

Test 2 Susceptibility of Each Stage of Peach Fruit Moth and Yellow Peach Moth to Low Temperature

Introduction

Of all life stages of peach fruit moth, *Carposina niponensis* WALSINGHAM and yellow peach moth, *Conogethes punctiferalis* (GUENÉE), two-day-old egg of peach fruit moth was the most resistant stage to methyl bromide fumigation for 2 hours at 15°C. The LD₅₀'s and LD₉₅'s for two-day-old eggs were 23.3 g/m³ and 33.3 g/m³, respectively. And it was estimated that the methyl bromide dose needed to attain 100% mortality of this stage would be approximately 50 g/m³ in a practical disinfestation standard (Part 1, Test 1 in this report).

On the other hand, phytotoxicity studies showed that the tolerance of 'Fuji' apples to methyl bromide fumigation varied depending on the length of period kept in cold storage prior to fumigation. That is, 'Fuji' apples stored for shorter periods after harvest were extremely susceptible to the fumigation even though at low doses, while no chemical injury or only a slight injury were observed on apples stored for a month or more at 1 to 0°C (Part 3, Test 1 in this report). Results of studies indicated that methyl bromide doses sufficiently high to attain 100% mortality of two-day-old peach fruit moth eggs, which were the most resistant stage to the fumigation (Part 2, Test 1 in this report), may cause chemical injury to apples, and that it would be difficult to control peach fruit moth and yellow peach moth by methyl bromide fumigation alone.

Other alternative methods must be introduced for apples destined for export to the United States. One such method is cold treatment in the place of methyl bromide fumigation. However, there are no such mortality test data available for peach fruit moth and yellow peach moth. Basic studies must therefore be conducted to evaluate the procedure as a potential quarantine pest control technique for apples infested with the two pests.

Cold treatments have been studied to control codling moth, *Cydia pomonella* (L.), oriental fruit moth, *Grapholitha molesta* (BUSCK) and navel orangeworm, *Amyelois transitella* WALKER.

NEWCOMER (1930) reported that exposures of codling moth eggs on apples to low temperatures ranging from -3.3 to 0.6°C for 5 days resulted in 50.1% mortality. He later reported 99.2% mortality of eggs on apples with 35 days exposure to -1.1 to 0°C and 100% mortality for young codling moth larvae in apples was obtained at -1.1 to 0°C for 35 days, respectively (NEWCOMER, 1930). Codling moth larvae in apples survived the entire period of 120 days in both Standard Cold storage (SC) and Controlled Atmosphere cold storage (CA; 2.5% CO₂, 1.0 to 1.5% O₂) at -0.6°C for 120 days (MOFFITT, 1971). All life stages of codling moth were exposed under the same conditions of SC or CA storage at -0.6°C for 30 to 133 days. The result showed that no one- and five-day-old eggs, immature larvae (14~18 days old) and adults were killed 100% with 30 days storage, while mature larvae were also killed 100% with 90 days storage. However, diapause larvae survived 133 days of SC and CA storage, and also, a few pupae survived storage of either type for 60

days (MOFFITT & ALBANO, 1972). Codling moth eggs oviposited on wax paper and first to fourth instar naked larvae were completely killed under the condition of SC storage at 0 °C for 21 days, however, fifth instar larvae survived (YOKOYAMA & MILLER, 1989). The order of tolerance of three embryonic stages of codling moth to low temperature (0°C) was red ring stage (three- to four-day-old) > white stage (one- to two-day-old) > blackhead stage (five- to six-day-old). Red ring stage eggs were 1.5 times more tolerant of low temperature than were white stage eggs and 2.5 times more tolerant than blackhead stage eggs. Thirty-six to 40 days exposure was required for complete mortality of red ring stage eggs on mature apples (MOFFITT & BURDITT, 1989). Of the tolerance of navel orangeworm and oriental fruit moth, larval stages were more tolerant than egg stages, and diapause larvae were the most tolerant of all life stages of the two pests (DUSTON et al., 1963; TEBBETS et al., 1978; YOKOYAMA & MILLER, 1989).

