Part 2 Plant Quarantine Treatments to Control Peach Fruit Moth, *Carposina niponensis* WALSINGHAM and Yellow Peach Moth, *Conogethes punctiferalis* (GUENÉE) on Apples for Export to The U.S.

Test 1 Susceptibility of Each Stage of Peach Fruit Moth and Yellow Peach Moth to Methyl Bromide Fumigation

Introduction

There are many reports on studies of plant quarantine treatments for internal fruit feeders of Lepidoptera such as the codling moth, *Cydia Pomonella* (L.) in cherries (ANTHON et al., 1975, 1977 ; GAUNCE et al., 1981), unshelled walnuts (NELSON et al., 1983 ; TEBBETS et al., 1986), nectarines (YOKOYAMA et al., 1987a, 1988, 1990a, 1990b ; WADDELL et al., 1989), apples (MOFFITT, 1971 ; MORGAN et al., 1974 ; GAUNCE et al., 1980), and pears (MACKIE et al., 1939), and oriental fruit moth, *Grapholitha molesta* (BUSCK) in nectarines (JOHNSON et al., 1942 ; YOKOYAMA et al., 1987b), respectively. In many countries, methyl bromide fumigation is widely used as a quarantine treatment against such fruit borers (MONRO, 1969a ; STOUT, 1983 ; California Department of Food and Agriculture, 1983 ; BOND, 1984 ; USDA, 1985).

In the above cited reports, the susceptibility to methyl bromide fumigation of egg stage of the codling moth and oriental fruit moth which might be present on/in the fruits at harvest was determined. The most resistant stage of these stages was determined and then the stages were used in large-scale mortality tests to evaluate if the proposed treatments were effective.

Susceptibility of each stage of codling moth or oriental fruit moth to methyl bromide fumigation in these reports showed that egg stages were more resistant than larval stages in both pests and that newly-laid eggs (24-48 hours) of the two pests were the most resistant stage (GAUNCE et al., 1980 ; TEBBETS et al., 1986 ; YOKOYAMA et al., 1987b ; WADDELL et al., 1989). However, there were no such susceptibility and mortality test data available for peach fruit moth, *Carposina niponensis* WALSINGHAM and yellow peach moth, *Conogethes punctiferalis* (GUENÉE) in apples.

Our objectives were to study the relationship between methyl bromide doses and mortalities for each stage of the two pests which may be present on/in apples at harvest and to select the most resistant stage to be used for methyl bromide fumigation in large-scale mortality tests.

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 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. They were reared on immature apples using method described by NARITA (1986b) at the Research Division, Yokohama Plant Protection Station, Ministry of Agriculture, Forestry and Fisheries (YPPS, MAFF). The test insects were prepared as follows;

a. 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 container which were maintained in the rearing room at 25°C, 70% R.H. with a 16L : 8D photoperiod. In order to facilitate observation of the number of eggs oviposited and hatched, six fruit with two threads of nylon wound around the middle were placed in a row in a plastic container (27 cm×30 cm×9 cm in size). Thirty mated females were released into the container, which was 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 after oviposition were considered one-day-old.

b. Eggs Oviposited on Wax Paper

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 and placed in a petri dish. The eggs were then maintained under the same conditions as the aforementioned apples to obtain the desired stages.

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 and placed in a petri dish. The pieces of wax paper were then placed on the stem 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).

Yellow Peach Moth

Test insects were obtained from the Laboratory of Applied Entomology, Faculty of Agriculture, University of Tokyo in May 1987 (Yayoi, Bunkyo-ku, Tokyo). 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), because KADOI & KANEDA (1990) found that the insect does not lay eggs on apples. Several immature apples were placed in two tea strainers which were tied face to face, which was then 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 one day and 4 days were considered as two-day-old and five-day-old, respectively.

b. Larvae Inoculated in Fresh Chestnuts

Larvae inoculated in fresh chestnuts were used for obtaining supplementary data. Fresh 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 larval instars were obtained (second instar-9 days; fifth instar-14 days). These infested chestnuts were stored at 15°C for 24 hours until fumigation.

c. Larvae Inoculated in Mature Apples

Five holes, 4 mm in diameter, were made in each apple. A larva reared in the same manner as above (b) was placed in each hole, which was then filled in with apple pulp and sealed with plastic tape. Apples thus prepared were stored at 15°C until fumigation.

3. Fumigation

A 29.5 ℓ fiber-glass fumigation chamber (26.0 cm×28.0 cm×41.0 cm in size) equipped with a circulation fan, ventilation apparatus, and ports for gas application and sampling, a manometer and temperature probes was used in the fumigation room and maintained at 15°C.

