Test 3 Susceptibility of Fifth Instar Diapause Larvae of Peach Fruit Moth Treated by Cold Storage to Methyl Bromide Fumigation

Introduction

Tolerance of all life stages of peach fruit moth, *Carposina niponensis* WALSINGHAM and yellow peach moth, *Conogethes punctiferalis* (GUENÉE) to low temperature showed that larval stages were more tolerant than egg stages in both pests and fifth instar diapause inducing larvae (diapause larvae) of peach fruit moth were the most tolerant of all life stages of the two pests (Part 2, Test 2 in this report). Complete kill of egg stages, which were susceptible to low temperature, would be obtained by the single cold storage treatment, while a few diapause larvae, the most tolerant stage to low temperature, would survive a treatment of 45 days storage at 1.5°C. Complete mortality of the surviving larvae might require three to five months storage (Part 2, Test 2 in this report). Fortunately, however, since larval stages were susceptible to methyl bromide fumigation (Part 2, Test 1 in this report), larvae surviving cold storage treatment could be killed with subsequent methyl bromide fumigation. Therefore, a combined cold storage and methyl bromide fumigation treatment was considered for controlling egg and larval stages of peach fruit moth and yellow peach moth.

Diapause larvae of codling moth were the stage of the insect most tolerant to environmental conditions (GEIER, 1963 ; PUTMAN, 1963) and has survived minimum temperatures of −28.9°C (NEWCOMER & WHITECOMB, 1924). Insect diapause is marked by a fall in the level of metabolism and a consequent reduction in the rate of respiration (LEES, 1955 ; GILMORE, 1965 ; HANSEN et al., 1968). The rate of respiration of a given stage of an insect has been linked to the susceptibility of that stage to methyl bromide. Those stages with a higher rate of respiration are generally those that are more susceptible. Toxicity of a fumigant depends mainly, if not completely, upon the rate of respiration of the insect (SUN, 1947 ; BOND, 1956 ; BOND et al., 1961 ; MONRO, 1969a). With the codling moth, the rate of CO_2 production (respiration) decreased to low levels within 5 to 7 days after formation of the cocoon (SELL et al., 1985). The diapause larvae, with a decreasing rate of respiration, were more tolerant to methyl bromide fumigation than non-diapause larvae (TEBBETS et al., 1986). Furthermore, the insect exposed to low temperature for long periods is capable of cold-hardiness or cold-acclimation by the accumulation of anti-freeze compounds (mostly glycerol). These insects were not easily killed in subsequent low temperature (MACPHEE, 1961 ; SMITH, 1970 ; BAUST & MILLER, 1972 ; CANNON, 1987). These data may indicate that diapause larvae surviving cold storage for long periods may have lower mortality in subsequent methyl bromide fumigation and in this case, methyl bromide dose must be increased to obtain complete kill of the larvae, if they aqcuire cold-acclimation.

Therefore, susceptibility tests of diapause larvae were conducted to determing if larvae of peach fruit moth become cold-acclimated. These consisted of mortality tests for diapause larvae of peach fruit moth by methyl bromide fumigation (without cold storage) and the combined cold storage and methyl bromide fumigation.

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

Thirty each 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 containers which were maintained in the rearing room at 25°C, 70% R.H. with a 16L : 8D photoperiod. Four to five mated females 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 were then placed on the stem end and calyx end of each mature apple placed in a row in plastic containers (37 cm×46 cm×16 cm in size). The containers were then 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. Methyl Bromide 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, and manometer and temperature probes was used in a fumigation room maintained at 15°C.

Apples infested with fifth instar diapause larvae of peach fruit moth were placed in the fumigation boxes and fumigated with methyl bromide at a dose of 12 g/m^3 (corresponding dose of LD_{50}'s ; Part 2, Test 1 in this report) for 2 hours at 15°C. The circulation fan was kept on throughout the fumigation. Fumigation was followed by one hour of exhausting using the ventilation apparatus. A gas chromatograph (FID : GC 2AF, Shimazu) was used to monitor gas concentrations at time intervals of 20, 60 and 120 minutes after the commencement of fumigation. A multi-channel automatic temperature recorder (Hybrid Recorder : AH, Chino) was used to monitor fruit pulp and air temperatures within fumigation boxes.

4. Cold Storage+Methyl Bromide Fumigation

Cold storage treatment was conducted in a 31.5 m^3 (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).

Infested fruit in a styrofoam box without a lid were stored overnight at 15°C before the commencement of cold storage and then placed in a 31.5 m^3 cold chamber for 18 days (corresponding storage period of LT_{95} ; Part 2, Test 2 in this report) at 0.5±0.5°C.

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

The infested fruit treated by cold storage were then gradually brought up to the fumigation temperature of 15°C in 3 days (at 5°C for 24 hours, 10°C for 24 hours and 15°C for 24 hours). Methyl bromide fumigation was conducted in the same manner as described above (3).

5. Determination of Mortality

Fumigated or cold storage treated apples were stored in the rearing room at 25°C, 70% R.H. with a 16L : 8D photoperiod. They were all cut for the determination of mortality. The single fumigated apples were cut for assessment after 3 to 7 days, and the combined cold storage and methyl bromide fumigation treated apples were cut and assessed after 3 days. The number of treated larvae for any given treatment was estimated by the number of larvae which remained viable in twelve fruit of the untreated control. The number of surviving larvae in cold storage was estimated by the number of surviving larvae in twenty-four fruit of the appropriate control which was provided for the combined treatment.

Results and Discussion

Table 2(3) presents the results of mortalities of fifth instar diapause larvae treated with the single methyl bromide fumigation (without cold storage treatment) or the combined cold storage and methyl bromide fumigation treatment.

The average per cent survival for 3 replications of the methyl bromide fumigation treatment was 44.6% and that in the combined treatment was 25.6%. The latter treatment was more effective than the former. The significance of differences between the two methods was compared by using the Chi-square test. A significant difference was observed between both treatments and it was confirmed that the surviving diapause larvae

Table 2(3). Effect of prior exposure to low temperatures of 0.5±0.5°C for 18 days on susceptibility of 5th instar diapause larvae of the peach fruit moth, *Carposina niponensis*, to subsequent methyl bromide fumigation at 12 g/m³ for 2 hours at 15°C.

Replicate	No. of larvae infested /apple	Methyl bromide fumigation				Cold storage+Methyl bromide fumigation				
		No. of apples tested	No. of larvae treated*	No. of larvae survived	Percent survival	No. of apples tested	No. of larvae treated*	No. of larvae survived (A)**	No. of larvae survived (B)	Percent survival (B / A × 100)
1	16.75±5.69	48	804	368	45.7	48	804	38	7	18.4
2	17.17±4.06	48	824	368	44.6	48	824	42	14	33.3
3	17.75±7.83	48	852	370	43.4	48	852	40	10	25.0

* Based on survival in appropriate control.

** Based on survival in 24 fruit of appropriate control which were additionally provided for the combined treatment.

treated by cold storage treatment were more susceptible to subsequent methyl bromide fumigation.

This test showed that the combined treatment was more effective than the single methyl bromide fumigation, and that there was no phenomena of cold-acclimation for diapause larvae. Therefore, doses of methyl bromide in the combined treatment could be considerably reduced in comparison with those of methyl bromide in the single fumigation treatment.