These data for these internal fruit feeders indicate that larval stages are more tolerant to cold treatment than are egg stages, and that diapause larvae are the most tolerant of the larval stages. These studies also indicate that 150 or more days may be required to attain complete mortality of diapause larvae.

Our objectives were to study the relationship between low temperatures, exposure periods and mortalities for all life stages of peach fruit moth and yellow peach moth which may be present on/in apples at harvest and to select the most tolerant stage of the two pests for use in cold treatments in large-scale mortality tests.

Materials and Methods

1. Test Fruit

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

2. Test Insects and Preparation of Infested Fruit

Peach Fruit Moth

Test insects were obtained from the Fruit Tree Research Station, Ministry of Agriculture, Forestry and Fisheries (FTRS, MAFF; Tsukuba City, Ibaraki Prefecture) in May 1987 and another strain of the insect was obtained from Aomori Apple Experiment Station, Aomori Prefecture (AAES, Aomori) in April 1988. They were reared on immature apples using methods described by NARITA (1986b) at the Research Division, Yokohama Plant Protection Station, Ministry of Agriculture, Forestry and Fisheries (YPPS, MAFF) and test insects were prepared as follows;

a. Eggs Oviposited on Immature 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.

Immature apples were placed in a row in a plastic container (27 cm × 30 cm × 9 cm in

size). Thirty mated females were released into the containers, which were then 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 containers and removed after 1, 3, and 5 days to obtain two-, four-, and six-day-old eggs, respectively. Eggs within 24 hours of oviposition were considered one-day-old.

b. Eggs Oviposited on Mature Apples

Mated females were prepared the same manner as above (a). In order to facilitate the counting of the number of eggs oviposited and hatched, the dents at the stem end and calyx end 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 containers, which were 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 containers and removed after 1, 3, and 5 days to obtain two-, four-, and six-day-old eggs, respectively. Eggs within 24 hours of oviposition were considered one-day-old.

c. Larvae Infested in Mature Apples

Mated females were prepared the same manner as above (a). Four to five mated females were allowed to oviposit for 24 hours on a piece of wax paper with 1.5 mm deep creases which was placed in a petri dish. The pieces of wax paper were then placed on the stem end or calyx end of each mature apple placed in a row in plastic containers (37 cm × 46 cm × 16 cm in size). The containers were placed in the rearing room and maintained at 25°C, 70% R.H. with a 16L : 8D photoperiod for non-diapause larvae and a 12L : 12D photoperiod for diapause inducing larvae (diapause larvae), respectively, until larvae of the desired instar could be obtained (first instar-4 days, third instar-10 days, fifth instar-19 to 20 days).

d. Larvae Infested in Immature Apples

Mated females were prepared the same manner as above (a). Immature apples were placed in a plastic container (27 cm × 30 cm × 9 cm in size). Thirty mated females were released into the containers, which were then 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 removed and containers were then placed in the rearing room and maintained 25°C, 70% R.H. with a 16L : 8D photoperiod for non-diapause larvae and a 12L : 12D photoperiod for diapause inducing larvae (diapause larvae), respectively, until larvae of the desired instar could be obtained (first instar-4 days, third instar-10 days, fifth instar-19 to 20 days).