Twelve larva-infested and six egg-infested apples were placed in the fumigation chamber by species, instar, and methyl bromide dose. Petri dishes carrying pieces of wax paper with eggs of peach fruit moth and of cheesecloth with eggs of yellow peach moth also were placed in the boxes by developmental stage, together with six 'Fuji' apples to fill up the empty space. Infested fruit or pieces of cheesecloth holding the eggs were stored

overnight (18 hours) at the fumigation temperature of 15°C prior to fumigation.

Seven different doses of methyl bromide were used to determine the mortality of all developmental stages in dose-mortality tests. The doses were determined in advance through preliminary tests. The circulation fan was kept on throughout fumigation. Fumigation was conducted for 2 hours at 15°C. Fumigation was followed by an hour of exhausting using the ventilation apparatus. A gas chromatograph (FID : GC 8AF, Shimazu) was used to monitor gas concentrations at time intervals of 20, 60 and 120 minutes after the commencement of fumigation, and a multi-channel automatic temperature recorder (Hybrid Recorder : AH, Chino) was used to monitor the fruit and air temperatures within the fumigation chambers.

4. Determination of Mortality

Peach Fruit Moth

In order to allow viable eggs to hatch, eggs infested in mature apples and pieces of wax paper were placed in plastic containers (37 cm × 46 cm × 16 cm in size) following fumigation and incubated in the rearing room at 25°C, 70% R.H. with a 16L : 8D photoperiod. The incubation periods were 8, 6, and 4 days, respectively, for two-, four-, and six-days-old eggs, after which they counted the number of eggs hatched under microscopes. Larva-infested apples were placed in plastic containers and maintained in the rearing room at 25°C, 70% R.H. with a 16L : 8D photoperiod for non-diapause larvae and a 12L : 12D photoperiod for diapause larvae. Apples infested with first and third instars were maintained in the rearing room long enough for the larvae to reach the fifth instar and those with fifth instars were maintained for 10 to 14 days and, then, they were all cut and assessed.

Yellow Peach Moth

A rearing box was prepared by attaching pieces of cardboard for cocooning larvae on the inner walls of a plastic container and placing fresh kernels of corn inside. Pieces of fumigated cheesecloth with eggs were placed on the kernel 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 artificially infested with fifth instars and fumigated were maintained in the aforementioned rearing room long enough for the larvae to develop into adults, and the number of adults was counted. Larvae which escaped from the apples during fumigation (including moribund larvae) were reared on chestnuts and the number of adult emerging was counted.

5. Statistical Analysis

Data for the responses to methyl bromide of all developmental stages were analyzed by the Probit procedure using FINNEY's formula (FINNEY, 1971). Linearity regression lines obtained from the statistical analysis were tested by the Chi-square test and the fiducial limits were calculated using FIELLER's formula (FINNEY, 1971). The LD_{50}'s and LD_{95}'s were considered to be significantly different when their 95% confidence limits did not 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(1)-1 shows results of the Probit analysis of methyl bromide dose-mortality response data for each stage of peach fruit moth. The regression lines for the various stages are shown in Figure 2(1)-1 (eggs on mature apples), Figure 2(1)-2 (eggs on wax paper), and Figure 2(1)-3 (larvae in mature apples), respectively.

The response of all egg stages of peach fruit moth to methyl bromide fumigation for 2 hours at 15°C showed that the LD_{50}' and LD_{95}'s for two-day-old eggs were 18.8 g/m^3 and 29.3 g/m^3 and those on wax paper were 23.3 g/m^3 and 33.3 g/m^3, respectively, and that both LD_{50}'s and LD_{95}'s for two-day-old eggs were higher than other egg stages. It could therefore be said that two-day-old eggs were the most resistant among egg stages.

As for larval stages, the LD_{50}'s and LD_{95}'s for each instar were 7.9 to 9.5 g/m^3 and 15.0 to 16.4 g/m^3, respectively. No significant differences were found in various instar's response to methyl bromide based on nonoverlap of 95% confidential limits of the LD_{50}'s and LD_{95}'s. However, when comparing fifth instar non-diapause and diapause larvae, which were the most likely to be found in 'Fuji' apples at harvest, mortality ratios for diapause larvae were slightly lower than those for non-diapause larvae at high doses. It

Table 2(1)-1. Estimated LD_{50} and LD_{95} values for egg and larval stages of the peach fruit moth, *carposina niponensis*, fumigated with methyl bromide for 2 hours at 15°C.

