Plant Quarantine Treatment of 'Fuji' Apples for Export to The United States

Fusao KAWAKAMI¹⁾, Shunji Motoshima²⁾, Kenji Miyamoto³⁾, Yukihiro Soma, Mitsusada Mizobuchi, Mieko Nakamura, Takashi Misumi⁴⁾, Kunio Sunagawa⁵⁾, Masao Moku⁶⁾, Toshiyuki Akagawa and Toshiyuki Kato

Chemical & Physical Control Laboratory, Research Division

Hiroshi AKIYAMA⁷⁾, Tetsuo IMAMURA⁸⁾, Masahiro TAO, Masashi KANEDA⁹⁾, Shunichiro SUGIMOTO¹⁰⁾, Masanori YONEDA, Hitoshi KADOI¹¹⁾, Hajime KATSUMATA¹²⁾, Hiroshi NAGAI¹³⁾, Motonori SASAKI and Fumihiko ICHINOHE¹⁴⁾

> Entmology Laboratory, Research Division Yokohama Plant Protection Station Ministry of Agriculture, Forestry and fisheries 1-16-10, Shinyamashita, Naka-ku, Yokohama, Japan 231

Kozo KAWASHIMA, Tsuguyoshi KUDO, Yoshiaki Osanai and Akira Saito¹⁵⁾

Division of Plant Protection, Aomori Apple Experiment Station 24, Fukutami, Botandaira, Kuroishi, Japan 036-03

Key words: quarantine pests, Insecta, *Carposina niponensis, Conogethes punctiferalis, Adoxophyes orana fasciata*, Arachnida, *Tetranychus viennensis, Tetranychus urticae, Tetranychus kanzawai*, quarantine treatments, methyl bromide fumigation, sorption, desorption, *residues, organic methyl bromide, inorganic bromide, standard cold storage, controlled atmosphere storage, cold tolerance, a combined treatment, phytotoxicity, Malus, apples*

- 7) Plant Protection Division, Agricultural Production Bureau, MAFF
- 8), 9), 10), 11), 14) Yokohama Plant Protection Station
- 13) Yokohama Plant Protection Station Kamaishi office
- 15) Aomori Agricultural Experiment Station

¹⁾ Kobe Plant Protection Station

²⁾ Yokohama Plant Protection Station Narita office

³⁾ Moji Plant Protection Station

⁴⁾ Yokohama Plant Protection Station Kawasaki office

^{5), 12)} Naha Plant Protection Station

⁶⁾ Yokohama Plant Protection Station Sakata office

Summary

1. Various disinfestation tests against such quarantine pests as peach fruit moth, *Carposina niponensis* WALSINGHAM, yellow peach moth, *Conogethes punctiferalis* (GUENÉE), summer fruit tortrix, *Adoxophyes orana fasciata* WALSINGHAM and hawthorn spider mite, *Tetranychus viennensis* ZACHER, kanzawa spider mite, *Tetranychus kanzawai* KISHIDA, which are unknown in the United States, and which may be present on or in Japanese 'Bagged and Unbagged Fuji' apples to be exported to the United States, were conducted in October 1987 to October 1990 for proving sufficient efficacy of quarantine treatments which have been required by the United States plant quarantine regulatory agency (USDA-APHIS).

2. Peach fruit moth has not been reported to have caused much damage in commercial orchards, owing to the introduction of fruit-bagging and through pest control. Although yellow peach moth is a serious pest of such fruits as peaches, plums, persimmons and chestnuts, it is rarely known to cause injury to apple fruits. Judging from the harvest time of 'Fuji' apples and the life cycle of the two pests in major production areas, the stages of the two pests that may be present on/in 'Bagged Fuji' and 'Unbagged Fuji' apples at harvest are eggs of all ages and larvae in the first through fifth instars. Fifth instar larvae will be developing to diapause condition (diapause larvae)(Part 1).

