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Methyl Bromide Fumigation for Quarantine Control of Persimmon Fruit Moth and Yellow Peach Moth on Japanese Persimmons

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Abstract : Susceptibility of mature larvae of persimmon fruit moth (PFM), *Stathmopoda masinissa* MEYRICK, and egg and larval stages of yellow peach moth (YPM), *Conogethes punctiferalis* (GUENÉE) to methyl bromide (MB) fumigation for 2 hours at 15 °C showed that mature larvae of PFM were the most resistant (LD_{50}: 15.4-16.4 g/m^3, LD_{95}: 23.9-26.1 g/m^3) of other stages of YPM (2-day-old egg; LD_{50}:13.5 g/m^3, LD_{95}: 19.4 g/m^3, 5-day-old egg; LD_{50}:4.1 g/m^3, LD_{95}: 8.4 g/m^3 and Larvae; LD_{50}: 5.0-7.7 g/m^3, LD_{95}: 8.8-12.3 g/m^3). A 100% mortality for mature larvae of PFM in Japanese persimmons "Fuyu", *Diospyros kaki* THUNB. in plastic field bins was attained with fumigation standard of 48 g/m^3 MB for 2 hours at 15℃ with 49.1-51.9% loading in large-scale mortality tests.
Key words : Insecta, *Stathmopoda masinissa*, *Conogethes punctiferalis*, quarantine treatment, methyl bromide, Japanese persimmons

Introduction

Japanese persimmons, *Diospyros kaki* THUNB. are produced mainly in the Central and Western regions in Japan which include such prefectures as Gifu, Aichi, Nara, Wakayama, Ehime and Fukuoka. The total weight of commercial crops for persimmons in 1989 was 265,700 tons. Today, 51.3 percent of the crop is produced in those areas (Statistics and Information Department, MAFF, 1990).

There is strong interest in exporting persimmons to overseas countries, especially, the United States and Australia. The two 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.

The two countries have quarantine regulations which prohibit importation of fresh persimmons, because of insects of quarantine significance including the persimmon fruit moth (PFM), *Stathmopoda masinissa* MEYRICK and yellow peach moth (YPM), *Conogethes punctiferalis* (GUENÉE) which do not exist in both countries. The quarantine regulations of both countries also require the development of disinfestation treatments against these target insects on or in fresh persim-

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mons for lifting the ban on fresh persimmons from Japan (YOSHIZAWA, 1990).

The objectives of this study were to determine the most resistant stage of all stages of the two pests which may be present on/in persimmons at harvest to methyl bromide (MB) fumigation, and to confirm if the most resistant stage could be killed completely by the disinfestation standard established.

Materials and Methods

1. Test Fruit

Test fruit, Japanese persimmons "Fuyu" were obtained from three Prefectural Experiment Stations of Gifu, Nara and Wakayama.

2. Test Insects

PFM: The stages of the pest which may be present in persimmons at harvest are mature larvae (third through fifth instar) from the life cycle of the pest (ODA & SUGIURA, 1961; OKUDAI, 1961; ITO, 1972; ODA, 1982; ODA, 1983; CLEARWATER, 1984) and the measurement of width of head capsule of the larvae in the tests and harvest time of persimmons "Fuyu" from early October to late December (Statistics and information Department, MAFF, 1990) in main production areas.

In mortality tests, naturally infested persimmons with third through fifth instar larvae collected from unsprayed orchards were obtained from the above mentioned Experiment Stations. This is because of mass-rearing technics for the insect have not been established in both artificial diets and persimmon fruit (ODA, 1982; CLEARWATER, 1984).

YPM: The stages of the pest which may be present on/in fruit at harvest are eggs and larvae from the life cycle (SHINKAJI, 1969; SINKAJI & Ito, 1969; SINKAJI, 1971; SINKAJI & OHO, 1970; SEKIGUCHI, 1974) and harvest time of persimmons "Fuyu" from early October to late December in main production areas. Test insects were obtained from the Laboratory of Applied Entomology, Faculty of Agriculture, University of Tokyo (Yayoi, Bunkyo-ku, Tokyo). They were reared on fresh chestnuts using methods described by HONDA et al.(1979).

The calyx of the fruit was removed and put a piece of woolen cloth on the dent of the fruit. A total of 150 male and female adults were placed in the cage and allowed to mate and oviposite overnight in the rearing room at 23 - 24℃ 60 - 65% R.H. with a 12L: 12D photoperiod. The eggs were taken out and maintained under the same condition for one or four days. The eggs thus reared were considered as two-day-old and five-day-old, respectively. Five holes, 4mm in diameter, were made in each persimmons. Each first through fifth instar larvae reared on/in fresh chestnuts was placed in each hole, which was then filled with cotton and sealed with plastic tape.

3. Fumigation

In susceptibility tests, a 29.5 ℓ fiber-glass fumigation box (57.5cm × 43.5cm × 52.0cm in size; equipped with circulation and ventilation apparatus) was used in the room maintained at 15°C. These egg-infested and larva-infested persimmons were placed in the fumigation box by species, instar, and MB dose. Infested fruit were stored overnight (18 hours) at 15℃ prior to fumigation. Several different doses of MB were used to determine the mortality of all developmental stages in dose-mortality tests. Fumigation was conducted for 2 hours at 15℃.

