Methyl Bromide Fumigation of Five Cultivars of Sweet Cherries as a Quarantine Treatment against Codling Moth

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SUMMARY. A quarantine treatment was developed against codling moth (*Cydia pomonella* L.) (Lepidoptera: Tortricidae) for the sweet cherry (*Prunus avium* L.) cultivars ('Garnet,' 'Brooks,' 'Tulare,' 'Lapins,' and 'Sweetheart') exported to Japan. Specific procedures were required to demonstrate treatment efficacy. Dose-mortality responses were measured for each cultivar at regularly incremented concentrations of methyl bromide for 2 hours at 43 °F (6 °C) compared to the dose-mortality curve of an approved cultivar under the same conditions. Based on the overlap of confidence limits, there were no significant differences between new and approved cultivars in group comparisons. In a confirmation test of

efficacy, fumigation with methyl bromide at 0.064 oz/ft³ (64 g·m⁻³) for 2 hours at 43 °F resulted in complete mortality for all codling moth larvae in the treated cultivars. By convention, fumigation at this or the following concentrations [0.048 oz/ft³ (48 g·m⁻³) for 2 h at 54 to 63 °F (12 to 17 °C); 0.040 oz/ft³ (40 g·m⁻³) for 2 h at 63 to 72 °F (17 to 22 °C); 0.032 oz/ft³ (32 g·m⁻³) for 2 h at 72 °F (22 °C) or above] were considered as an efficacious quarantine treatment against the codling moth in sweet cherries. Except for 'Brooks,' fumigation did not significantly influence fruit firmness, soluble solids, or titratable acids. Reduction in fruit and stem quality was more associated with temperature than with methyl bromide concentration. 'Tulare,' 'Lapins,' and 'Sweetheart' could be fumigated with minimal change in fruit quality.

The codling moth (*Cydia pomonella*) (Lepidoptera: Tortricidae), is a quarantine pest for sweet cherries (*Prunus avium*) entering Japan and the Republic of Korea. The Ministry of Agriculture, Forestry and Fisheries–Japan (MAFF–Japan) has approved methyl bromide fumigation as a treatment for sweet cherries against the codling moth based on research by Anthon et al. (1975, 1977) and Moffitt et al. (1977, 1983, 1992). At the time this study was initiated, the approved cultivars were 'Bing,' 'Lambert,' 'Van,' and 'Rainier.'

For an additional cultivar to obtain approval for importation into Japan, MAFF–Japan required comparisons of probit regressions from concentration-mortality data of codling moth larvae to methyl bromide fumigation in the proposed cultivars with an already approved cultivar (Moffitt et al., 1992). If the proposed cultivars were no different from the approved cultivar, as demonstrated by overlap in confidence intervals (CIs) of lethal concentrations for mortality at the selected n percentage level (LC_ns, lethal concentration of fumigant that resulted in n% mortality), a required large-scale confirmation test for efficacy followed using one of the cultivars from the group of selected cultivars. The treatment was deemed efficacious if no larvae survived.

Since the completion of this study, the United States has challenged Japan, in the court of the World Trade

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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) has been accepted. Thus, this report presents the results of the final concentration-response comparisons with codling moth larvae in five additional cultivars of sweet cherry and the results of the required large-scale confirmation tests of methyl bromide fumigation with 0.064 oz/ft³ (64 g·m⁻³) for 2 h at 43 °F (6 °C), the accepted treatment.

Materials and methods

TEST CHERRIES. Cultivars of sweet cherries studied were 'Bing,' an early to midseason cherry with a mahogany skin obtained from Washington and central California; 'Garnet,' an early season cherry with a red skin obtained from central California; 'Brooks,' an early season cherry with pink and red streaking on a light straw skin, obtained from central California; 'Tulare,' a very early season cherry with a bright red skin obtained from central California; 'Lapins,' a late season cherry with mahogany skin from Washington; 'Sweetheart,' a late season cherry with red skin from north-central Washington; and 'Lambert,' a mid- to late-season cherry with red skin from Washington. The field-run cherries used in the fumigations were slightly immature in order to provide firmness during infestation.