Stage	Number tested*	LD_{50} (95% FL) (g/m^3)	LD_{95} (95% FL) (g/m^3)
Eggs on mature apples			
2-day-old	3,622	18.8(14.7-22.3)	29.3(25.0-40.6)
4-day-old	6,274	12.7(10.8-14.1)	24.7(22.8-27.5)
6-day-old	5,384	13,8(11.4-16.0)	20.7(17.9-28.0)
Eggs on wax-paper sheets			
1-day-old	900	15,9(10.0-23.0)	23.3(18.8-58.9)
2-day-old	532	23.3(19.6-28.8)	33.3(28.1-52.7)
3-day-old	2,013	20.8(19.1-23.6)	31.4(27.3-40.0)
4-day-old	1,043	18.0(17.4-18.5)	26.3(25.5-27.2)
6-day-old	786	14.5(5.2-22.3)	25.3(19.5- **)
Larvae in mature apples			
1st instar	639	8.3(6.5- 9.4)	15.5(13.6-19.5)
3rd instar	665	8.8(7.4- 9.9)	15.0(13.4-18.0)
5th instar (non-diapause)	698	9.5(8.8-10.3)	16.1(15.3-17.2)
5th instar (diapause)	827	7.9(−0.1-10.3)	16.4(13.8-25.1)

* Total number of test insects fumigated in 7 dose levels.

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

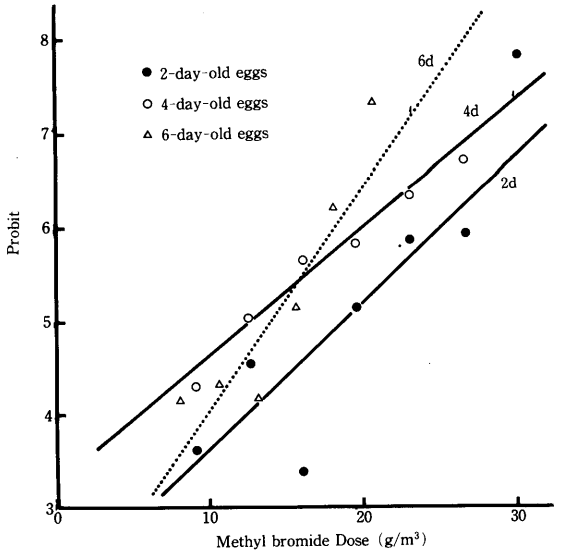

Fig. 2 (1)-1. Dose/response lines estimated by Probit regression for 2-, 4-, and 6-day-old eggs of the peach fruit moth, *Carposina niponensis*, on mature 'Fuji' apples fumigated with methyl bromide for 2 hours at 15°C.

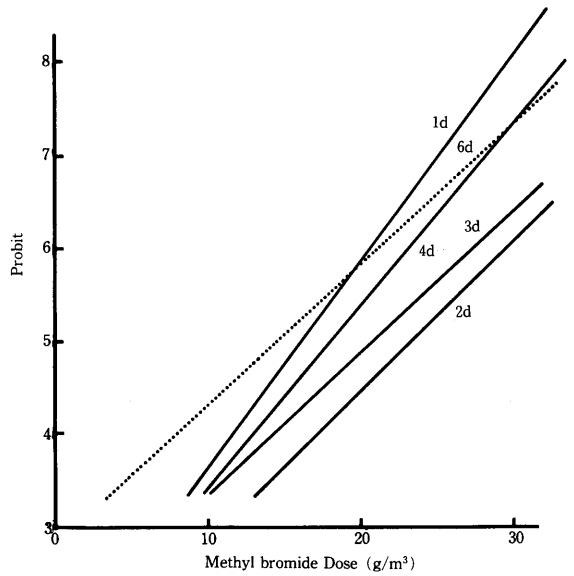

Fig. 2 (1)-2. Dose/response lines estimated by Probit regression for 1-, 2-, 3-, 4-, and 6-day-old eggs of the peach fruit moth, *Carposina niponensis*, on wax paper sheets fumigated with methyl bromide for 2 hours at 15°C.