3. Studies on the susceptibility of all stages of the two pests to methyl bromide fumigation (for 2 hours at 15°C) showed that egg stages were less susceptible than larval stages in both pests and that the egg stages of the two pests were 2.2 times more resistant than larval stages. Two-day-old eggs of peach fruit moth were the most resistant stage (LD_{50} : 23.3 g/m³, LD_{95} : 33.3 g/m³) of all stages. It was estimated that a practical dose of methyl bromide which was sufficient to attain 100% mortality of the two-day-old eggs would be approximately 50 g/m³ or more when the fumigation was carried out for 2 hours at 15°C. A methyl bromide dose sufficiently high to attain 100% mortality for two-day-old peach fruit moth eggs may cause chemical injury on apples (Part 4 : Test 1, Part 5 : Test 1). Therefore, methyl bromide fumigation alone would not be developed as quarantine treatments for the pests in question (Part 2 : Test 1).

4. Studies on the susceptibility of all stages of peach fruit moth and yellow peach moth to low temperatures (at 1.5°C) showed that the larval stages were less susceptible than egg stages in both pests. Six-day-old eggs in eggs of all ages and fifth instar diapause larvae in larval stages of the peach fruit moth were the most resistant stages. The LT_{95} 's for six-day-old eggs and fifth instar larvae were 26.2 days and 32.4 days, respectively. Therefore, the stage most resistant to low temperature was the fifth instar diapause larvae of peach fruit moth. No significant difference in susceptibility was observed between Standard Cold storage and CA storage for either six-day-old eggs or fifth instar diapause larvae of peach fruit moth. Fifth instar diapause larvae of peach fruit moth. Storage treatment for 45 days at 1.5 ± 0.5 °C. Complete

mortality would require longer exposure periods of more than 150 days. Therefore, cold storage alone would not successfully eliminate these pests from apples, and a combined cold storage and methyl bromide fumigation treatment would be developed as a disinfestation method (Part 2 : Test 2).

5. Fifth instar diapause larvae of peach fruit moth exposed to low temperature for long periods are capable of developing cold-hardiness and may have lower mortality in subsequent methyl bromide fumigation because of a decreasing rate of respiration. Mortality tests were conducted for confirming on acquirement of cold-hardiness by using fifth instar diapause larvae of peach fruit moth in the single methyl bromide fumigation and the combined cold storage and methyl bromide fumigation. Studies showed that the combined treatment is more effective than the single methyl bromide fumigation treatment; no effect of cold acclimation was observed on cold-treated diapause larvae; and doses of methyl bromide in the single fumigation treatment (Part 2: Test 3).

6. Tolerance of 'Fuji' apples to methyl bromide fumigation varied depending on the length of time kept in cold storage prior to fumigation. 'Fuji' apples stored for shorter periods after harvest were extremely susceptible to the fumigation even at low doses, while no or only slight injury was observed on apples stored for a month or more at -1 to 0°C and then fumigated (Part 4 : Test 1).

7. Tests for methyl bromide sorption and its penetration showed that approximately 20% of initial dose was absorbed by export cartons, packing materials and fruit. The gas was mainly absorbed by carton boxes and polyethylene fruit caps, while a relatively small amount of the gas was absorbed by plastic field bins. Gas concentrations inside both export cartons and plastic field bins reached uniformity in 10 minutes after injection of the gas. The gas penetrated satisfactorily into the carton boxes with a vent ratio of 0.74% and plastic field bins with many small vents. Gas concentrations inside both containers during exhaust declined rapidly and were $1.0 \text{ mg}/\ell$ in 10 minutes in export cartons and 0.2 mg/ ℓ in 5 minutes in plastic containers. Both fruit containers worked well for methyl bromide fumigation (Part 5).

8. The following quarantine procedures and two disinfestation standards for export of 'Fuji' apples were established on the basis of data from Part 2 : Test 1, 2, and 3, Part 4 and Part 5, respectively. Six-day-old peach fruit moth eggs, which are 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 peach fruit moth diapause larvae, which are the most resistant to low temperature of all larval stages and which are the least susceptible to methyl bromide fumigation, will be completely killed by subsequent fumigation with methyl bromide at lower doses as the second step of the treatment.

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 $0.5 \pm 0.5^{\circ}$ 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).

