# Part 3 Mortality Confirmation Tests for Summer Fruit Tortrix, *Adoxophyes orana fasciata* WALSINGHAM, Hawthorn Spider Mite, *Tetranychus viennensis* ZACHER, Kanzawa Spider Mite, *Tetranychus kanzawai* KISHIDA and Two-Spotted Spider Mite, *Tetranychus urticae* KOCH by a Combined Cold Storage and Methyl Bromide Fumigation Treatment Established for Export of 'Fuji' Apples to The U.S.

## Introduction

The United States plant quarantine regulatory agency required mortality tests for summer fruit tortrix, *Adoxophyes orana fasciata* WALSINGHAM, hawthorn spider mite, *Tetranychus viennensis* ZACHER and kanzawa spider mite, *Tetranychus kanzawai* KISHIDA, which are unknown species in the United States. Hence, mortality tests were conducted to confirm if the species could be killed by two disinfestation standards (Part 2, Test 4 in this report) established for peach fruit moth, *Carposina niponensis* WALSINGHAM and yellow peach moth, *Conogethes punctiferalis* (GUENÉE). And at the same time, another species of two-spotted spider mite, *Tetranychus urticae* KOCH, which is sometimes found on the calyx end of 'Bagged Fuji' apples and exists widely in the United States, was also tested in mortality tests for confirming the efficacy of the disinfestation standards.

## Materials and Methods

### 1. Test Insects

#### Summer Fruit Tortrix

The second generation larvae of the insect feed on leaves and the surface of fruit. The stage that may be found on 'Fuji' apples at harvest in the third instar larva (diapause inducing larva) (TSUGAWA, 1986). Test insects were obtained from the Aomori Apple Experiment Station, Aomori Prefecture (AAES, Aomori). They were reared on artificial diet using methods described by TAMAKI et al. (1966) and YAMAYA et al. (1972) in a rearing room at 20°C, 70% R.H. with a 12L : 12D photoperiod at the Research Division, Yokohama Plant Protection Station, Ministry of Agriculture, Forestry and Fisheries (YPPS, MAFF). Diapause larvae were put in petri dishes or in the stalk cavity of apples which were placed in nylon-meshed bags. The bags were then placed in wooden or plastic field bins (31.8 cm × 63.5 cm × 32.0 cm in size, 0.062 $m^3$ in capacity) with filler apples.

#### Hawthorn Spider Mite

The hawthorn spider mite has not been reported to cause damage to apple fruit in commercial orchards in major apple production areas. The stage that may be found on 'Fuji' apples at harvest is overwintering adults (TAKAFUJI et al., 1981 ; KITAMURA, 1986 ;

WAKO, 1986). Overwintering adult mites under the bark of cherry were collected on the campus of the Faculty of Horticulture, Chiba University (Matsudo City, Chiba Prefecture). They were put on leaves of potted plum for mass-production and reared on the window side of the room. Plum or cherry leaves infested with mites were then placed in small plastic containers and reared in the rearing room at 23°C, 50 to 60% R.H. with a 10L : 14D photoperiod (WAKO, 1986) at YPPS, MAFF. These infested leaves were put on apples and the apples then placed in wooden field bins (31.8 cm×63.5 cm×32.0 cm in size, 0.062 $m^3$ in capacity) together with filler apples.

**Kanzawa Spider Mite**

Life cycle or seasonal occurrence on apple fruit or rearing methods for kanzawa spider mite have not studied since there were no reports for the mite to be found on apple fruit or to cause injury to apple fruit in Japan. Therefore, all stages of the mite were used for tests.