Yellow Peach Moth

Test insects were obtained from the Laboratory of Applied Entomology, Faculty of Agriculture, University of Tokyo (Yayoi, Bunkyo-ku, Tokyo) in May 1987. They were reared at the YPPS, MAFF on fresh chestnuts or corn using methods described by HONDA (1979). The test insects were prepared as follows ;

a. Eggs Oviposited on Cheesecloth

Yellow peach moth eggs oviposited on cheesecloth were used as described by HONDA (1979), since KADOI & KANEDA (1990) found that yellow peach moth would not oviposit on apple fruits. Several immature apples were placed in two tea strainers which were tied face to face, wrapped in a piece of cheesecloth and hung from the top of a stainless steel cage (30 cm×30 cm×30 cm in size). A total of 150 male and female adults were placed in the cage and allowed to mate and oviposit overnight in a sunroom without controlling either the temperature or humidity. The eggs laid on the cheesecloth were taken out the following morning and were considered one-day-old. The eggs were further maintained in the rearing room at 23°C, 70% R.H. with a 15L : 9D photoperiod. The eggs thus reared for 1 day and 4 days were considered as two-day's old and five-day's old, respectively.

b. Larvae Inoculated in Mature Apples

Chestnuts, with a knife cut in the middle, were placed in a row in plastic containers (21 cm×24 cm×10 cm in size). A piece of cheesecloth with eggs oviposited in the same manner as above (a) was placed on the chestnuts and the containers were maintained in the rearing room at 23°C, 70% R.H. with a 15L : 9D photoperiod for non-diapause larvae and a 12L : 12D photoperiod for diapause inducing larvae (diapause larvae), respectively, until the desired instars were obtained (second instar-9 days, fifth instar-14 days). Five holes, 4 mm in diameter, were made in each apple. Second and fifth instar larvae reared in chestnuts were placed in each hole, which was then filled with apple pulp and sealed with plastic tape. Apples thus prepared were stored for 24 hours at 15°C until cold treatment.

3. Cold Storage Treatments

All cold treatments for eggs and larvae were conducted using 30 ℓ fiber-glass treatment chambers (26.0 cm×28.0 cm×41.0 cm in size) placed in a 31.5 m³ (4.3 m×3.2 m×2.3 m in size) cold chamber (temperature adjustment of ±0.5°C, 60 to 90% R.H., 4 defrosting cycles per day).

Each chamber was prepared for CA and SC treatment. Each chamber was connected with silicone tube to allow a steady flow of the desired concentration of CA gas (O₂ : 2%, CO₂ : 2%, N₂ : 96%) and air which is used in commercial cold storage (TSUGAWA, 1984 ; KUDO, 1985), respectively, from cylinders into the chambers. CA gas and air were fed into a scrubbing bottle filled with distilled water to maintain pressure and humidity. Fruit infested with eggs and larvae of peach fruit moth were placed in styrofoam boxes without the lid. Yellow peach moth eggs oviposited on cheesecloth and larvae infested in apples and chestnuts were placed in plastic boxes. Both the containers were put into the aforementioned cold chamber. These were treated at 1.5±0.5°C or 0.5±0.5°C, 70% R.H. The eggs and larvae were then removed from each chamber at regular intervals after treatment for assessment of mortality.

A multi-channel automatic temperature recorder (Hybrid Recorder : AH, Chino) was used to monitor air temperature, surface temperature of fruit, and fruit pulp temperature during the treatment. Temperature probes were calibrated in ice water at 0°C.

4. Determination of Mortality

Peach Fruit Moth

Following cold treatment, apples infested with eggs and fifth instar non-diapause larvae were maintained in the rearing room at 25°C, 70% R.H. with a 16L : 8D photoperiod. Those infested with fifth instar diapause larvae were also maintained in the rearing room at the same temperature and relative humidity, but with a 12L : 12D photoperiod.

Eggs oviposited on mature apples were observed under microscopes after 3 to 4 days to count the total number of eggs and the number of eggs hatched. Both mature and immature apples were cut and assessed after 30 days when larvae from hatched eggs were expected to have reached the fifth instar. Immature apples infested with fifth instar larvae were cut and assessed after 3 to 10 days.

