconco Corp., Kansas City, Mo.), each with a continuously circulating fan on the inside top of the chamber. Chambers were in a walk-in controlled environment room

maintained at 50 °F. Infested apples were held in each chamber within a perforated metal basket designed to allow flow of the fumigant and to be a 50% load factor (volume/volume) when filled. Temperatures were monitored using a personal computer with thermistors (Techni-Systems, Chelan, Wash.). For each chamber, a thermistor was inserted into one apple in the center of the load. Concentrations of methyl bromide were determined using gas chromatography, as was done for the confirmatory tests.

Apples were infested with codling moth larvae as described above. Methyl bromide concentrations of 0 (nonfumigated controls), 0.008, 0.016, 0.024, 0.032, 0.040 oz/ft³ (8, 16, 24, 32, 40 g·m⁻³) were examined for each apple cultivar in each of the four tests. Each test consisted of 45 apples infested with a total of 135 larvae. One complete test of each dosage for two cultivars was treated weekly. An untreated control was included with each test. All fumigations were conducted with a 50% load factor (volume: volume) of about 17 lb (7.7 kg) at normal atmospheric pressure for a 2-h exposure period followed by a 2-h aeration period. Treated fruit were then stored in optimum rearing conditions at about 75 °F, 60% to 80% RH with a 16/8 h light/dark photoperiod) until evaluation.

The untreated control for a given replication was evaluated the day of fumigation and treated infested apples were evaluated 4 d after fumigation. In a treatment evaluation, all fruit were dissected to obtain live larvae. To determine delayed mortality, all moribund larvae were placed, with immature apples as a food source, in plastic

cups [4.3 inch (11 cm) diameter, 2.5 inch (6.5 cm) high] with perforated lids and held in an environmental room at about 75 °F. The numbers of larvae treated were estimated using the number of live larvae found in the untreated controls for that cultivar (estimated total treated number = initial treated number - [initial number of controls - number of live controls]).

MAFF-Japan required a demonstration of no cultivar differences in insect mortality from variable fumigation concentrations. As specified in the research guidelines (Government of Japan, 1998; Waddell et al., 1990), the concentration-mortality data were analyzed assuming the probit (i.e., normal) mode; responses were compared at the LC₅₀. Although applicable to any LC_n, the LC₅₀ is the usual and most reliable response level for comparison (Robertson and Preisler, 1992). We used POLO-PC (LeOra Software Inc., 1987) to estimate parameters of LC₅₀s, LC₉₀s, and LC₉₅s for comparison of apple cultivars. Each response ratio at LC_n (e.g., LC₅₀ cultivar x / LC₅₀ of cultivar y) and its 95% CI (confidence interval) was computed with parameters from POLO-PC. The parameters (slope, intercept, SE of slope, SE of intercept, covariance of the slope and intercept, heterogeneity factor) were then used in a program to run the S-Plus program (MathSoft, 1997). If the lower and upper 95% CIs bracket 1.0, then the LC_xs were not significantly different ($P > 0.05$) (Robertson and Preisler, 1992; Robertson and Yokoyama, 1998).

If there were no significant differences among the cultivars, the dose-mortality data were pooled and TableCurve (Jandel Scientific, San

Table 2. Average air and fruit temperatures for the confirmatory tests during 55-d cold storage at 36 °F (2.2 °C) based on 6-h readings.

Year	Cultivar	Life stage	Test	Temp °F (°C) ^z	
				Air	Fruit
1993	Delicious	Egg	1	37.8 (3.2)	37.9 (3.3)
			2	37.9 (3.3)	38.0 (3.4)
		Larva	1	38.5 (3.6)	38.4 (3.5)
			2	37.9 (3.3)	37.8 (3.2)
			3	37.7 (3.2)	37.5 (3.1)
1997	Fuji	Egg	1	36.5 (2.5)	36.8 (2.7)
			2	36.3 (2.4)	36.2 (2.3)
		Larva	1	32.5 (2.7)	36.6 (2.6)
			2	37.0 (2.8)	36.5 (2.5)
			3	36.8 (2.7)	36.6 (2.6)

^zAll SE temperatures were 0.09 °F (0.05 °C) or less.

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 confirmatory tests for apple cultivars.

