Test 4 Large-Scale Mortality Tests for Six-Day-Old Eggs and Fifth Instar Diapause Larvae of Peach Fruit Moth by a Combined Cold Storage and Methyl Bromide Fumigation Treatment

Introduction

Susceptibility of all life stages of peach fruit moth, *Carposina niponensis* WALSINGHAM and yellow peach moth, *Conogethes punctiferalis* (GUENÉE) to methyl bromide fumigation showed that two-day-old eggs of peach fruit moth were the most resistant stage. And it was estimated that a practical dose of methyl bromide which was sufficient to attain 100% mortality of two-day-old eggs of peach fruit moth would be approximately 50 g/m^3 or more when the fumigation was conducted for 2 hours at 15°C (Part 2, Test 1 in this report).

On the other hand, 'Fuji' apples stored for shorter period after harvest were extremely susceptible to methyl bromide fumigation and doses of methyl bromide sufficiently high to attain 100% mortality of two-day-old eggs of peach fruit moth may cause chemical injury to apples (Part 4, Test 1 in this report). However, no or only a slight injury, was observed on apples stored for 30 days or more prior to fumigation at such low temperature as −1 to 0°C (Part 4, Test 1 in this report).

Susceptibility studies of all life stages of the two pests to low temperatures showed that larval stages were more resistant than egg stages of both pests ; six-day-old eggs of all egg stages and fifth instar diapause larvae of all larval stages of peach fruit moth were more resistant stages in those life stages. Fifth instar diapause larvae of the peach fruit moth were the most resistant to low temperatures of all life stages of the two pests.

Cold storage periods of 120 to 150 days at 0°C would be required for complete mortality of fifth instar diapause larvae of the peach fruit moth (Part 2, Test 2 in this report). However, the diapause larvae surviving cold storage treatment for a certain periods have become more susceptible to the subsequent methyl bromide fumigation (Part 2, Test 3 in this report).

Therefore, a combined cold storage and methyl bromide fumigation treatment was deviced, whereby six-day-old peach fruit moth eggs, which were the most resistant of all ages of eggs to low temperature, will be completely killed by cold storage as the first step of the treatment. Fifth instar diapause larvae of peach fruit moth, which is the most resistant to low temperature of larval stages, will survive the single cold storage treatment (Part 2, Test 2 in this report). This stage was the least susceptible to methyl bromide fumigation and will be killed 100% by subsequent methyl bromide fumigation (Part 2, Test 1 in this report) as the second step of the treatment.

Two disinfestation standards of the combined cold storage and methyl bromide fumigation were thus established on the basis of basic test data for attaining sufficient quarantine security. Large-scale mortality tests were conducted with two practical standards to confirm if six-day-old peach fruit moth eggs would be killed 100% by the single cold storage treatment and if fifth instar peach fruit moth diapause larvae would be killed 100% by the combined cold storage and methyl bromide fumigation treatment.

Materials and Methods

1. Test Fruit

Medium size (36 per box) 'Fuji' apples produced in Hirosaki City, Aomori Prefecture and stored at −1 to 0°C after harvest were obtained from a packing house and stored at 1.5°C before testing.

2. Test Insects and Preparation of Infested Fruit

Test insects were obtained from the Fruit Tree Research Station, Ministry of Agriculture, Forestry and Fisheries (Tsukuba City, Ibaraki Prefecture) in May 1987 and another strain of the insect was obtained in April 1988 from Aomori Apple Experimental Station, Aomori Prefecture (AAES, Aomori).

They were reared on immature apples using methods described by NARITA (1986b) and test insects were prepared as follows; fruit infested with six-day-old eggs were prepared at the Research Division, Yokohama Plant Protection Station, Ministry of Agriculture, Forestry and Fisheries (YPPS, MAFF). Fruit infested with fifth instar diapause larvae were prepared at the both AAES, Aomori and YPPS, MAFF; ie. infested fruit with first instar larvae were transported to YPPS, MAFF from AAES, Aomori, and reared until the desired fifth instar larval stages were reached.