Yellow Peach Moth

A rearing box was prepared by attaching pieces of cardboard for cocooning larvae on the inner walls of a plastic container (37 cm×46 cm×16 cm in size) and placing fresh kernels of corn inside. Pieces of cold-treated cheesecloth with eggs were placed on the kernels and incubated in the rearing room at 23°C, 70% R.H. with a 15L : 9D photoperiod. The number of cocoons in the box and larvae in the corn were then counted. Apples and fresh chestnuts infested with second and fifth instar larvae were maintained in the aforementioned rearing room for the larvae to develop into adults and the number of adults emerging was counted.

5. Statistical Analysis

Data for the response of all developmental stages to low temperatures and storage periods were analyzed by the Probit analysis on the basis of FINNEY's formula (FINNEY, 1971). Linearity regression lines obtained from the statistical analysis were tested by the Chi-square test and ranges of fiducial limits were calculated using FILLER's formula (FINNEY, 1971). Significant differences between LT_{50} 's and LT_{95} 's were evaluated on the basis of failure of 95% fiducial limits to overlap.

The Probit calculation was made using a computer program provided by Professor Akira Sakuma, Tokyo Medical and Dental College and modified by YPPS, MAFF.

Results and Discussion

1. Peach Fruit Moth

Table 2(2)-1 shows the result of the Probit analysis of the low temperature-mortality response data obtained of the SC and CA storage at $1.5\pm 0.5^\circ\text{C}$. The regression lines for various stages are shown in Figure 2(2)-1 (eggs on immature apples), Figure 2(2)-2 (eggs on mature apples), Figure 2(2)-3 (fifth instar larvae in immature apples) and Figure 2(2)-4 (fifth instar larvae in mature apples), respectively.

The responses of all egg stages of peach fruit moth to low temperatures showed that the LT_{50} 's and LT_{95} 's for six-day-old eggs treated by SC storage at $1.5\pm 0.5^\circ\text{C}$ were 18.3 days and 25.6 days and those treated by CA storage at $1.5\pm 0.5^\circ\text{C}$ were 15.9 days and 26.2

days, respectively. Both the LT_{50} 's and LT_{95} 's for six-day-old eggs were greater than for two- and four-day-old eggs in SC and CA storage. The statistical analysis, therefore, revealed significant differences in LT_{50} 's and LT_{95} 's among the various stages, and showed that six-day-old eggs were more tolerant than two- and four-day-old eggs in SC and CA storage. There were no significant differences in the tolerance of six-day-old eggs to SC and CA storage because the LT_{50} 's and LT_{95} 's for both storages were roughly the same. It could therefore be said that there is no difference in efficacy for six-day-old eggs between SC and CA storage treatments.

A comparative study of fifth instar diapause and non-diapause larvae of peach fruit moth to low temperature of $1.5 \pm 0.5^\circ\text{C}$ showed that LT values for diapause larvae were 10.3 to 10.7 days (LT_{50}) and 22.1 to 32.4 days (LT_{95}) in SC storage and 10.8 (LT_{50}) and 28.1 days (LT_{95}) in CA storage, while those for non-diapause were 8.1 to 9.2 days (LT_{50}) and 16.4 to 20.8 days (LT_{95}) in SC storage and 9.1 days (LT_{50}) and 20.5 days (LT_{95}) in CA storage, respectively, and that diapause larvae were more tolerant than non-diapause larvae.

Table 2(2)-2 shows the result of the Probit analysis for larval stages of peach fruit moth treated by SC storage at $0.5 \pm 0.5^\circ\text{C}$.

The low temperature-mortality response data for larvae treated by SC storage at $0.5 \pm 0.5^\circ\text{C}$ showed that the mortalities of fifth instar diapause larvae were clearly lower than for

Table 2(2)-1. Estimated LT_{50} and LT_{95} values for eggs on immature and mature apples, and 5th instar larvae of the peach fruit moth, *Carposina niponensis*, in immature and mature apples stored for different periods in Standard Cold storage (SC) and Controlled Atmosphere storage (CA) at $1.5 \pm 0.5^\circ\text{C}$.