TEST INSECTS. Third instar codling moths were obtained from a colony reared at the USDA-ARS Yakima Ag-

ricultural Research Laboratory, Yakima, Wash., and maintained on a soya-wheat germ-starch artificial diet (Toba and Howell, 1991) at 81 °F (27 °C), 40% to 50% relative humidity (RH), with a 16/8 h light/dark photoperiod. Third instar codling moths were removed from artificial diet and placed separately on individual cherries (one larva per cherry). Infested cherries were held overnight (12 to 18 h) at 75 °F (24 °C), 60% to 70% RH, with a 16/8 h light/dark photoperiod.

COMPARISON TESTS. The purpose of the comparison tests was to demonstrate that, among the examined cultivars, larval mortality rates were the same at specific fumigant concentrations. The proposed cultivars were tested in different years (Table 1). All fumigations were conducted in 7.5-gal (28.3-L) fiberglass chambers (model 55300 vacuum desiccator; Labconco Corp., Kansas City, Mo.) each equipped with a circulating fan which operated continuously throughout the exposure period. Chambers were in a walk-in controlled environment room, and the temperatures in the chambers were maintained by the temperature in the room. For each chamber, infested cherries were held in cardboard cylinder [7 inch (17 cm) diameter × 3 inch (8 cm) h] with cloth ends placed inside of a perforated metal basket; this arrangement allowed circulation of the fumigant at a 50% load factor (fruit volume/chamber volume).

Temperatures were monitored during fumigations using a personal computer-controlled temperature re-

corder equipped with thermocouples. For each chamber, a thermocouple was inserted into one cherry placed in the center of the load. Concentrations of methyl bromide were monitored by gas chromatography with electron capture (HP-5890 series II gas chromatograph; Hewlett-Packard, Avondale, Pa.) (Moffitt et al., 1992; Sell et al., 1988).

Proposed cultivars were tested with an accepted cultivar (Table 1). Each cultivar had three test cycles, except 'Sweetheart' which had two test cycles. MAFF-Japan required that the concentrations of methyl bromide to be 0.000 (untreated controls), 0.032, 0.036, 0.040, 0.044, 0.048 oz/ft³ (or 0, 32, 36, 40, 44 and 48 g·m⁻³, respectively). Each treatment cycle, including the untreated controls, had 200 cherries infested with 200 larvae, except for the third cycle for 'Brooks' and 'Tulare' which consisted of 150 cherries infested with 150 larvae for all treatments. One complete cycle of every concentration for each cultivar was treated in any one week. All fumigations were conducted with a 2-h exposure period at normal atmospheric pressure, a 2-h aeration period, a 50% load factor (fruit volume/chamber volume) of 17 lb (8 kg) for 'Garnet' and 'Bing,' and 19 lb (9 kg) of fruit for the remaining cultivars.

The untreated controls were evaluated on the day of fumigation to determine number of live larvae. After treatment, the treated larvae in the cherries were retained inside the cardboard containers and placed at 75 °F, 70% to 80% RH, with a 16/8 h light/

Table 1. Mortality of third instar codling moth in cherry cultivars fumigated with methyl bromide at different concentrations for 2 h at 43 °F; the approved cultivar is the MAFF-Japan standard to which the new cultivar is compared (10⁻³ oz/ft³ = 1.0 g·m⁻³).

Season	Cultivar	Status ^y	Infested	Treated ^x	Dead ^z (%)				
					Concn (10 ⁻³ oz/ft ³)				
					32	36	40	44	48
1993	Bing	Approved	600	527	60.0	63.6	67.9	77.2	86.7
	Garnet	New	600	538	---	63.4	74.9	85.7	88.8
1995	Garnet	Approved	550	500	55.6	62.0	69.8	72.3	---
	Brooks	New	550	492	47.4	48.4	52.2	67.9	72.0
	Tulare	New	550	506	42.3	46.8	56.1	54.7	68.2
Early 1996	Bing	Approved	600	492	81.3	89.0	---	94.1	98.2
	Lapins	New	600	515	77.1	81.7	92.6	91.1	94.4
Late 1996	Lambert	Approved	600	535	46.2	61.5	65.2	70.7	76.8
	Sweetheart	New	400	342	60.2	77.2	82.7	---	---

^zPercent dead = (number treated minus number alive/number treated) 100%.