Cultivar	Test	Untreated control			Treatment		
		Infested (no.)	Survivor (no.)	Survivor (%)	Infested (no.)	Treated ^y (no.)	Survivor (no.)
Delicious	1	760	469	61.7	4,940	3,048	0
	2	760	554	72.9	5,700	4,155	0
	3	760	617	81.2	5,700	4,628	0
	Total	2,280	1,640	77.1	16,340	11,831	0
Fuji	1	800	600	75.0	3,200	2,400	0
	2	800	484	60.5	3,200	1,936	0
	3	800	505	63.1	4,000	2,524	0
	4	800	570	71.3	4,000	2,852	0
	Total	3,200	2,159	67.5	14,400	9,712	0

^zMethyl bromide fumigation with 0.056 oz/ft³ (56 g·m⁻³) for 2 h at 50 °F (10 °C).

^yNumber of treated larvae estimated by the percent survival of the untreated controls: Treated number = (infested number) (% control survival).

Rafael, Calif.) was used to screen ninety potential mathematical models for the best fit of the mortality-dosage response.

Results

CONFIRMATORY TESTS—COLD STORAGE. Temperatures averaged more than 36 °F during the storage periods with fruit temperatures higher for the 'Delicious' (1993 tests) than for the 'Fuji' apples (1997 tests) (Table 2). Egg hatch of the untreated controls was 82.4% for the 'Delicious' and 89.9% for the 'Fuji' cultivars. No egg hatch was observed among 46,091 treated eggs in the 'Delicious' test and 7,235 treated eggs in the 'Fuji' test. Treatment efficacy would be equally effective at lower temperatures (Moffitt and Burditt, 1989b).

CONFIRMATORY TESTS—COLD STORAGE WITH METHYL BROMIDE FUMIGATION. The confirmatory tests using the two components resulted in no survivors for both 'Delicious' and 'Fuji' cultivars (Table 3). Control survivorship (mean ± SE) was 77.1 ± 5.7% for 'Delicious' and 67.5 ± 3.9% for 'Fuji.'

Many of the treated larvae were black, suggesting death from the 55-d cold storage at 36 °F. Only six moribund larvae were observed in the 'Fuji' tests, and all larvae were dead within 2 weeks of fumigation without pupation.

The recovery of larvae infesting 'Fuji' apples in the controls was between 60 and 75% compared to 62 and 81% of the 'Delicious' or 94.4% for the 'Fuji' apples used in the comparison tests. 'Fuji' apples for the confirmatory tests were organically grown and laboratory analysis (by the Washington State Department of Agriculture) showed no insecticide residues. Washing the fruit did not improve the infestation rates. Temperatures of fruit infested with larvae during cold storage were consistent with those infested with eggs (Table 2).

COMPARISON TESTS—COLD STORAGE. The cold treatment began when internal fruit temperature about 36 °F, about 11 h after the fruit were placed in the cold room, and was concluded after 42 d. Mean (±SE) temperatures were 4.28 ± 0.04 °F (2.38 ± 0.02 °C) for air and 36.39 ± 0.14 °F (2.44 ±

0.08 °C) for fruit. Nearly all the eggs were dead after 4 weeks of cold treatment and no eggs survived beyond 5 weeks (Table 4).

The best model describing the dose-mortality response was the sigmoid curve equation ($r^2 = 0.9816$; $n = 42$; fit SE = 36.5; $F = 674.8$) $y = a + b / (1 + e^{-(x-c)/d})$, where x is the duration of cold exposure to 36 °F in days, y = the number dead, and a , b , c , and d = fitted model parameters (±SE) 114.03 (±25.09), 690.4 (±29.0), 11.7 (±0.5), and 4.0 (±0.4), respectively, when treated population = 800 eggs.

COMPARISON TESTS—METHYL BROMIDE FUMIGATION. The average ±SE fruit temperatures during fumigation were 50.2 ± 0.0 °F (10.1 ± 0.0 °C) and no fruit temperature exceeded 51.4 °F (10.8 °C). Mortality was directly related to concentration with no mortality rate less than 99.2% for the highest dosage at 0.040 oz/ft³ (40 g·m⁻³) (Table 5). Numerically, codling moth response to methyl bromide fumigation of the six cultivars was similar at LC₅₀, LC₉₀, and LC₉₅ (Table 6). All 95% CIs for response ratios at these

Table 4. Survival of codling moth eggs (n = 580 to 800) on six apple cultivars during cold treatment at 36 °F (2.2 °C) from weekly observations.