a. Six-Day-Old Eggs Oviposited on Mature Apples

Thirty each of males and females were placed in a plastic cylinder 15 cm in height and 9 cm in diameter. The test insects were allowed to mate for 24 hours in the containers which were maintained in the rearing room at 25°C, 70% R.H. with a 16L : 8D photoperiod. In order to facilitate the counting of the number of eggs oviposited and hatched, the stalk cavity and calyx end of the apples were filled with melted wax. Six apples thus prepared were placed in a plastic container (27 cm × 30 cm × 9 cm in size). Thirty mated females were released into the container, which was placed in the rearing room, and allowed to oviposit for 24 hours at 25°C, 70% R.H. with a 16L : 8D photoperiod. The females were then removed from the container. The apples were left in the container in the rearing room and removed after 5 days to obtain six-day-old eggs.

b. Fifth Instar Diapause Infested in Mature Apples

Four to five mated females, which were prepared the same manner as above (a), were allowed to oviposit for 24 hours on a piece of wax paper with 1.5 cm deep creases which was placed in a petri dish. The pieces of wax paper (20 eggs per piece) were then placed on the stalk cavity and calyx end of each mature apple placed in a row in a plastic container (37 cm × 46 cm × 16 cm in size). The containers were placed in the rearing room and maintained for 19 to 20 days at 25°C, 70% R.H. with a 12L : 12D photoperiod until fifth instar larvae could be obtained.

3. Disinfestation Treatment Standards

Disinfestation standards to provide quarantine security for complete mortality of six-

day-old eggs and fifth instar diapause larvae of peach fruit moth on/in 'Fuji' apples to be exported to the United States were determined with consideration of the following items obtained from basic tests ; ie. a : a complete kill for six-day-old eggs of peach fruit moth, which were the most resistant stage of all egg stages, was achieved in 30 days to 36 days by Standard Cold storage (SC) or Controlled Atmosphere storage (CA) at 1.5±0.5°C (Part 2, Test 2 in this report), b : The LD_{95}'s was 16.4 g/m³ for fifth instar diapause larvae of peach fruit moth, which were the most resistant stages of the larval stages (Part 2, Test 1 in this report), c : methyl bromide sorption rates in loading factors for export were 3.7% and 17.7 to 16.3% of initial doses, respectively, for apples and export carton boxes including packing materials (Part 5, Test 2 in this report). d : additional doses are added in proportion to sorption to the surface of the chamber or possible gas leakage during fumigation and to provide an additional degree of quarantine security. The following two treatment schedules of combined cold storage and methyl bromide fumigation were established for 'Fuji' apples to be exported to the United States.

Six-Day-Old Peach Fruit Moth Eggs

Cold storage : Standard Cold storage at 0.5±0.5°C for 40 days or more, fruit in plastic field bins.

Fifth Instar Peach Fruit Moth Diapause Larvae

Standard 1 : Cold storage (Standard Cold storage at 0.5±0.5°C for 40 days or more, fruit in plastic field bins)+Methyl bromide fumigation (Methyl bromide at 38 g/m³ for 2 hours at 15°C or above with 40% or less loading, fruit packed in export cartons).

Standard 2 : Cold storage (Standard Cold storage at 0.5±0.5°C for 40 days or more, fruit in plastic field bins)+Methyl bromide fumigation (Methyl bromide at 48 g/m³ for 2 hours at 10°C or above with 50% or less loading, fruit in plastic field bins).

Large-scale mortality tests were conducted under less favorable conditions than the treatment schedule established to attain maximum efficacy for export to the United States ; ie. for cold storage, the temperatures were chosen at 1.5°C or 0.5°C, which was higher than the −1 to 0°C of commercial cold storage and the treatment periods were chosen for 37 to 40 days, which were shorter than those for the standards. For fumigation, doses of methyl bromide (29.0 to 35.4 g/m³ at 15°C and 39.1 to 44.4 g/m³ at 10°C) were applied as less than those of the standards. Minimum temperatures of 15°C or 10°C and maximum permissible loading rate of more than 40% at 15°C and more than 50% at 10°C were chosen.

4. Cold Storage

In the experiments conducted in May to July 1989, apples infested with eggs and fifth instar larvae were placed separately in molded paper packs and packed in two layers into styrofoam boxes (41 cm×48 cm×23 cm in size), which were then sealed. Styrofoam boxes and molded paper packs were used with a view to simulate a gradual decline in temperature to avoid adversely affecting the efficacy of the treatment.