9. Large-scale mortality tests were conducted to demonstrated that six-day-old peach fruit moth eggs, which were most resistant to low temperatures of all ages of eggs, and fifth instar peach fruit moth diapause larvae, which were most resistant of all stages to low temperature and which were most resistant of all larval stages to methyl bromide fumigation, would be completely killed by either of the two standards established. Treatment conditions less favorable for maximum efficacy were chosen in the tests in order to attain quarantine security. For cold storage, the temperatures were set at 1.5°C or 0.5°C, which are higher than the -1 to 0°C of commercial cold storage, and the treatment periods were set for 37 to 40 days, which are shorter than the 40 days or more of the standards. For fumigation, doses (29.0 to 35.4 g/m³ at 15°C and 39.1 to 44.4 g/m³ at 10°C) of methyl bromide less than those of the standards, minimum temperatures and maximum permissible loading rates were used. The results of the test showed that a total of 62,322 six-day-old eggs (7 replicates of cold storage) and a total of 55,851 fifth instar diapause larvae (Standard 1: 11 replicates of cold storage, and 23 replicates of methyl bromide fumigation) and a total of 69,284 larvae (Standard 2: 9 replicates of cold storage and 18 replicates of methyl bromide fumigation) were completely killed (Part 2: Test 4), and that no injury was observed with respect to 'Bagged Fuji' and 'Unbagged Fuji' apples treated with either of the two standards (Part 4: Test 2).

10. Mortality tests were also conducted to confirm if summer fruit tortrix, hawthorn spider mite, kanzawa spider mite and two-spotted spider mite, Tetranychus urticae Koch could be killed 100% by the two standards established. The results of test showed that the four species of pests were completely killed (Part 3).

11. Organic methyl bromide and inorganic bromide residues were determined for 'Fuji' apples treated by the combined cold storage and methyl bromide fumigation treatments established and stored under conditions of simulating commercial export to the United States. The analysis for organic methyl bromide was conducted periodically by using method of headspace analysis. Organic methyl bromide in apples with Standard 1 and Standard 2 declined rapidly to less than 0.001 ppm in 12 to 14 days and 13 to 14 days after fumigation, respectively, and there should be no detectable residues in apples when unloaded at ports of entry in the United States. Inorganic bromide residues were determined by using a bromide ion-selective electrode. The levels were 2.6 ppm (applied dose of 34.4 g/m^3) and 2.8 to 4.2 ppm (applied dose of 44.4 to 48.9 g/m^3), respectively, for Standard 1 and Standard 2. The levels in unfumigated control apples were 0.2 to 0.4 ppm. This level is below the US accepted tolerance of 5 ppm (Part 6).

12. To attain maximum efficacy, disinfestation in commercial practice will be carried out

in more favorable conditions than those that were used in this series of mortality tests. For cold treatment, the temperature of 0°C or below will be used, which is lower than the $0.5\pm0.5^{\circ}$ C used in the tests. For fumigation, methyl bromide doses of 38 g/m^3 at 15° C or 48 g/m^3 at 10° C will be applied, which are higher than those used in the tests. Therefore, either of the two standards of the combined treatment will provide 100% mortality of all stages of peach fruit moth, yellow peach moth, summer fruit tortrix, hawthorn spider mite, kanzawa spider mite and two-spotted spider mite that may be present on/in 'Fuji' apples, and that they will provide quarantine security.

Introduction

In Japan, apples, *Malus pumila* MILLER var. *domestica* SCHNEIDER, are produced mainly in Nagano Prefecture, the central mountain area and the Tohoku area which includes such prefectures as Aomori, Iwate, Fukushima and Yamagata. The total weight of commercial crops for apples in 1989 was 1,045,000 tons. Today, 95 percent of the crop is produced in those areas, especially in Aomori and Nagano Prefectures. 'Fuji' variety, the most widely planted in Japan, is 50.7 percent (530,000 tons) of the total in the main production areas (Statistics and Information Department, Ministry of Agriculture, Forestry and Fisheries, 1990).

Today, the fruit tree industry in Japan is confronted with a very difficult situation due to lower domestic prices, caused by an increase in productivity of domestic horticultural crops and a large amount of imported fruits. Hence, there is strong interest in exporting high quality 'Fuji' apples to overseas countries. Many countries, however, impose severe restrictions, such as prohibitions or restrictions on the import of Japanese produce to protect their own agricultural products from injurious pests which may invade with imported agricultural products. To export Japanese horticultural crops to these countries, quarantine treatments against target pests must be developed.