^yStatus with MAFF-Japan; approved cultivar or a new cultivar proposed for import from United States.

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

^wNot recorded.

Table 2. Comparisons of lethal concentrations (LC_n = LC of fumigant that resulted in $n\%$ mortality) of methyl bromide at 50% and 95% mortality levels of third instar codling moth larvae between MAFF-Japan approved and proposed imported cherry cultivars fumigated at 43 °F (6 °C) (10^{-3} oz/ft³ = 1.0 g·m⁻³).

Season	Cultivar	No. larvae	Concn (10 ⁻³ oz/ft ³) ^z		
			LC ₅₀ (95%CI)	LC ₉₀ (95%CI) ^z	Slope ±SE ^y
1993	Bing	2,635	29.5 (16.9–33.7)	56.6 (48.0–119.0)	4.523 ± 0.427
	Garnet	2,152	32.3 (30.5–33.6)	48.2 (46.7–50.2)	7.382 ± 0.677
1995	Brooks	2,460	35.6 (25.0–39.4)	64.2 (54.0–114.2)	0.045 ± 0.005
	Tulare	2,530	37.5 (31.0–41.1)	71.2 (58.9–122.5)	0.038 ± 0.004
	Garnet	1,460	31.7 (26.6–34.6)	75.2 (65.8–94.7)	0.029 ± 0.005
Early 1996	Lapins	2,557	22.5 (8.5–28.0)	41.1 (37.5–50.2)	4.884 ± 0.529
	Bing	1,968	22.5 (7.8–27.9)	37.3 (32.8–43.2)	5.837 ± 0.639
Late 1996	Sweetheart	1,026	27.4 (23.3–30.1)	60.0 (54.2–71.4)	3.769 ± 0.551
	Lambert	2,675	32.6 (28.5–34.9)	63.7 (55.3–86.6)	4.392 ± 0.407

^zCI = confidence interval.

^ySlope = change in insect mortality per unit concentration.

dark photoperiod for 3 d. After the 3-d holding period, the infested cherries were dissected and all live larvae counted. Moribund larvae were held to determine subsequent mortality. The number of larvae treated for a given cultivar was based on the number of live larvae in the untreated controls for that cultivar (Moffitt et al., 1992). Following the MAFF-Japan protocol, percent mortality was determined for each treatment based on the accumulated totals among the cycles, then the concentration-mortality responses were estimated using the POLO-PC computer program (LeOra Software, 1987). LCs were considered not to differ significantly if their 95% CIs overlapped (Moffitt et al., 1992).

CONFIRMATION TESTS. By agreement, confirmation of efficacy for one of the treatment schedules would qualify all treatments for quarantine. For each test cycle, 3,000 to 4,000 third instar codling moths were used to infest the cherries along with 400 cherries infested with 400 larvae as untreated controls. The infested cherries were placed in 1.0-gal (3.8-L) cylindrical cartons with the solid ends replaced with cloth to allow circulation of the fumigant. Each carton held 200 infested cherries and was held overnight (12 to 18 h) at 75 °F, 60% to 70% RH, with a 16/8 h light/dark photoperiod to allow the larvae to enter the fruit before treatment. Each cultivar had three cycles; for each replication: 'Garnet' used 16, 17, and 20 cartons; 'Tulare' used 20, 20, and 17 cartons; 'Sweetheart' used 20, 15, and 16 cartons. For a test, one or two cartons were placed in polyethylene bags with uninfested cherries used to fill each bag around the cartons to reach the

desired load factor of 50%. The infested cherries were then conditioned 24 h at the planned treatment temperature of 43 °F. The untreated controls were evaluated for larval survival on the day of fumigation. The number of larvae treated was based on the number of survivors in the untreated control for each replication.

By agreement, confirmation of efficacy for one of the treatment schedules would qualify all treatments for quarantine. Thus, infested cherries were treated with methyl bromide at 0.064 oz/ft³ (64 g·m⁻³) for 2 h at a planned temperature of 43 °F and at normal atmospheric pressure, as was done with previous cultivars (Moffitt et al., 1992). All fumigations were conducted in a 34-ft³ (0.96-m³) fumigation chamber equipped with a circulating fan which operated continuously throughout the exposure period. The chamber was enclosed in a containment structure equipped with a combination cooling-heating system to maintain a specific temperature [± 1.8 °F (1 °C)]. Air and gas tightness tests of the chamber were done before the chamber to demonstrate that the chamber sealed properly before the confirmation tests.