Treatment (wk)	Survival (%)					
	Braeburn	Fuji	Gala	Golden Delicious	Granny Smith	Jonagold
Control	90.6	83.5	81.5	83.6	82.0	69.0
1	72.4	58.0	59.3	70.8	64.5	64.0
2	34.1	36.0	33.5	39.6	24.6	18.8
3	6.1	7.0	7.3	10.6	2.3	3.1
4	0.1	0.1	0.0	0.3	0.2	0.0
5	0.0	0.0	0.0	0.0	0.0	0.0
6	0.0	0.0	---	0.0	0.0	---

^zWeekly observation discontinued.

concentration levels bracketed 1.0, indicating no significant differences among the cultivars (Table 7).

The best model describing the concentration–mortality response was the sigmoid curve equation ($r^2 = 0.9923$; $n = 36$; fit $SE = 17.6$; $F = 1383.3$) $y = a + b / (1 + e^{-(x-c)/d})$

where x is the concentration of methyl bromide ($\text{g}\cdot\text{m}^{-3}$), y = the number dead, and a , b , c and d = fitted model parameters ($\pm SE$) = -22.54 (± 19.92), 578.6 (± 26.7), 14.9 (± 0.6), and 6.9 (± 0.6), respectively, when treated population = 500.

Discussion

CONFIRMATORY TESTS. Much of the previous work on cold storage of apples involved temperatures near freezing (Moffitt and Albano, 1972; Moffitt and Burditt, 1989b; Newcomer, 1936). Because apples are commercially stored between 32 and 35.6 °F

(0 and 2 °C) (Kupferman, 1997), we selected to test at the warmer end of the temperature range where mortality should be the lowest. Thus, the cold storage component, a minimum of 55 d at 36 °F or below, allows some flexibility according to cultivar.

Our tests showed that the cold storage component was effective in the control of codling moth eggs on mature apples. Earlier et al. (1989b) recommended this procedure for controlling codling moth eggs after they conducted a test on 35,203 red-ring stage eggs with no survivors. Observations of dissected fruit after methyl bromide fumigation suggested that the cold storage component also eliminated many of the infesting larvae. Others reported similar results. Moffitt (1988) observed a 5.6% survival rate for fifth instars held at 36.5 °F (2.5 °C) for 53 d. Yokoyama and Miller (1989) recorded 67.2% survival for fifth in-

stars held at 32 °F (0 °C) for 21 d. Morgan et al. (1974) killed all first and second instars after 35 d of cold storage at -31.1 °F. Quarantine security, however, requires complete elimination of all life stages. Thus, the methyl bromide fumigation provides the additional margin of security needed to export to Japan and Korea.

In previous experiments, Moffitt (1971) reported complete efficacy against codling moth larvae fumigated for 2 h at 0.032 oz/ft³ (32 g·m⁻³) at about 75 °F followed by 30 d of standard cold storage at 40 to 45 °F (4.4 to 7.2 °C). Similarly, Morgan et al. (1974) observed that no codling moth larvae survived after fumigation for 2 h with 0.032 oz/ft³ (32 g·m⁻³) at 62.6 °F (17 °C) followed by 35 d of cold storage at 31.1 °F (-0.5 °C). Our research, however, has demonstrated that cold storage for 55 d at 36 °F followed by a 2-h methyl bromide fumigation at 0.056

Table 5. Mortality of fifth instar codling moth larvae in six apple cultivars fumigated with methyl bromide for 2 h at 40 °F (10 °C). For each cultivar, 540 larvae on 180 apples were treated at each dosage.