Stage	SC storage			CA storage		
	Number tested*	LT_{50} (95% FL) (days)	LT_{95} (95% FL) (days)	Number tested*	LT_{50} (95% FL) (days)	LT_{95} (95% FL) (days)
Eggs on immature apples						
2-day-old	4,206	8.5(7.6- 9.3)	16.9(15.0-19.9)	4,206	8.3(7.6- 9.0)	17.9(16.1-20.4)
4-day-old	2,610	10.4(8.6-12.1)	18.9(15.8-25.4)	2,610	11.0(7.6-14.0)	22.5(17.2-40.7)
6-day-old	1,608	18.3(17.0-19.5)	25.6(23.6-29.1)	1,608	15.9(14.7-16.9)	26.2(24.0-29.4)
Eggs on mature apples						
2-day-old	1,529	—	—	1,917	11.2(4.2-17.3)	22.4(15.1- **)
4-day-old	1,968	10.0(8.5-11.2)	17.0(15.0-20.7)	1,761	10.0(9.4-10.6)	19.1(18.3-20.2)
6-day-old	1,135	—	—	1,467	—	—
5th instar larvae in immature apples						
non-diapause	1,620	8.1(7.7- 8.5)	16.4(15.3-17.8)	1,620	9.1(7.5-10.5)	20.5(17.2-26.6)
diapause	2,406	10.3(9.0-11.6)	22.1(19.1-27.1)	2,406	10.8(8.0-13.3)	28.1(21.9-44.1)
5th instar larvae in mature apples						
non-diapause	1,626	9.2(8.2-10.1)	20.8(18.3-24.6)			
diapause	970	10.7(8.8-12.5)	32.4(26.5-43.7)			

* Total number treated with cold storage in 6 to 7 storage periods.

** 95% fiducial limits could not be calculated because the slope was not significant.

— Probit analysis was not conducted because of the high mortalities observed at relatively short exposure periods.

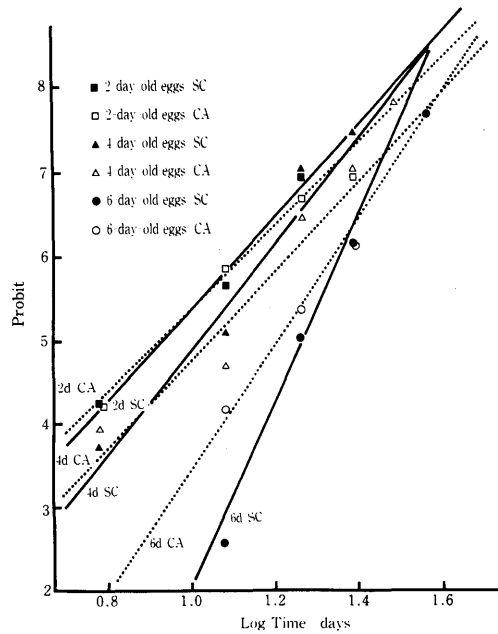


Fig. 2 (2)-1. Time/response lines estimated by Probit regression for 2-, 4- and 6-day-old eggs of the peach fruit moth, *Carposina niponensis*, on immature 'Fuji' apples stored in Standard Cold storage (SC) and Controlled Atmosphere storage (CA) at $1.5 \pm 0.5^\circ\text{C}$.