Kanzawa spider mite were reared on kidney bean leaves at 25°C, 60% R.H. with a 16L : 8D photoperiod at the Research Division, Yokohama Plant Protection Station. Larva, first and second nymph and adult stages on the leaves were counted before testing. Each stage of the mite were dropped on the stalk cavity of apples by a slight tapping with fingers. The leaves with some mites were also put on the apples. The leaves infested with eggs with moist sanitary cottons for avoiding dry condition. The infested apples with each stage were then put in a plastic container (20 cm×25 cm×10 cm in size) and stored for about 24 hours at 10°C or 15°C before testing. Apples infested with egg in the untreated lot were stored at 25°C and the number of hatching egg in the untreated lot was counted to estimate that of the treated lot.

**Two-Spotted Spider Mite**

The stage of the mite on apples at harvest is the overwintering stage and they are sometimes found in the calyx end of 'Bagged Fuji' apples (SUGAWARA et al., 1967 ; GOTO et al., 1981 ; KITAMURA, 1986). Naturally-infested 'Fuji' apples for the tests were obtained from AAES, Aomori. The infested fruit were placed in wooden field bins (31.8 cm×63.5 cm×32.0 cm in size) together with filler apples.

**2. Disinfestaion Standards**

**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/$m^3$ 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/$m^3$ for 2 hours at 10°C or above with 50% or less loading, fruit in plastic field bins).

**3. Combined Cold Storage and Methyl bromide Fumigation Treatment**

Test fruits in wooden field bins 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., 4 defrosting cycles per day and treated at 0.5±0.5°C for 30 to 40 days. A multi-channel automatic temperature recorder (Hybrid Recorder: AH, Chino) was used to monitor air and fruit pulp temperatures during the treatment. The temperature recorder and probes were calibrated in ice water at 0°C.

Following cold storage, infested test fruit for subsequent methyl bromide fumigation were prepared as follows; ie. in fumigation with Standard 1, each apple was covered with a meshed polyethylene fruit cap and packed in a corrugated carton box (38 cm×44.7 cm×25 cm in size, 0.043 $m^3$ in capacity; 36 fruit, ca. 10 kgs) with six fiberglass screen-covered vents (4 vents: 2 cm×5 cm, 2 vents: 4 cm×2 cm; vent ratio of 7.4%) on four sides and with two layers. 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. Five of these cartons (ca. 50 kgs) were stored for 3 days at the fumigation temperature of 15°C. 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; 70 to 80 fruit, ca. 20 kgs) with many vents on four sides without packing materials. Four bins were stored for 3 days at the fumigation temperature of 10°C. Infested apples with kanzawa spider mite were placed in small plastic containers and then stored for 3 days at 10°C or 15°C.

These infested apples were placed in a 0.52$m^3$ stainless steel fumigation box (0.9 m×0.66 m×0.86 m in size) equipped with 0.86 $m^3$/min. circulation and ventilation apparatus, graduated dispenser, ampoule breaker and vaporizer for methyl bromide application, ports for gas sampling, temperature probe and manometer. Fumigation was conducted for 2 hours at 15°C or 10°C with applied doses (33.8 to 38.0 g/$m^3$ at 15°C, 43.0 to 48.0 g/$m^3$ at 10°C) of methyl bromide as shown in Table 3-1 (summer fruit tortrix), Table 3-2 (hawthorn spider mite), Table 3-3 (kanzawa spider mite) and Table 3-4 (two-spotted spider mite). Methyl bromide enclosed in an ampoule was applied by using the built-in ampoule breaker or a graduated dispenser.

Fumigations for all kanzawa spider mites, and a part of summer fruit tortrixes and hawthorn spider mites were conducted using 29.5 ℓ fiber-glass fumigation chamber (26.0 cm×28.0 cm×41.0 cm in size) equipped with circulation and ventilation apparatus, and ports for gas application and sampling, manometer and temperature probes. These fumigation chambers were placed in the fumigation room maintained at 15°C or 10°C. Fumigation was conducted for 2 hours at 15°C or 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 fumigation were monitored by using a gas chromatograph (FID: GC 8AF, Shimazu) 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.