In the experiments conducted in December 1989 to July 1990, infested apples with eggs and fifth instar larvae were placed separately into wooden field bins (31.8 cm×63.5 cm×32.0 cm in size) which had been lined with a nylon-meshed bag to prevent larvae escaping. These containers were placed in the 31.5 m^3 cold chamber (4.3 m×3.2 m×2.3 m in size) with temperature adjustment of ±0.5°C, 60 to 90% R.H., and 4 defrosting cycles per day. A multi-channel automatic temperature recorder (Hybrid Recorder: AH, Chino) was used to monitor air and fruit pulp temperatures during the treatment. Temperature recorder and probes were calibrated in ice water at 0°C.

5. Fumigation

Following cold storage, apples infested with fifth instar diapause larvae were fumigated with one of the following two methods; ie. in fumigation with Standard 1, each apple covered with a meshed polyethylene fruit cap was packed with two layers (36 fruit, ca. 10 kgs) in a corrugated carton box (38 cm×44 cm×25 cm in size, 0.043 m^3 in capacity) with six fiberglass screen-covered vents (4 vents: 2 cm×5 cm, 2 vents: 4 cm×2 cm; vent ratio of 0.74%) on four sides. One macerated paper sheet was placed on the bottom of the carton and between the first and second layer, and one polyethylene meshed sheet was placed on the top of the first layer. Export cartons were sealed with sealing tape. These apples were stored for 3 days at the fumigation temperature of 15°C. Five of these cartons (ca. 50 kgs) were then placed in a 0.52 m^3 stainless steel fumigation chamber (0.9 m×0.66 m×0.86 m in size) equipped with a 0.86 m^3/min. circulation and ventilation apparatus, a graduated dispenser, and an ampoule breaker and vaporizer for methyl bromide application, ports for gas sampling, temperature probes and manometer to make a load of 40%.

In fumigation with Standard 2, infested apples were transfered to plastic field bins (31.8 cm×63.5 cm×32.0 cm in size, 0.062 m^3 in capacity) with many vents on four sides (70 to 80 fruit, ca. 20 kgs) without packing materials. Four bins were placed in the above mentioned fumigation box to make a load of 50% and stored for 3 days at the fumigation temperature of 10°C.

Methyl bromide enclosed in an ampoule was applied by using a built-in ampoule breaker or a graduated dispenser. Fumigation was conducted for 2 hours at 15°C or 10°C with applied doses of methyl bromide are shown in Table 2(4)-2, Table 2(4)-3 and Table 2(4)-7 (29.0 to 35.4 g/m^3 at 15°C and 39.1 to 44.4 g/m^3 at 10°C). The built-in circulation apparatus was kept on for the first 30 minutes and then used with an automatic timer (on: 0.5 minutes, off: 2.5 minutes) throughout the fumigation. Gas concentrations and air and fruit pulp temperatures during the fumigation were monitored by using a gas chromatograph (FID: GC 8AF, Simazu) and a multi-channel automatic temperature recorder (Hybrid Recorder: AH, Chino). Following fumigation, the air-fumigant mixture was exhausted for one hour at 15°C or 10°C by using the built-in ventilation apparatus.

6. Determination of Mortality

Cold-treated apples infested with eggs were maintained in the rearing room at 25°C, 70% R.H. with a 16L: 8D photoperiod. Eggs were observed under microscopes after 4 days to count the number of eggs hatched. The apples were cut and assessed after 30 days

when emerged larvae were expected to have reached the fifth instar. Cold-treated and subsequently fumigated apples infested with larvae were maintained in the rearing room at 25°C, 70% R.H. with a 12L : 12D photoperiod after 3 to 7 days. They were then all cut and assessed.

Results and Discussion

1. Fruit Temperatures During Cold Storage

The average fruit temperature, for six-day-old eggs anf fifth instar diapause larvae of peach fruit moth, in the tests conducted in May to July 1989 was 2°C (2.5°C maximum, 1.5°C minimum), which was 2°C higher than in commercial storage. Most temperature recordings were in the range of 2.0±0.5°C. It took as long as 168 hours for apples to drop from the rearing room temperature of 25°C to 1.5°C or below owing to use of styrofoam boxes.