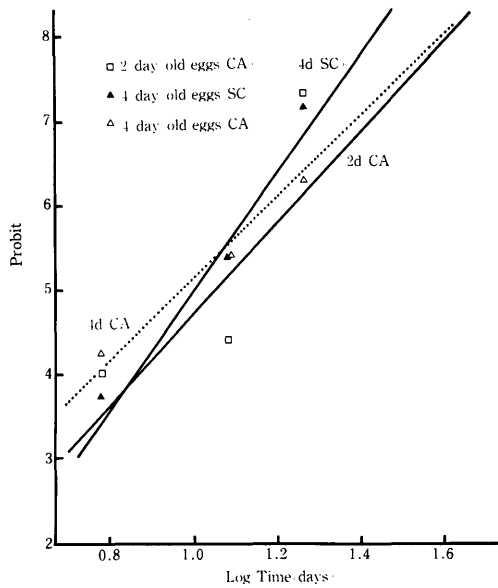


Fig. 2 (2)-2. Time/response lines estimated by Probit regression for 2- and 4-day-old eggs of the peach fruit moth, *Carposina niponensis*, on mature 'Fuji' apples stored in Standard Cold storage (SC) and Controlled Atmosphere storage (CA) at $1.5 \pm 0.5^\circ\text{C}$.

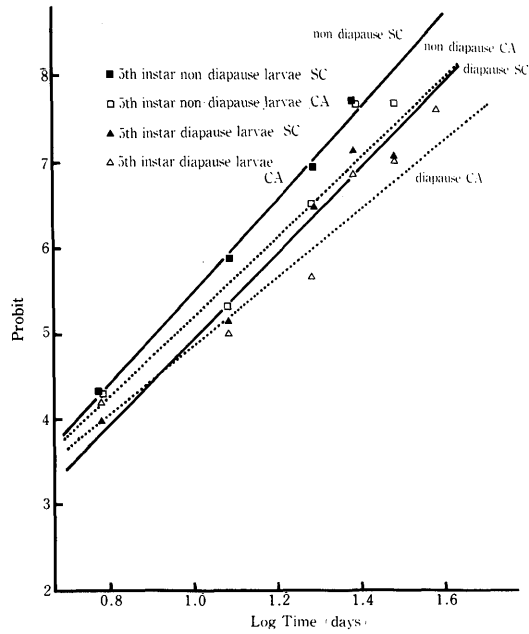


Fig. 2 (2)-3. Time/response lines estimated by Probit regression for 5th instar non-diapause and diapause larvae of the peach fruit moth, *Carposina niponensis*, in immature 'Fuji' apples stored in Standard Cold storage (SC) and Controlled Atmosphere storage (CA) at $1.5 \pm 0.5^\circ\text{C}$.

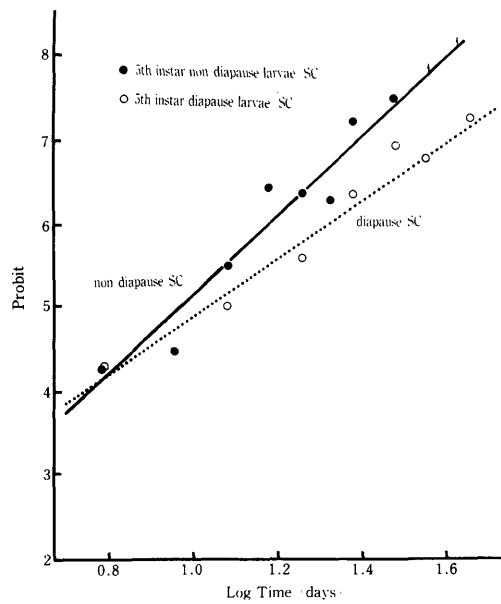


Fig. 2 (2)-4. Time/response lines estimated by Probit regression for 5th instar non-diapause and diapause larvae of the peach fruit moth, *Carposina niponensis*, in mature 'Fuji' apples stored in Standard Cold storage (SC) at $1.5 \pm 0.5^\circ\text{C}$.

Table 2(2)-2. Mortality data for larval stages of the peach fruit moth, *Carposina niponensis*, in mature apples stored for different periods in Standard Cold storage at $0.5 \pm 0.5^\circ\text{C}$.