**4. Evaluation of Mortality**

After fumigation, infested fruit were held for 3 days at 25°C, 70% R.H. with a 16L: 8D

photoperiod and assessed under microscopes. Mortalities of the hawthorn spider mite, kanzawa spider mite and two-spotted mite were assessed after 7 days.

## Results

### 1. Fruit Temperatures During Cold Storage and Fruit Temperatures and Gas Concentrations During Fumigation

Fruit temperatures and gas concentrations during the treatment are shown in Table 3-1 (summer fruit tortrix), Table 3-2 (hawthorn spider mite), Table 3-3 (kanzawa spider mite) and Table 3-4 (two-spotted spider mite), respectively.

The average fruit temperature during cold storage was 0.5°C (1.4°C maximum, −0.4°C minimum) and most temperature recordings were in the range of 0.5±0.5°C. Fruit temper-

**Table 3-1.** Fruit temperatures and gas concentrations in the combined treatment of cold storage at 0.5±0.5°C for 37 to 40 days and methyl bromide fumigation at a dose of 33.8 g/m³ for 2 hours at 15°C (Standard 1) and doses of 43.8 to 48.0 g/m³ for 2 hours at 10°C (Standard 2) for the summer fruit tortrix, *Adoxophyes orana fasciata*.

| Standard | Replicate | Cold storage | | Methyl bromide fumigation | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Temperature (°C) | | Dose (g/m³) | Gas concentration (mg/ℓ) | | Temperature (°C) | |
| | | Max. | Min. | | 30 | 120 min. | Chamber | Fruit |
| Standard 1 | 1 | 0.9 | −0.2 | 33.8** | 34.0 | 30.2 | 16.1 | 14.8 |
| | 2 | 0.8 | 0.2 | 33.8** | 34.0 | 30.7 | 15.8 | 14.8 |
| Standard 2 | 1 | 1.2 | 0.3 | 43.8* | 47.0 | 43.6 | 10.9 | 9.9 |
| | 2 | 1.3 | 0.2 | 44.0* | 48.4 | 44.1 | 11.1 | 10.0 |
| | 3 | 1.2 | 0.3 | 48.0** | 57.7 | 51.5 | 10.8 | 10.1 |

* A 29.5 ℓ fumigation chamber was used.
** A 0.52 m³ fumigation chamber was used.

**Table 3-2.** Fruit temperatures and gas concentrations in the combined treatment of cold storage at 0.5±0.5°C for 30 to 40 days and methyl bromide fumigation at a dose of 38.0 g/m³ for 2 hours at 15°C (Standard 1) and at doses of 43.8 to 48.8 g/m³ for 2 hours at 10°C (Standard 2) for the hawthorn spider mite, *Tetranychus viennensis*.

| Standard | Replicate | Cold storage | | Methyl bromide fumigation | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Temperature (°C) | | Dose (g/m³) | Gas concentration (mg/ℓ) | | Temperature (°C) | |
| | | Max. | Min. | | 30 | 120 min. | Chamber | Fruit |
| Standard 1 | 1 | 1.2 | 0.0 | 38* | 40.0 | 36.3 | 16.1 | 15.0 |
| | 2 | 1.3 | 0.1 | 38* | 39.8 | 36.3 | 15.8 | 15.0 |
| | 3 | 1.2 | 0.1 | 38* | 39.6 | 35.7 | 15.1 | 14.9 |
| Standard 2 | 1 | 1.2 | 0.1 | 43.8** | 47.0 | 43.6 | 10.9 | 9.9 |
| | 2 | 1.3 | 0.1 | 44.4** | 52.7 | 43.8 | 11.1 | 10.0 |
| | 3 | 1.2 | 0.1 | 48.0* | 57.7 | 51.5 | 10.8 | 10.1 |

* A 29.5 ℓ fumigation chamber was used.
** A 0.52 m³ fumigation chamber was used.