The United States has quarantine regulations which prohibit importation of fresh apples from Japan. This is because of insects of quarantine significance including the peach fruit moth, *Carposina niponensis* WALSINGHAM, yellow peach moth, *Conogethes punctiferalis* (GUENÉE), summer fruit tortrix, *Adoxophyes orana fasciata* WALSINGHAM, hawthorn spider mite, *Tetranychus viennensis* ZACHER and kanzawa spider mite, *Tetranychus viennensis* ZACHER and kanzawa spider mite, *Tetranychus kanzawai* KISHIDA which do not exist in the United States. United States quarantine regulations require the development of disinfestation treatments against these target insects on or in fresh apples and for submission of scientific data, as conditions for lifting the ban on fresh apples from Japan (YOSHIZAWA, 1990).

Various studies were mainly conducted in December 1987 to December 1990 to develop effecting quarantine treatments for the target quarantine pests on 'Fuji' apples with these treatments to cause phytotoxic injury to the treated apples. Much of this work was conducted in collaboration between the Chemical & Physical Control Laboratory and the Entomology Laboratory, Research Division, Yokohama Plant Protection Station, Ministry of Agriculture, Forestry and Fisheries (YPPS, MAFF) and the Division of Plant Protection, Aomori Apple Experiment Station, Aomori Prefecture (AAES, Aomori).

Here we report the results of various studies. These reports present experimental data which demonstrate the efficacy of quarantine treatments for fresh apples; ie. Part 1; Stages of Peach Fruit Moth, Carposina niponensis WALSINGHAM and Yellow Peach Moth, Conogethes punctiferalis (GUENÉE) Which May be Present on/in 'Fuji' Apples at Harvest, Part 2; Plant Quarantine Treatment 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, Test 2: Susceptibility of Each Stage of Peach Fruit Moth and Yellow Peach Moth to Low Temperature, Test 3: Susceptibility of Fifth Instar Diapause Larvae of Peach Fruit Moth Treated by Cold Storage to Methyl Bromide Fumigation, 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), 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., Part 4; Phytotoxicity of 'Fuji' Apples Fumigated with Methyl Bromide, (Test 1: Tolerance of 'Fuji' Apples to Methyl Bromide Fumigation, Test 2; Confirmation Tests for Chemical Injury of 'Fuji' Apples in Practical Methyl Bromide Fumigation), Part 5; Methyl Bromide Gas Penetration, Sorption and Desorption under Disinfestation Standards for Export to The U.S., (Test 1: Methyl Bromide Sorption, Test 2: Methyl Bromide Gas Sorption, Penetration and Desorption in Practical Fumigation), Part 6; Residue Analysis for Organic Methyl Bromide and Inorganic Bromide in Fumigated 'Fuji' Apples.

Acknowledgements

We extend our sincere thanks and appreciation to Dr. Kazuo TAKAGI (Fruit Tree Research Station, MAFF, Tsukuba, Ibaraki Prefecture), to Dr. Hiroshi NARITA (Akita Fruit-Tree Experiment Station, Hirashika, Akita Prefecture) and Dr. Toshio OKU (Fruit Tree Research Station, Morioka Branch Station, MAFF, Morioka, Iwate Prefecture) for providing peach fruit moth or for their advice of the mass-production for test insects, and to associate professor Hiroshi HONDA (Faculty of Agriculture, Tokyo University, Tokyo) for providing yellow peach moth and for his advice of the mass-production for test insects, and to Mr. Shiro NAKAGAKI (Ibaraki-Ken Horticultural Experiment Station, Ibaraki Prefecture) for providing hawthorn spider mite, and to Dr. Shichiro TSUCHIYA (Fruit Tree Research Station, Morioka Branch Station, MAFF, Morioka, Iwate Prefecture) for storing test apples and for his advice of apple quality assessments, and to Dr. Kaichi FURUHASHI (Shizuoka Prefectural Citrus Experiment Station) and Mr. Makoto MINAMISHIMA (Nagano Prefectural Experiment Station) for providing kanzawa spider mite, and to Mr. Isao HIRAMATSU, Miss Satoko NISHIKAWA, Mr. Takanori TSUKAMOTO and Mr. Hideaki KISHINO (Import & Export Inspection Division, Yokohama Plant Protection Station, MAFF, Yokohama) for laboratory assistance in mortality determinations in large-scale mortality tests. We are also grateful to Mr. Takushi OBATA (Japan Plant Quarantine Association, Tokyo) and Dr. Harold R. MOFFITT (Yakima Agricultural Research Laboratory, USDA-ARS, Yakima, Wash.) for reviewing the manuscript.