Temperatures were monitored

during fumigations using a personal computer-controlled temperature recorder equipped with thermocouples. Five temperature probes were used, one each in the air in the upper and lower parts of the chamber and three in cherries in the load. Concentrations of methyl bromide were monitored by gas chromatography. Four gas sampling tubes were used, one each in the upper and lower parts of the chamber and two in the load.

After treatment, the infested cherries were placed at 75 °F, 70% to 80% RH, with a 16/8 h light/dark photoperiod for 3 d. After the 3-d holding period, the treated cherries were dissected and examined for live larvae. Moribund larvae were held on immature apples to determine delayed mortality. Survival data were accumulated from the cycles; complete mortality was the criterion used to demonstrate efficacy.

PHYTOTOXICITY TESTS. All fumigations were conducted in 7.5-ft³ (28.3-L) fiberglass chambers in the same manner as described for the comparative efficacy studies. Cultivars, temperature conditions and methyl bromide concentrations are given in Table 4. Each

Table 3. Survival of codling moth larvae in cherries fumigated with 0.064 oz/ft³ (64 g·m⁻³) methyl bromide for 2 h at 43 °F (6 °C); accumulated totals from three cycles for each cultivar.

Season	Cultivar	No. larvae		
		Infested	Treated ^z	Survivors
1994	Garnet	11,475	10,200	0
1996	Tulare	11,400	10,587	0
1997	Sweetheart	10,200	7,700	0

^zNo. treated = (no. infested) (no. recovered in control/no. infested in control).

Table 4. Effects of 2-h fumigation with methyl bromide on quality of cherry cultivars

Cultivar	Fumigation			Soluble Titratable		Color				Visual rating ^z	
	Dosage (10 ⁻³ oz/ft ³)	temp (°F)	Firmness (N)	solids (%)	acidity (% malic)	Fruit		Stem		Fruit	Stem
						L	hue	L	hue		
Garnet	64	43	7.6 a ^y	17.5 a	0.70 a	31.8 a	16.3 a	37.8 a	81.5 a	--- ^x	---
	Control	---	6.8 b	17.2 a	0.67 b	32.8 ab	15.7 b	38.3b	82.1b	---	---
	48	54	6.7 a	17.4 a	0.63 a	33.4 a	15.7 a	38.1 a	88.4 a	---	---
	Control	---	6.6 a	17.4 a	0.63 a	34.2 b	14.0 b	40.6 b	96.3b	---	---
	40	63	6.9 a	17.2 a	0.65 a	35.4 a	15.1 a	39.2 a	79.1 a	---	---
	Control	---	6.6 a	16.9 b	0.62 b	35.7 a	13.9 b	41.5 b	83.3 b	---	---
	32	72	6.7a	16.7 a	0.70 a	34.6 a	15.5 a	41.5 a	89.4 a	---	---
	Control	---	6.3 b	16.7 a	0.63 b	34.9 a	13.4 b	44.0 b	83.3b	---	---
Brooks	64	43	8.5 a	20.2 a	0.86 a	23.7 a	15.9 a	22.8 a	91.3a	---	---
	Control	---	8.2 a	19.2b	0.90 b	22.2 b	15.3 a	22.5 a	94.1b	---	---
'Tulare'	64	43	7.7 a	18.8 a	0.81 a	24.6 a	12.7 a	22.4 a	95.6a	---	---
	Control	---	7.4 a	18.7 a	0.80 a	23.4 b	12.4 a	26.8 b	98.7b	---	---
'Lapins'	64	43	6.8 a	21.0 a	0.67 a	25.8 a	13.5 a	21.2 a	63.3 a	1.5 a	1.7 b
	Control	---	6.9 a	20.8 a	0.68 a	25.2 a	14.9 a	25.6 b	84.8 b	1.5 a	1.5 a
Sweetheart	64	43	6.8 a	22.9 a	0.90 a	39.5 a	2.4 a	30.6 a	86.7 a	1.4 a	1.5 a
	Control	---	7.5 a	23.1 a	0.88 a	29.8 b	12.0 b	32.7 a	90.2 a	1.3 a	1.2 b

^zVisual fruit and stem rating on a scale of 1 to 3 (1 = good, 2 = acceptable, 3 = poor).