**Table 3-3.** Fruit and air temperatures and gas concentrations in the combined treatment of cold storage at 0.5±0.5°C for 37 to 40 days and methyl bromide fumigation at a dose of 38 g/m³ for 2 hours at 15°C (Standard 1) and at a dose of 48 g/m³ for 2 hours at 10°C (Standard 2) for the kanzawa spider mite, Tetranychus kanzawai.

| Standard | Replicate | Cold storage | | Methyl bromide fumigation | | | |
|---|---|---|---|---|---|---|---|
| | | Temperature (°C) | | Dose (g/m³) | Concentration (mg/ℓ) | | Temperature (°C) |
| | | Max. | Min. | | 30 | 120min. | Fruit |
| Standard 1 | 1 | 1.2 | 0.0 | 38 | 39.4 | 35.7 | 14.0-14.4 |
| | | | | 38 | 40.3 | 35.6 | 13.8-14.1 |
| | | | | 38 | 38.4 | 34.5 | 15.0-15.3 |
| | 2 | 1.4 | −0.4 | 38 | 38.4 | 34.0 | 13.4-14.3 |
| | | | | 38 | 38.0 | 35.6 | 13.4-13.8 |
| | | | | 38 | 37.0 | 34.7 | 13.4-13.8 |
| | 3 | 1.4 | −0.4 | 38 | 38.8 | 35.5 | 13.6-14.5 |
| Standard 2 | 1 | 1.2 | 0.0 | 48 | 57.1 | 49.4 | 9.8-10.3 |
| | | | | 48 | 57.2 | 49.5 | 9.6-10.1 |
| | 2 | 1.4 | −0.4 | 48 | 55.3 | 47.7 | 10.2-10.6 |
| | | | | 48 | 56.1 | 48.3 | 9.8-10.4 |
| | 3 | 1.4 | −0.4 | 48 | 55.1 | 48.7 | 9.6-10.6 |

**Table 3-4.** Fruit temperatures and gas concentrations in the combined treatment of cold storage at 0.5±0.5°C for 37 to 40 days and methyl bromide fumigation at doses of 34.1 to 34.8 g/m³ for 2 hours at 15°C (Standard 1) and at doses of 43.1 to 44.4 g/m³ for 2 hours at 10°C (Standard 2) for the two-spotted spider mite, *Tetranychus urticae*.

| Standard | Replicate | Cold storage | | Methyl bromide fumigation | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Temperature (°C) | | Dose (g/m³) | Concentration (mg/ℓ) | | Temperature (°C) | |
| | | Max. | Min. | | 30 | 120 min. | Chamber | Fruit |
| Standard 1 | 1 | 0.8 | −0.1 | 34.0 | 32.2 | 28.5 | 15.5 | 14.8 |
| | 2 | 1.0 | 0.1 | 34.5 | 32.9 | 28.8 | 16.1 | 15.0 |
| | 3 | 1.0 | −0.2 | 34.4 | 32.8 | 30.5 | 15.0 | 14.8 |
| | 4 | 0.9 | −0.1 | 34.1 | 32.8 | 29.5 | 16.0 | 14.9 |
| | 5 | 0.9 | −0.1 | 34.2 | 32.8 | 28.7 | 15.8 | 14.6 |
| | 6 | 1.0 | 0.0 | 34.8 | 33.2 | 30.0 | 16.1 | 14.8 |
| Standard 2 | 1 | 0.9 | −0.1 | 43.1 | 47.5 | 39.3 | 11.0 | 10.0 |
| | 2 | 0.9 | −0.2 | 44.2 | 47.3 | 37.9 | 11.1 | 9.8 |
| | 3 | 0.9 | −0.2 | 43.9 | 48.3 | 43.0 | 11.0 | 9.6 |
| | 4 | 0.8 | −0.2 | 43.0 | 48.2 | 42.5 | 10.9 | 9.8 |
| | 5 | 0.9 | −0.2 | 44.4 | 48.0 | 41.7 | 10.8 | 9.9 |
| | 6 | 0.8 | −0.1 | 43.8 | 48.3 | 41.9 | 11.0 | 10.0 |