Fruit pulp temperatures during cold storage conducted in December 1989 to July 1990 are shown in Table 2(4)-1 (six-day-old eggs of peach fruit moth), Table 2(4)-2 (Standard 1 : fifth instar diapause larvae of peach fruit moth) and Table 2(4)-3 (Standard 2 : fifth instar diapause larvae of peach fruit moth).

The temperature was set at 0.5±0.5°C, but most temperature recordings were in the 0.5±0.5°C range (1.2°C maximum, −0.3°C minimum). It took 50 hours on the average (96 hours maximum, 32 hours minimum) for apples to drop from the rearing room temperature of 25°C to cold storage of 0.5°C or below.

2. Methyl Bromide Gas Concentrations and Temperatures During Fumigation

Methyl bromide gas concentrations and temperatures monitored during fumigation for fifth instar diapause larvae of peach fruit moth were as follows;

Table 2(4)-4 shows records obtained from the tests conducted in May to July 1989. The average of residual gas concentrations in export carton boxes 2 hours after fumigation were 24.4 mg/ℓ and 28.8 mg/ℓ for initial doses of 30 g/m^3 and 35 g/m^3, respectively. Sorption ratios were 18.6% and 17.9% of initial doses, respectively. These monitoring records were similar to the results of data from 'Methyl Bromide Penetration in Practical Fumigation' (Part 5, Test 2 in this report).

Data from tests conducted in December 1989 to July 1990 are shown in Table 2(4)-2 and Table 2(4)-3, respectively, for Standard 1 and Standard 2.

The average of residual gas concentrations 2 hours after fumigation were 28.9 mg/ℓ (24.4 to 30.7 mg/ℓ range) and 42.5 mg/ℓ (37.9 to 45.8 mg/ℓ range), for export carton boxes in Standard 1 and for plastic field bins in Standard 2, respectively. Methyl bromide sorption ratios were 13.2% (for the export carton boxes, fruit and packing materials) in Standard 1 and 2.6% (for the field bins and fruit) in Standard 2, respectively.

Fruit temperatures during fumigation were 14.6 to 15.0°C in Standard 1 and 9.3 to 10°C in Standard 2, respectively, which were lower than established for the two Standards.

Table 2(4)-1. Fruit temperatures recorded in Standard Cold storage treatment at 0.5±0.5°C for 38 to 39 days for 6-day-old eggs of the peach fruit moth, *Carposina niponensis* (December 1989 to July 1990).

Replicate	Period in cold storage (days)	Fruit temperature (°C)	
		Maximum	Minimum
1	39	0.9	−0.2
2	39	1.0	0.0
3	38	0.9	−0.1
4	38	0.9	0.0
5	38	1.0	0.0
6	38	0.9	−0.2

Table 2(4)-2. Fruit temperatures and methyl bromide gas concentrations recorded in the combined treatment of Standard Cold storage at 0.5±0.5°C for 38 to 40 days and methyl bromide fumigation at dose of 29.0 to 35.4 g/m³ for 2 hours at 15°C for 5th instar diapause larvae of the peach fruit moth, *Carposina niponensis* (Standard 1 : December 1989 to July 1990).

Cold treatment			Methyl bromide Fumigation					
Replicate	Fruit temperature (°C)		Replicate	Dose (g/m³)	Gas concentration (mg/ℓ)		Temperature (°C)	
	Max.	Min.			0	120 min.	Chamber	Fruit
1	0.9	−0.2	1-1	33*	34.0	30.2	16.1	14.8
			-2	29*	25.9	—	16.0	15.0
2	0.9	−0.1	2-1	29*	25.9	24.4	15.6	14.7
			-2	29*	27.8	25.0	15.5	14.7
3	0.9	−0.2	3-1	33*	32.5	29.6	15.0	14.8
			-2	33*	32.7	29.7	15.2	14.7
4	0.8	−0.2	4-1	33*	34.0	30.7	15.8	14.8
			-2	33*	32.7	29.7	15.6	14.9
5	0.8	−0.1	5-1	34.0	32.2	28.5	15.5	14.8
			-2	33.8	32.1	28.6	15.3	14.8
6	1.0	0.1	6-1	34.5	32.9	28.8	16.1	15.0
			-2	33.7	31.6	27.8	16.3	14.8
7	1.0	−0.2	7-1	34.4	32.8	30.5	15.0	14.8
			-2	34.3	32.8	30.5	15.3	14.7
8	0.9	−0.1	8-1	34.1	32.8	29.5	16.0	14.9
9	0.9	−0.1	9-1	34.2	32.8	28.7	15.8	14.6
			-2	33.5	32.4	28.4	16.0	14.8
10	1.0	0.0	10-1	34.8	33.2	30.0	16.1	14.8
			-2	35.4	32.5	29.2	16.0	14.8