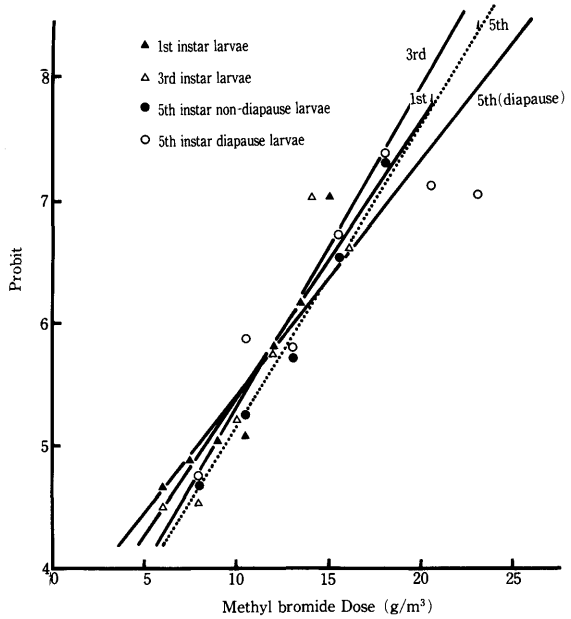

Fig. 2 (1)-3. Dose/response lines estimated by Probit regression for 1st, 3rd, and 5th instar non-diapause and diapause larvae of the peach fruit moth, *Carposina niponensis*, in mature 'Fuji' apples fumigated with methyl bromide for 2 hours at 15°C.

Table 2(1)-2. Estimated LD_{50} and LD_{95} values for egg and larval stages of the yellow peach moth, *Conogethes punctiferalis*, fumigated with methyl bromide for 2 hours at 15°C.

Stage	Number tested*	LD_{50} (95% FL) (g/m^3)	LD_{95} (95% FL) (g/m^3)
Eggs on cheesecloth			
1-day-old	2,109	—	—
2-day-old	3,344	10.5(9.6-11.3)	15.0(13.9-16.9)
5-day-old	5,925	—	—
Larvae in mature apples			
5th instar (non-diapause)	420	5.4(5.1- 5.6)	7.9(7.7- 8.4)
5th instar (diapause)	420	5.2(**)	6.8(**)
Larvae in chestnuts			
2nd instar	1,127	—	—
5th instar (non-diapause)	1,078	—	—
5th instar (diapause)	875	—	—

* Total number of test insects fumigated in 7 dose levels.
** 95% fiducial limits could not be calculated because the slope was not significant.
— Probit analysis was not calculated because of high mortality ratios at low doses used.

could therefore be said that diapause larvae were the most resistant instar among larval stages.

A comparative study of all stages of peach fruit moth to methyl bromide fumigation for 2 hours at 15°C showed that egg stages were clearly more resistant than larval stages, because the LD_{50}'s for two-day-old eggs were 2.5 times higher than those for larval stages. It could therefore be said that two-day-old eggs were the most resistant among all stages of peach fruit moth.

These results were similar to those previously reported (GAUNCE et al., 1980 ; TEBBETS et al., 1986 ; YOKOYAMA et al., 1987a ; WADDELL et al., 1989) on studies of methyl bromide against codling moth and oriental fruit moth.

2. Yellow Peach Moth

Table 2(1)-2 shows results of the Probit analysis of dose-mortality response data for each stage of yellow peach moth.

Parts of the dose-mortality response data were not subjected to the Probit analysis, since one- and five-day-old eggs oviposited on cheesecloth and second and fifth instar larvae (non-diapause and diapause) inoculated in chestnuts showed high mortalities at relatively low doses. The LD_{50}'s for each stage were 10.5 g/m^3, 5.4 g/m^3 and 5.2 g/m^3, for two-day-old eggs on cheesecloth, fifth instar non-diapause larvae in mature apples and fifth instar diapause larvae. Two-day-old eggs were 1.9 to 2.0 times more resistant than fifth instar larvae.

When comparing the susceptibility of all stages of peach fruit moth and yellow peach moth to methyl bromide fumigation for 2 hours at 15°C, the LD_{50}'s for egg stages of the two pests were 2.0 to 2.5 times higher than for larval stages. The LD_{50}'s and LD_{95}'s for two-day-old eggs of peach fruit moth were 23.3 g/m^3 and 33.3 g/m^3, respectively, while those of yellow peach moth were 10.5 g/m^3 and 15.0 g/m^3, respectively and both LD values for peach fruit moth were 2.2 times higher than those for yellow peach moth. It could therefore be said that the most resistant stage to methyl bromide fumigation for 2 hours at 15°C was the two-day-old egg of peach fruit moth among all stages of the two pests which may be present on/in 'Fuji' apples at harvest.

It was estimated that a practical dose of methyl bromide which was sufficient to attain 100% mortality of the two-day-old eggs of peach fruit moth would be approximately 50 g/m^3 or more when the fumigation was carried out for 2 hours at 15°C with consideration of various fumigation elements, such as LD_{95}'s (33.3 g/m^3), gas absorption ratio to items to be fumigated and an additional dose for a guarantee of quarantine security.