A 0.52 m³ fumigation chamber was used.

atures during fumigation were 13.4 to 15.3°C and 9.6 to 10.6°C for Standard 1 and Standard 2, respectively. The average of residual gas concentrations were 31.1 mg/ℓ (36.3 mg/ℓ maximum, 28.5 mg/ℓ minimum) and 45.2 mg/ℓ (51.5 mg/ℓ maximum, 37.9 mg/ℓ minimum) for Standard 1 with initial doses of 33.8 to 38 g/m³ and Standard 2 with initial doses of 43.0 to 48.0 g/m³, respectively.

## 2. Mortality

The evaluation of mortalities are shown in Table 3-5 (summer fruit tortrix), Table 3-6 (hawthorn spider mite), Table 3-7 (kanzawa spider mite) and Table 3-8 (two-spotted spider mite).

### Summer Fruit Tortrix

A total of 1,043 larvae in 2 replicates were killed 100% by Standard 1, while a total of 700 larvae in 3 replicates were killed 100% by Standard 2.

**Table 3-5.** Mortality of 3rd instar diapause larvae of the summer fruit tortrix, *Adoxophyes orana fasciata*, stored for 37 to 40 days in cold storage at 0.5±0.5°C followed by methyl bromide fumigation at a dose of 33.8 g/m³ for 2 hours at 15°C (Standard 1) and at doses of 43.8 to 48.0 g/m³ for 2 hours at 10°C (Standard 2).

| Replicate | Standard 1 | | | Standard 2 | | |
|---|---|---|---|---|---|---|
| | No. of larvae treated | No. of larvae survived | Percent mortality | No. of larvae treated | No. of larvae survived | Percent mortality |
| 1 | 500 | 0 | 100 | 300 | 0 | 100 |
| Cont. | 150 | 150 | 0 | 100 | 100 | 0 |
| 2 | 543 | 0 | 100 | 200 | 0 | 100 |
| Cont. | 150 | 150 | 0 | 100 | 100 | 0 |
| 3 | — | — | — | 200 | 0 | 100 |
| Cont. | — | — | — | 100 | 100 | 0 |
| Total | 1,043 | 0 | 100 | 700 | 0 | 100 |

**Table 3-6.** Mortality of overwintering adults of the hawthorn spider mite, *Tetranychus viennensis*, stored for 30 to 40 days in standard cold storage at 0.5±0.5°C followed by methyl bromide fumigation at a dose of 38.8 g/m³ for 2 hours at 15°C (Standard 1) and at doses of 43.8 to 48.0 g/m³ for 2 hours at 10°C (Standard 2).

| Replicate | Standard 1 | | | Standard 2 | | |
|---|---|---|---|---|---|---|
| | No. of adults treated | No. of adults survived | Percent mortality | No. of adults treated | No. of adults survived | Percent mortality |
| 1 | 305 | 0 | 100 | 712 | 0 | 100 |
| 2 | 497 | 0 | 100 | 405 | 0 | 100 |
| 3 | 381 | 0 | 100 | 381 | 0 | 100 |
| Cont. | 239 | 231 | 3.3 | 224 | 208 | 7.1 |
| Total | 1,183 | 0 | 100 | 1,498 | 0 | 100 |

**Table 3-7.** Combined treatment tests: Mortality of the kanzawa spider mite, *Tetranychus kanzawai*, stored for 37 to 40 days in cold storage at 0.5±0.5°C followed by methyl bromide fumigation at a dose of 38 g/m³ for 2 hours at 15°C (Standard 1) and at a dose of 48 g/m³ for 2 hours at 10°C (Standard 2).