Instar	Exposure (days)	Number tested	Number dead	Percent mortality
2nd instar	6	239	50	20.9
	9	239	100	41.8
	12	239	204	85.3
	15	239	232	97.1
	18	239	239	100
	Cont.	239	0	0
3rd instar	6	239	0	0
	9	239	44	18.4
	12	239	178	74.5
	15	239	227	95.0
	18	239	238	99.6
	Cont.	239	0	0
4th instar	6	239	0	0
	10	239	121	50.6
	14	239	216	90.4
	19	239	239	100
	22	239	238	100
	Cont.	239	0	0
5th instar (diapause)	6	239	17	7.1
	10	239	144	60.3
	14	239	213	89.1
	18	239	227	95.0
	23	239	237	99.2
	Cont.	239	0	0

other larval stages and fifth instar larvae were more tolerant to low temperature than other larval stages. When comparing the low temperature-mortality response data which were used for the Probit analysis of Table 2(2)-1 for six-day-old eggs and fifth instar diapause larvae of peach fruit moth, a 100% mortality for six-day-old eggs was achieved with 36 day exposure at 1.5°C , while a 98.7% mortality of fifth instar diapause larvae was obtained from the treatment of 45 days in SC storage at 1.5°C . Such high mortalities for the larvae were not expected even at longer periods of the exposure.

These data indicated that the fifth instar diapause larva was the most tolerant stage to low temperatures of all life stages of the peach fruit moth. These results are similar to previous reports on mortality studies of codling moth, oriental fruit moth and navel orangeworm (DUSTON, 1963; MOFFITT, 1971; MOFFITT *et al.*, 1972; TEBBETS *et al.*, 1978; YOKOYAMA *et al.*, 1989). Furthermore, there was no significant difference in the susceptibility of fifth instar diapause larvae to SC or CA storage.

Table 2(2)-3. Mortality data for 1- and 5-day-old eggs of the yellow peach moth, *Conogethes punctiferalis*, on cheesecloth for different exposure periods in Standard Cold storage (SC) and Controlled Atmosphere storage (CA) at $1.5 \pm 0.5^\circ\text{C}$.

Stage	SC storage					CA storage			
	Exposure (days)	Number tested	Percent mortality	Percent hatched	Number larval entries	Number tested	Percent mortality	Percent hatched	Number larval entries
Eggs									
1-day-old	3	567	88.1	—	62	529*	45.1	—	170
	6	604	99.8	—	1	361	98.8	—	4
	9	478	100	—	0	494*	100	—	0
	13	380*	100	—	0	336	100	—	0
	15	551	100	—	0	563	100	—	0
	18	513*	100	—	0	516*	100	—	0
	21	465	100	—	0	646*	100	—	0
	Cont. A	301	—	92.3	278	301	—	92.4	278
	Cont. B	648	—	58.5	379	648	—	58.5	379
5-day-old	3	852	35.2	—	392	892	21.0	—	500
	6	994	94.0	—	42	1,011	82.4	—	126
	9	1,037	100	—	0	1,039	97.9	—	15
	13	1,097	100	—	0	1,170	100	—	0
	15	1,176	100	—	0	1,195	100	—	0
	18	1,354	100	—	0	1,382	100	—	0
	21	1,542	100	—	0	2,380	100	—	0
	Cont.	776	100	28.9	551	776	—	28.9	551

* Based on survival in untreated Control B.

2. Yellow Peach Moth

Table 2(2)-3 shows results of the low temperature-mortality response data for egg stages of yellow peach moth on cheesecloth treated by SC or CA storage at $1.5 \pm 0.5^\circ\text{C}$. All one-day-old eggs were killed after 9 days in either SC or CA storage. Five-day-old eggs required 9 days in SC storage or 13 days in CA storage to attain 100% mortality.

Table 2(2)-4 shows the low temperature-mortality response data for second and fifth instar larvae infested in mature apples treated by SC storage at $0.5 \pm 0.5^\circ\text{C}$. Both larval stages were killed 100% after only 6 days in SC storage. It could therefore be said from these data that eggs of all ages are much more susceptible to low temperatures than are larval stages. The low temperature-mortality response data were not subjected to the Probit analysis since all egg and larval stages of yellow peach moth were killed within an extremely short period of exposure and not enough data were available for analysis.