* A graduated dispenser was used for methyl bromide application.

Table 2(4)-3. Fruit temperatures and methyl bromide gas concentrations recorded in the combined treatment of Standard Cold storage at 0.5±0.5°C for 37 to 40 days and methyl bromide fumigation at doses of 39.1 to 44.4 g/m³ for 2 hours at 10°C for 5th instar diapause larvae of the peach fruit moth, *Carposina niponensis* (Standard 2 : December 1989 to July 1990).

Cold treatment			Methyl bromide fumigation					
Replicate	Fruit temperature (°C)		Replicate	Dose (g/m³)	Gas concentration (mg/ℓ)		Temperature (°C)	
	Max.	Min.			30	120 min.	Chamber	Fruit
1	0.8	−0.1	1-1	39.1	43.2	38.7	10.8	9.8
			-2	44.1	49.2	44.6	10.8	9.8
2	1.0	−0.2	2-1	44.2	48.8	45.3	10.8	9.3
			-2	44.4	48.5	45.8	10.5	9.3
3	0.9	−0.2	3-1	43.9	48.3	43.0	11.0	9.7
			-2	43.6	49.8	45.1	11.0	10.0
4	0.8	−0.1	4-1	43.6	48.2	42.5	10.9	9.8
			-2	43.0	49.9	43.3	11.1	9.8
5	0.9	−0.2	5-1	43.9	48.0	41.7	10.8	9.9
			-2	44.4	48.8	43.2	10.6	10.0
6	0.8	−0.1	6-1	44.0	48.3	41.9	11.0	10.0
			-2	43.8	50.2	43.2	11.0	10.0
7	0.9	−0.3	7-1	44.4	47.5	39.3	11.0	10.0
8	0.9	−0.2	8-1	44.2	47.3	37.9	11.2	9.8
			-2	44.0	46.4	39.2	11.0	9.8
9	1.2	0.1	9-1	43.8	47.0	43.6	10.9	9.9
			-2	44.4	52.7	43.8	11.1	10.0
			-3	44.0	52.9	44.4	10.8	9.9

Table 2(4)-4. Methyl bromide concentrations recorded in a 0.52 m³ fumigation chamber. Fumigation at doses of 30 g/m³ and 35 g/m³ for 2 hours at 15°C with 40% loading (Standard 1 : May to July 1989).

Gas sampling points	Dose (g/m³) Gas concentration (mg/ℓ) & Fruit temperature (°C)							
	30 g/m³				35 g/m³			
	10	30	120 min.	Fruit temp.	10	30	120 min.	Fruit temp.
Replication 1				15				15
Air space	26.9	26.0	23.9		33.3	31.7	29.3	
Top (in carton)	26.6	25.8	23.2		32.1	31.4	28.9	
Bottom (in carton)	26.2	25.6	23.4		32.1	31.2	28.3	
Replication 2				15				15
Air space	29.2	27.6	25.8		33.4	31.5	28.9	
Top (in carton)	28.5	27.3	25.1		33.0	31.3	28.6	
Bottom (in carton)	28.3	27.3	25.1		32.9	31.4	28.4	

3. Mortality

Six-Day-Old Peach Fruit Moth Eggs Treated by Cold Storage

Results of the mortality tests for six-day-old peach fruit moth eggs treated by cold storage alone are shown in Table 2(4)-5 (tests in May to July 1989) and Table 2(4)-6 (tests in December to July 1990). A total number of 1,518 eggs treated at 1.5±0.5°C for 40 days in a 1 replicate test conducted in May to July 1989 and that of 60,804 eggs treated at 0.5± 0.5°C for 38 to 39 days in a 6 replicate test conducted in December 1989 to July 1990 were completely killed. It was confirmed that a grand total of 62,322 six-day-old peach fruit moth eggs was killed by the cold storage treatment alone in tests with 7 replicates.