| Replicate | Stage | Standard 1 | | | | Standard 2 | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Apples (no.) | Treated (no.) | Surv. (no.) | Surv. (%) | Apples (no.) | Treated (no.) | Surv. (no.) | Surv. (%) |
| 1 | Lavae | 5 | 528 | 0 | 0 | 5 | 531 | 0 | 0 |
| | 1st nymph | 5 | 205 | 0 | 0 | 5 | 197 | 0 | 0 |
| | 2nd nymph | 5 | 419 | 0 | 0 | 5 | 521 | 0 | 0 |
| | Adult | 5 | 277 | 0 | 0 | 5 | 271 | 0 | 0 |
| 2 | Lavae | 5 | 542 | 0 | 0 | 5 | 566 | 0 | 0 |
| | 1st nymph | 5 | 434 | 0 | 0 | 5 | 404 | 0 | 0 |
| | 2nd nymph | 5 | 473 | 0 | 0 | 5 | 447 | 0 | 0 |
| | Adult | 5 | 276 | 0 | 0 | 5 | 287 | 0 | 0 |
| 3 | Egg | 1 | 346 | 0 | 0 | 1 | 338 | 0 | 0 |

Hatching rate of eggs in untreated control lot is 278/285=97.5%.

**Table 3-8.** Mortality of overwintering adults of the two-spotted spider mite, *Tetranychus urticae*, for 37 to 40 days in cold storage at 0.5±0.5°C followed by methyl bromide fumigation at doses of 34.0 to 34.8 g/m³ for 2 hours at 15°C (Standard 1) and at doses of 43.0 to 44.4 g/m³ for 2 hours at 10°C (Standard 2).

| Replicate | Standard 1 | | | | Standard 2 | | | |
|---|---|---|---|---|---|---|---|---|
| | No. of apples infested | No. of adults treated | No. of adults survived | Percent mortality | No. of apples infested | No. of adults treated | No. of adults survived | Percent mortality |
| 1 | 36 | 153 | 0 | 100 | 10 | 1,206 | 0 | 100 |
| Cont. | 20 | 88 | 73 | 17.1 | 5 | 595 | 431 | 27.6 |
| 2 | 36 | 233 | 0 | 100 | 11 | 1,907 | 0 | 100 |
| Cont. | 20 | 136 | 121 | 11.0 | 5 | 872 | 715 | 18.0 |
| 3 | 36 | 141 | 0 | 100 | 11 | 1,802 | 0 | 100 |
| Cont. | 20 | 80 | 68 | 15.0 | 5 | 786 | 589 | 25.1 |
| 4 | 3 | 233 | 0 | 100 | 10 | 1,312 | 0 | 100 |
| Cont. | 3 | 225 | 162 | 28.0 | 5 | 674 | 533 | 20.9 |
| 5 | 3 | 154 | 0 | 100 | 4 | 1,881 | 0 | 100 |
| Cont. | 3 | 159 | 118 | 25.0 | 3 | 1,215 | 826 | 32.0 |
| 6 | 2 | 176 | 0 | 100 | 23 | 69 | 0 | 100 |
| Cont. | 2 | 168 | 116 | 31.0 | 15 | 49 | 41 | 16.3 |
| Total | 116 | 1,090 | 0 | 100 | 69 | 8,177 | 0 | 100 |

## Hawthorn Spider Mite

A total of 1,183 adults in 3 replicates were killed 100% by Standard 1, while a total of

1,498 adults in 3 replicates were killed 100% by Standard 2.

**Kanzawa Spider Mite**

A total of 346, 1,070, 639, 892 and 553 by Standard 1 and that of 338, 1,097, 601, 968 and 558 by Standard 2 were in each 3 replicate were killed 100% for eggs, larvae, first and second nymphs and adults, respectively.

**Two-Spotted Spider Mite**

A total of 1,090 adults in 6 replicates were killed 100% by Standard 1, while a total of 8,177 adults in 6 replicates were killed 100% by Standard 2.

These mortality tests showed that summer fruit tortrix, hawthorn spider mite, kanzawa spider mite and two-spotted spider mite that may be found on 'Fuji' apples at harvest were killed completely by the two standards established for export of 'Fuji' apples to the United States.