A comparative study of the susceptibility of egg stages of peach fruit moth and yellow peach moth to low temperatures showed that complete kill of six-day-old eggs, which were the most tolerant stage of all egg stages, was achieved in 30 to 36 days with SC and CA storage at $1.5 \pm 0.5^\circ\text{C}$. Only 13 days of the treatment was sufficient to attain 100% kill of one- and five-day-old eggs of yellow peach moth. It could therefore be said that six-day-

Table 2(2)-4. Mortality data for 2nd and 5th instar larvae of the yellow peach moth, *Conogethes punctiferalis*, in mature 'Fuji' apples stored for different exposure periods in Standard Cold storage at $0.5 \pm 0.5^\circ\text{C}$.

Instar	Exposure (days)	Number tested	Number dead	Percent mortality
2nd instar	2	133	111	83.4
	4	133	133	100
	6	133	133	100
	8	133	133	100
	10	133	133	100
	Cont.	133	0	0
5th instar non-diapause	2	147	107	72.8
	4	147	144	98.0
	6	147	147	100
	8	147	147	100
	10	147	147	100
	Cont.	147	0	0
5th instar diapause	2	191	158	82.7
	4	191	191	100
	6	191	191	100
	8	191	191	100
	10	191	191	100
	Cont.	191	0	0

old eggs of peach fruit moth were more tolerant to low temperatures than all egg stages of the two pests. Fifth instar diapause larvae of peach fruit moth were more tolerant than other larval stages of the two pests.

When comparing the susceptibility of six-day-old eggs with fifth instar diapause larvae of peach fruit moth, the LT_{95} 's were 26.2 days and 32.4 days, respectively, for six-day-old eggs and fifth instar larvae, and fifth instar larvae were more tolerant than six-day-old eggs. It could therefore be said that fifth instar diapause larvae of peach fruit moth were most tolerant to low temperature of all life stages of the two pests which may be present on/in 'Fuji' apples at harvest. Fifth instar diapause larvae of peach fruit moth suffered 98.7% mortality by cold storage treatment for 45 days at $1.5 \pm 0.5^\circ\text{C}$. Complete mortality of surviving diapause larvae in cold storage would require much longer exposure periods (more than 150 days) as shown in previous reports on codling moth and oriental fruit moth (DUSTON, 1963; MOFFITT, 1971; MOFFITT *et al.*, 1972). Therefore, neither methyl bromide fumigation nor cold storage alone has proved successful in eliminating the pests from apples and combined cold storage and methyl bromide fumigation treatment should be considered as an alternative disinfestation method for internal fruit feeders.

In overseas countries, such an approach has been used to control fruit flies in fresh fruit such as apples, apricots, cherries, grapes, nectarines, peaches, pears and avocados and light brown apple moth complex, *Epiphyas* spp., in apples and pears (STOUT, 1983; BOND, 1984;

USDA, 1985). DRAKE et al., (1988) reported that fumigation with 32 g/m³ methyl bromide for 2 hours at 23.9 to 25.6°C followed by a minimum 60 days of storage at -0.56°C in either SC or CA storage destroyed all live larvae of codling moth in apples with no phytotoxicity to 'Red Delicious' and 'Golden Delicious' apples, although unacceptable damage to the fruit occurred when apples were fumigated with 48 g/m³ methyl bromide. MORGAN (1974) also reported that all codling moth larvae in apples were killed by fumigation with 32 g/m³ methyl bromide for 2 hours at 17°C followed by 31 to 35 days of storage at -0.5°C and that the combined treatment of fumigation and cold storage did not injure several cultivars of apples.

These reports showed that the combined treatment of cold storage followed by methyl bromide fumigation or methyl bromide fumigation followed by cold storage may be used to control peach fruit moth and yellow peach moth in apples, since methyl bromide doses in a combined treatment could be reduced in comparison with the doses in the single fumigation treatment.