Cultivar	Concn [oz/ft ³ (g·m ⁻³)]	No. of larvae		Mortality	
		Treated ^z	Alive	No. dead ^y	(%)
Braeburn	0.008 (8)	499	377	122	24.4
	0.016 (16)	499	256	243	48.7
	0.024 (24)	499	114	385	77.2
	0.032 (32)	499	29	470	94.2
	0.040 (40)	499	4	495	99.2
Fuji	0.008 (8)	510	438	72	14.1
	0.016 (16)	510	249	261	51.2
	0.024 (24)	510	117	393	77.1
	0.032 (32)	510	38	472	92.6
	0.040 (40)	510	2	508	99.6
Gala	0.008 (8)	513	415	98	19.1
	0.016 (16)	513	308	205	40.0
	0.024 (24)	513	108	405	79.0
	0.032 (32)	513	18	495	96.5
	0.040 (40)	513	4	509	99.2
Granny Smith	0.008 (8)	498	384	114	22.9
	0.016 (16)	498	225	273	54.8
	0.024 (24)	498	120	378	75.9
	0.032 (32)	498	22	476	95.6
	0.040 (40)	498	0	498	100.0
Jonagold	0.008 (8)	502	388	114	22.7
	0.016 (16)	502	237	265	52.8
	0.024 (24)	502	78	424	84.5
	0.032 (32)	502	19	483	96.2
	0.040 (40)	502	2	500	99.6
Delicious	0.008 (8)	502	429	73	14.5
	0.016 (16)	502	235	267	53.2
	0.024 (24)	502	107	395	78.7
	0.032 (32)	502	20	482	96.0
	0.040 (40)	502	1	501	99.8

^zNumber treated = number alive in untreated controls for each cultivar.

^yNumber dead = number treated minus number alive.

Table 6. Concentration–mortality responses, based on probit^z models, of fifth instar codling moth larvae in six apple cultivars fumigated for 2 h with five concentrations at 50 °F (10 °C), November 1996 to February 1997 (1.0 g·m⁻³ = 0.001 oz/ft³).

Cultivar	n	Slope ±SE ^y	LC ₅₀ (95% CI) ^x (g·m ⁻³)	LC ₉₀ (95% CI) (g·m ⁻³)	LC ₉₅ (95% CI) (g·m ⁻³)
Braeburn	2,495	3.78 ± 0.14	13.9 (8.9–18.2)	30.4 (22.6–59.5)	37.9 (26.9–91.0)
Delicious	2,510	4.57 ± 0.15	14.6 (11.8–17.3)	27.9 (23.1–37.9)	33.5 (26.9–49.1)
Fuji	2,550	4.37 ± 0.15	14.9 (12.5–17.6)	35.9 (29.2–50.5)	35.9 (29.2–50.5)
Gala	2,565	4.33 ± 0.14	15.0 (9.0–20.5)	29.5 (21.3–71.7)	35.9 (25.1–110)
Granny Smith	2,490	3.93 ± 0.14	13.7 (9.0–17.7)	29.0 (21.9–60.0)	35.9 (26.0–78.3)
Jonagold	2,510	4.17 ± 0.14	13.4 (9.8–16.6)	27.1 (21.4–41.8)	33.2 (25.3–57.5)

^zProbit regressions were estimated with the probit option of POLO-PC (LeOra Software Inc., 1987). Cultivars did not differ in response at LC₅₀, LC₉₀, or LC₉₅.

^yChange in insect mortality (proportion) per unit dose.

^xRange at 95% confidence interval.

oz/ft³ (56 g·m⁻³) at 50 °F or above is effective in control of codling moth larvae in mature apples. The two-component treatment for codling moth is suitable for all apple cultivars including 'Braeburn,' 'Fuji,' 'Gala,' 'Jonagold,' and 'Granny Smith' and should be the accepted treatment for these cultivars exported to Japan and Korea. Additional cultivar testing is unnecessary. Furthermore, this treatment is compatible with commercial operations in which fruit can be marketed while in cold storage and remain chilled during fumigation.

COMPARISON TESTS. Comparison tests demonstrate that each component of the treatment surpassed quarantine security levels. The cold treatment component, a minimum of 55 d at 36 °F or below, was effective against codling moth eggs for all tested cultivars by 5 weeks. The methyl bromide component, fumigation for 2 h at 50 °F or above, was effective (>99% mortality) at 0.040 oz/ft³ (40 g·m⁻³) with no previous cold treatment (Table 5).

One would not expect cultivar differences in egg mortality due to cold storage. Codling moth eggs are laid on the surface of the apple and there are no known differences among cultivars in the release by the epidermis of toxic or protective chemicals at cold temperatures. Cold exposure would be the same regardless of cultivar. Indeed, no significant differences were observed among the cultivars.