Table 2(4)-5. Mortality of 6-day-old eggs of the peach fruit moth, *Carposina niponensis*, on 'Fuji' apples stored for 38 to 39 days in Standard Cold storage at 1.5±0.5°C (May to July 1989).

Stage	Exposure (days)	Number tested	Number dead	Percent mortality	Percent natural mortality
6-day-old eggs	40	1,518	1,518	100	—
	Cont.	1,858	441	—	23.7

Table 2(4)-6. Mortality of 6-day-old eggs of the peach fruit moth, *Carposina niponensis*, on 'Fuji' apples stored for 38 to 39 days in Standard Cold storage at 0.5± 0.5°C (December 1989 to July 1990).

Replicate	Control				Treatment			
	No. of apples infested	No. of eggs infested	No. of eggs hatched	Percent hatched	No. of apples infested	No. of eggs treated*	No. of eggs hatched	Percent mortality
1	20	2,016	1,458	72.9	190	13,851	0	100
2	18	1,486	1,161	64.5	102	6,579	0	100
3	50	4,964	4,015	80.3	310	24,893	0	100
4	10	594	461	46.1	30	1,383	0	100
5	23	1,185	916	38.8	100	3,982	0	100
6	32	2,115	1,800	56,2	180	10,116	0	100
Total	153	12,360	9,811	—	912	60,804	0	100

* Based on percent hatched in control.

Fifth Instar Peach Fruit Moth Diapause Larvae Treated by The Combined Cold Storage and Methyl Bromide Fumigation Treatment

Results of the mortality tests for fifth instar peach fruit moth diapause larvae treated by the combined cold storage and methyl bromide fumigation treatment are shown in Table 2(4)-7 (tests in May to July 1989 with Standard 1), Table 2(4)-8 (tests in December 1989 to July 1990 with Standard 1) and Table 2(4)-9 (tests in December 1989 to July 1990 with Standard 2).

A total of 55,851 fifth instar peach fruit moth diapause larvae treated by Standard 1 (Cold storage: Standard Cold storage at 0.5±0.5°C for 34 to 40 days+Methyl bromide

Table 2(4)-7. Mortality of 5th instar diapause larvae of the peach fruit moth, *Carposina niponensis*, in 'Fuji' apples stored for 34 days in Standard Cold storage at 1.5± 0.5°C followed by fumigation with methyl bromide at doses of 30 g/m³ or 35 g/m³ for 2 hours at 15°C with 40% loading (Standard 1 : May to July 1989).

Replicate	Dose (g/m³)	Number tested	Number dead	Percent mortality	Percent natural mortality
1	30	323	323	100	—
2	35	340	340	100	—
	Cont.	340	0	—	0
3	30	311	311	100	—
4	35	326	326	100	—
	Cont.	266	0	—	0
Total	Treat.	1,300	1,300	100	—
	Cont.	606	0	0	0

Table 2(4)-8. Mortality of 5th instar diapause larvae of the peach frmuit moth, *Carposina niponensis*, in 'Fuji' apples stored for 38 to 40 days in Standard Cold storage at 0.5±0.5°C followed by methyl bromide fumigation at doses of 29.0 to 35.4 g/m³ for 2 hours at 15°C with 40% loading (Standard 1 : December 1989 to July 1990).

Control			Combined treatment					
			Replicate					
No. of apples infested	No. of larvae infested	No of. larvae infested per apple	Cold storage	Fumigation	No. of apples infested	No. of larvae treated*	No. of larvae survived	Percent mortality
52	954	18.3	1	1-1	108	1,976	0	100
				-2	108	1,976	0	100
52	1,075	20.7	2	2-1	180	3,726	0	100
				-2	180	3,726	0	100
50	514	10.3	3	3-1	180	1,854	0	100
				-2	180	1,854	0	100
50	511	10.2	4	4-1	178	1,816	0	100
				-2	176	1,795	0	100
50	941	18.8	5	5-1	180	3,384	0	100
				-2	180	3,384	0	100
50	989	19.8	6	6-1	180	3,564	0	100
				-2	180	3,564	0	100
50	583	11.7	7	7-1	180	2,106	0	100
				-2	180	2,106	0	100
50	722	15.4	8	8-1	160	2,464	0	100
50	1,023	20.8	9	9-1	180	3,744	0	100
				-2	180	3,744	0	100
50	1,037	21.3	10	10-1	180	3,834	0	100
				-2	180	3,834	0	100
Total								
504	8,349	—	10	19	3,250	54,551	0	100

* Based on survival in control.