^yMeans in a column within cultivars not followed by a common letter are significantly different ($P < 0.05$).

^xNot recorded.

treatment was replicated three times. After treatment, treated and control cherries were transported to the USDA-ARS Laboratory in Wenatchee, Wash., and held at 1.8 °F (1 °C) for evaluation.

At intervals of 0 and 14 d, a 4.4-lb (2000-g) sample of cherries was removed from each methyl bromide treatment and replication, and the quality was determined. Quality evaluations consisted of determinations of objective color of fruit and stems, firmness, soluble solids, titratable acidity and subjective scores for fruit and stem condition. Objective color of fruit and stems was determined with The Color Machine (Pacific Scientific, Silver Spring, Md.), using the Hunter L, a, b system and calculated hue values (Hunter and Harold, 1987). Fruit firmness was determined using a TA-XTs texture analyzer (Texture Technologies, Scarsdale, N.Y.) equipped with a 3-mm probe. Soluble solids of the fruit was determined by an Abbe-type refractometer with sucrose scale calibrated at 68 °F (20 °C). Acids were titrated to pH 8.2 with 0.1 N NaOH and expressed as percentage of malic acid. Subjective scores for fruit and stem condition were determined by laboratory personnel familiar with cherry grades. Fruit and stems were rated subjectively on a scale from 1 to 3 (1 = good, 2 = acceptable, 3 = poor) and mean values reported. Analysis of variance was determined by MSTAT (Michigan State University, 1988) based on

significant F test and means were separated using Tukey's studentized range test (HSD).

Results and discussion

COMPARISON TESTS. In the concentration-response mortality studies, proposed cultivars were compared with approved cultivars (Table 1). In 1996, early season 'Lapins' was compared with 'Bing,' and late season 'Sweetheart' was compared with 'Lambert.' For all these tests, mortality rates increased with methyl bromide concentration, yet none had complete efficacy at 0.048 oz/ft³ (48 g·m⁻³).

The concentration-mortality responses of codling moth in the cherry cultivars were generally similar, particularly for LC₅₀ (Table 2). Concentration-response tests between approved and proposed cultivars need to be conducted at the same time to be valid. Variations in seasonal conditions, agricultural practices, and quality of reared larvae preclude across-time comparisons. Thus, based on the overlap of confidence limits among the cultivars within the season tested, there were no significant differences in LC₅₀ and LC₉₀. Moffitt et al. (1992) had similar observations for 'Bing' and 'Rainier' cherries. Likewise, Waddell et al. (1990) reported similar LC₅₀ values for five cherry cultivars in New Zealand.

MAFF-Japan required that probit analysis, without supporting documen-

tation or references, be used for the cultivar comparisons, although goodness-of-fit tests were not required. In the analysis, the probit model assumes that mortality is linear with log (concentration). There were several problems with this approach. First, the assumption was made that the current treatment concentration was near the minimum required for an efficacious treatment. However, the treatment concentration may be beyond the minimum so that even if the LC₅₀s of two cultivars may differ, they both would be efficacious at the treatment level. Second, before the probit model is used, the data should be demonstrated to fit the model. However, other models may be more precise, such as the logit (Waddell et al., 1988). If the intent is to compare mortality response based on cultivar, then the best model should be used. Other researchers have questioned the use of probit models for cultivar comparisons. Maindonald et al. (1992) suggested that log (concentration) should not be routinely used as the explanatory variable, and they demonstrated that LC estimates change based on the assumption of either using linear or power curves. Third, because the concentrations are set by the protocol, there is no quarantine that the resulting mortality rates will be in the range suitable for calculating the LC₅₀s and LC₉₀s. Measured mortalities may be above or below those ranges. Fourth, at the 95% CI, the

predicted LC_{50} s often show much variation (Table 2). When the range is expressed as a percentage based on the predicted mean [$\% = (100)(\text{range}) / \text{predicted}$], the values range from 7.2% to 125.4%. This variation in predicted LC_{50} s has been observed in other studies (Waddell et al., 1988; Yokoyama et al., 1987). Finally, the same cultivar can produced different probit slopes (e.g., Table 2). This indicates that factors other than cultivar influence the concentration-mortality response. Unless it is known what these factors are and what is the degree of their influence, it would be better not to use the probit models for comparisons.