As for the fumigation component, any concentration–mortality bioassay includes unavoidable intrinsic variables. By necessity and because of the labor involved, bioassays are likely to be done at different times. However, time is a known variable that affects bioassay results (Robertson and Preisler, 1992). To confound inter-

pretation of results of exclusionary bioassays even further, natural variation in response of insects to toxicants and various methods of control has been extensively documented (Robertson et al., 1995). Finney (1964) stated that, because of natural variation, responses of a group of insects tested at any one time will never be exactly the same as responses of another tested at either the same time or at a different time, regardless of the extent to which bioassay techniques are standardized.

Failure of biometrical methods in commodity treatments have been caused by investigators not recognizing that the hypothesis that LC_ns of choice could be tested with the methods described by Robertson and Preisler (1992). In the past, commercial programs, such as SAS, have been used rather than biometrical programs, such as GENSTAT (Alvey et al., 1983) or S-Plus (MathSoft Inc., 1997). For example, Yokoyama et al. (1987a, 1987b, 1988, 1990) analyzed nectarine data with the SAS PROBIT procedure (SAS Institute Inc., 1985). This version of SAS did not compute the parameters necessary for tests of hypotheses of equality (equal slopes, equal

intercepts) or parallelism (equal slopes). Even worse, the variance–covariance matrix was not calculated. Thus, significant differences among LC_ns could not be identified. Although the current version of SAS PROBIT includes the variance–covariance matrix and corrected calculations of the confidence limits, hypotheses of parallelism, equality, and relative response cannot be done easily or efficiently.

In our study, the best descriptive mathematical model for both components of the quarantine treatments was the sigmoid curve model, $y = a + b / (1 + e^{-(x-c)/d})$.

Although probit analysis is often used to evaluate concentration–response regressions, it may not always provide the best fit and, thus, may not be appropriate in comparing efficacy in these types of quarantine studies. Robertson and Preisler (1992) have discussed this problem in more detail.

The fumigation concentration–mortality response for 'Delicious' was not significantly different from that of any other cultivar. Thus, the results of this study showed that eggs and fifth instar codling moth life stages were equally susceptible to cold treatment

Table 7. Results of hypotheses tests for differences among apple cultivars in response to methyl bromide fumigation for codling moths. If the 95% confidence interval (CI) of the response ratio overlaps 1.0 (i.e., the lower 95% is <1.0), the hypothesis that the lethal concentrations (LCs) are significantly different must be rejected (Robertson and Preisler, 1992).

Cultivar	Response ratio (95% CI)		
	LC ₅₀	LC ₉₀	LC ₉₅
Braeburn	1.04 (0.8–1.4)	1.1 (0.3–7.8)	1.14 (0.008–12)
Fuji	1.11 (0.8–1.4)	1.1 (0.6–2.1)	1.08 (0.4–2.7)
Gala	1.12 (0.8–1.8)	1.1 (0.4–2.8)	1.08 (0.4–2.7)
Delicious	1.09 (0.8–1.4)	1.03 (0.5–2.0)	1.01 (0.5–2.1)
Granny Smith	1.02 (0.7–1.8)	1.1 (0.5–2.4)	1.08 (0.5–2.2)
Jonagold	1.0	1.0	1.0

and methyl bromide fumigation, respectively, for 'Golden Delicious' and 'Delicious,' compared with 'Braeburn,' 'Gala,' 'Fuji,' 'Granny Smith,' and 'Jonagold' apples. Therefore, all of the treatment schedules presently approved for 'Golden Delicious' and 'Delicious' apples would be effective for 'Braeburn,' 'Gala,' 'Fuji,' 'Granny Smith,' and 'Jonagold.'

POLO-PC and the statistical methods described by Robertson and Preisler (1992) have been used to demonstrate that apple cultivars do not differ significantly in treatment efficacy against codling moth. Requirements of international trade and regulatory agencies must be based on state-of-the-art statistical examination (Robertson et al., 1994). For apples, cultivar testing is unneeded. Instead, a generic approach should suffice to meet export requirements. The same conclusion has been made for fumigation of nectarines infested with codling moth (Robertson and Yokoyama, 1998).

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