Table 2(4)-9. Mortality of 5th instar diapause larvae of the peach fruit moth, *Carposina niponensis*, in 'Fuji' apples stored for 37 to 40 days in Standard Cold storage at 0.5±0.5°C followed by methyl bromide fumigation at doses of 39.1 to 44.4 g/m³ for 2 hours at 10°C with 50% loading (Standard 2: December 1989 to July 1990).

Control			Combined treatment					
			Replicate					
No. of apples infested	No. of larvae infested	No. of larvae infested per apple	Cold storage	Fumigation	No. of apples infested	No. of larvae treated*	No. of larvae survived	Percent mortality
50	971	19.4	1	1-1	160	3,040	0	100
				-2	160	3,040	0	100
50	954	19.1	2	2-1	240	4,548	0	100
				-2	240	5,548	0	100
50	776	15.5	3	3-1	240	3,724	0	100
				-2	240	3,724	0	100
50	905	18.1	4	4-1	240	4,344	0	100
				-2	240	4,344	0	100
50	757	15.1	5	5-1	200	3,028	0	100
				-2	200	3,028	0	100
50	897	17.9	6	6-1	240	4,296	0	100
				-2	240	4,296	0	100
50	892	17.8	7	7-1	147	2,616	0	100
50	1,254	25.1	8	8-1	200	5,020	0	100
				-2	200	5,020	0	100
40	584	14.1	9	9-1	233	3,401	0	100
				-2	233	3,401	0	100
40	493	12.3		-3	233	2,866	0	100
Total								
480	8,483	—	9	18	3,886	69,284	0	100

* Based on survival in control.

fumigation: 29.0 to 35.4 g/m³ of methyl bromide at 14.6 to 15.0°C for 2 hours) in 11 replicates of cold storage and 23 replicates of fumigation tests conducted in May to July 1989 and December 1989 to July 1990 were killed completely by the treatment. A total of 69,284 larvae treated by Standard 2 (Cold storage: Standard Cold storage at 0.5±0.5°C for 37 to 40 days+Methyl bromide fumigation: 39.1 to 44.4 g/m³ of methyl bromide at 9.3 to 10°C for 2 hours) in 9 replicates of cold storage and 18 replicates of fumigation tests conducted in December 1989 to July 1990 were also killed completely by the combined cold storage and methyl bromide fumigation treatment.

These results were obatained from tests with less favorable conditions than those of the disinfestation standards established for export to the United States. In commercial practice, apples are stored without packing materials in field bins and cooled to −1 to 0°C within as short period as possible. Such a rapid fall in fruit temperature would be a favorable condition for attaining a complete kill of insects. Hence, it could be said that complete mortality of insects in a commercial treatment would probably be obtained within a shorter period than that obtained from these large-scale mortality tests. It would

be also possible to attain 100% mortality with lower doses of methyl bromide than those used in the large-scale mortality test since it was confirmed that surviving fifth instar diapause larvae treated by cold storage were more susceptible to subsequent methyl bromide fumigation (Part 2, Test 3 in this report).

The combined cold storage and methyl bromide fumigation treatment in commercial practice will be conducted under more favorable conditions for attaining effective quarantine security than those used in the large-scale mortality tests ; ie. fruit temperatures of 0°C or below in cold storage and methyl bromide doses of 38 g/m^3 at 15°C or 48 g/m^3 at 10°C are to be applied commercial practice. It is, therefore, believed that all stages of peach fruit moth and yellow peach moth which may be present on/in 'Fuji' apples at harvest would be completely killed by the combined cold storage and methyl bromide fumigation treatment established for export to the United States and that the two standards would be sufficiently guarantee quarantine security.