CONFIRMATION TESTS. When fumigated with methyl bromide at 0.064 oz/ft³ (64 g·m⁻³) for 2 h at 43 °F, none of the treated codling moth larvae survived (Table 3). Delayed mortality of treated moribund larvae occurred as anticipated and all larvae were dead within 30 d after fumigation.

The accepted schedule for export of American sweet cherries ('Bing,' 'Lambert,' 'Van,' and 'Rainier') to Japan is 0.064 oz/ft³ at 2 h for 43 to 54 °F (12 °C); 0.048 oz/ft³ at 2 h for 54 to 63 °F (12 to 17 °C); 0.040 oz/ft³ (40 g·m⁻³) at 2 h for 63 to 72 °F (17 to 22 °C); 0.032 oz/ft³ (32 g·m⁻³) at 2 h for 72 °F or above (Moffitt et al., 1992). Based on the results of these confirmation tests of efficacy, all of the proposed cultivars of sweet cherry meet quarantine security when treated with methyl bromide by any of the above schedules.

PHYTOTOXICITY. Fumigation of sweet cherries with methyl bromide did not significantly influence fruit firmness, except for 'Garnet' cherries which was reduced by 10.5% for highest dose and 6% for the lowest dose (Table 4). Soluble solids and titratable acids were reduced for 'Brooks' and 'Garnet' cherries after fumigation, but the differences in both of these quality factors were dose dependent for 'Garnet' cherries. Exposure to methyl bromide generally reduced objective fruit and stem quality for all sweet cherry cultivars and increased visual pitting particularly for 'Brooks' and 'Tulare' cultivars (Table 4). Fumigation resulted in a darker color (reduced Hunter L values) for both fruit and stems of 'Garnet' cherries, particularly with the two highest methyl bromide concentrations. As fumigation temperature increased from 43 to 63 °F, fruit and stem color quality for 'Brooks' and 'Tulare' cultivars declined, particu-

larly of 'Brooks.' 'Tulare' sweet cherries, however, can probably be fumigated with methyl bromide with only minimal losses in fruit quality. The color (L and hue) and visual fruit condition of 'Lapins' cherries were similar between methyl bromide fumigated and control fruit. 'Sweetheart' cherry fruit color was changed with the use of methyl bromide (reduced L and hue values), but visual fruit quality was not reduced. This change in L and hue values for 'Sweetheart' cherries suggested that methyl bromide exposure produced darker red colored fruit than nontreated fruit. Stem color (L and hue) of 'Lapins' cherries was reduced as a result of fumigation with methyl bromide. Both Hunter L and hue values were less for fumigated than nonfumigated fruits indicating a darker, less green colored stem for methyl bromide treated fruit. Visual evaluation of the stems of 'Lapins' cherries agreed with objective color and indicated a less desirable stem condition with the use of methyl bromide. This reduction in stem color was also evident when stems were graded visually (1.5 vs. 1.7) and would be apparent to the consumer. Stem color of 'Sweetheart' cherries was not influenced with methyl bromide fumigation, but visual stem condition was reduced (1.2 vs. 1.5). This slight difference in stem color may be unnoticed by anyone other than individuals experienced in stem color evaluation. Use of methyl bromide as a fumigant can reduce the quality (firmness, color, etc.) of all cultivars of sweet cherries, but not to the extent that consumer quality would be in doubt. Time and temperature of fumigation had a more deleterious influence on fruit and stem quality than did the methyl bromide fumigation, and the higher the temperature and longer the exposure time, the lower the quality.

WTO DECISION. In 1998, the United States won a decision from the WTO that further cultivar testing for efficacy of quarantine treatments was not necessary once Japan accepted a commodity species. This decision was upheld in 1999 after an appeal by Japan. Hence, the process described in this paper will no longer be used. However, much effort went into demonstrating that the listed cultivars for Japan were protected by an efficacious quarantine treatment and that this research should be documented for future reference.

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