In large-scale mortality tests, fruit infested with third through fifth instar larvae of the PFM

from the Experiment Stations of Gifu, Nara and Wakayama were put in meshed bags and then placed in a plastic bin (0.046 m^3). These fruit were stored overnight (18 hours) at the fumigation temperature of 15°C. These four bins were then placed in a 1 m^3 stainless steel fumigation chamber equipped with a 7.5 m^3/minute circulation and 1.1 m^3/minute ventilation apparatus. MB enclosed in an ampoule was applied by using a built-in ampoule breaker. Fumigation was conducted for 2 hours at 15℃ at a dose of 48g/m^3 with 49.1 - 51.9% loading.

The built-in circulation apparatus was kept on for the first 30 minutes, and then an automatic timer was used (on: 0.5 minutes, off: 2.5 minutes) through the fumigation. Gas concentrations were monitored by the interference refractometer (Riken-18 and 21, Riken) at time intervals of 10, 30, 60 and 120 minutes after the commencement of fumigation. Fumigation was followed by an hour of exhausting using the ventilation apparatus.

4. Determination of Mortality

To allow viable eggs to hatch, the YPM eggs infested persimmons were placed in plastic containers following fumigation and incubated in the rearing room at 20 - 23℃, 60 - 65% R.H. with a 12L:12D photoperiod. The incubation periods were 5 - 7 and 1 - 3 days, respectively, for two- and five-day-old eggs, after which they counted the number of eggs hatched under microscopes. Persimmons infested with larval stages of the YPM and the PFM were maintained in the same conditions of egg stages. They were then cut and assessed after 4 days. Moribund larvae were also put on a sliced persimmon and assessed after further 3 days in susceptible tests. Mortality of the mature larvae were assessed after 4 days in large-scale mortality tests.

5. Statistical Analysis

Data for the responses to MB of all developmental stages were analyzed by the Probit procedure using FINNEY's formula (FINNEY, 1971). Linearity regression lines obtained from the statistical analyses were tested by the Chi-square test and the fiducial limits were calculated using FILLER's formula (FINNEY, 1971). The LD_{50}'s and LD_{95}'s were considered to be significantly different when their 95% fiducial limits did not overlap.

Results and Discussion

1. Susceptibility to MB Fumigation

Table 1 shows results of the Probit analyses of MB dose-mortality response data for each stage of the PFM and the YPM. The susceptibility of all stages of the two pests to MB fumigation for 2 hours at 15°C showed that the LD_{50}' and LD_{95}'s for larval stage of the PFM were 15.4 - 16.4 g/m^3 and 23.9 - 26.1 g/m^3, respectively and no significant differences in susceptibility was observed among third, fourth and fifth instar larvae, while the LD_{50}' and LD_{95}'s of the PFM were 13.5 g/m^3 and 19.4 g/m^3 (two-day-old eggs), 4.1 g/m^3 and 8.4 g/m^3 (five-day-old eggs) and 5.0 g/m^3 and12.3 g/m^3 (first through fifth instar larvae), respectively. It could therefore be said that the most resistant stage was mature larvae of the PFM among all stages of the two pests which may be present on/in Japanese persimmons at harvest.

Table 1. Estimated LD_{50} and LD_{95} values for mature larvae of the persimmon fruit moth, *Stathmopoda masinissa* and for egg and larval stages of the yellow peach moth, *Conogethes punctiferalis* fumigated with methyl bromide for 2 hours at 15℃.

Pest & Stage	Number tested[a]	LD_{50} (95%FL) (g/m^3)	LD_{95} (95%FL) (g/m^3)
Persimmon fruit moth			
3rd instar larvae[b]	641	15.4 (14.7-16.0)	23.9 (22.8-25.4)
4th[b]	1,059	16.1 (14.9-17.2)	24.4 (22.6-27.3)
5th[b]	1,351	16.4 (15.9-16.9)	26.1 (25.1-27.5)
Yellow peach moth			
2-day-old eggs[c]	1,039	13.5 (13.0-14.0)	19.4 (18.8-20.0)
5-[b]	1,208	4.1 (3.7- 4.6)	8.4 (7.4- 9.9)
1st instar larvae[b]	833	6.0 (5.1- 6.1)	9.7 (8.5-12.0)
2nd[b]	888	5.8 (5.5- 6.1)	8.8 (8.4- 9.3)
3rd[c]	986	7.7 (6.4- 9.3)	12.3 (10.3-17.4)
4th[b]	994	5.9 (5.5- 6.2)	11.6 (10.9-12.5)
5th[b]	1,034	5.0 (4.7- 5.3)	9.8 (9.3-10.5)

a Total number of test insects fumigated in 5-6 dose levels.
b Three replicates.
c Two replicates.

Table 2. Estimated LD_{50} and LD_{95} values for mature larvae of the persimmon fruit moth, *Stathmopoda masinissa* collected in different production areas fumigated with methyl bromide for 2 hours at 15℃.

Production area	Number tested[a]	LD_{50} (95%FL)[b] (g/m^3)	LD_{95} (95%FL)[b] (g/m^3)
Nara	931	14.8 (14.2-15.4)	27.8 (25.9-30.6)
Wakayama	1,330	15.3 (13.8-16.9)	25.7 (22.2-33.3)
Gifu	790	15.6 (14.9-16.3)	30.1 (27.6-33.7)

a Total number of test insects fumigated in 5-6 dose levels.
b Three replicates.

Table 2 shows results of the Probit analyses for mature larvae of the PFM collected in Gifu, Nara and Wakayama prefectures. No significant differences in susceptibility was also observed among mature larvae collected in different production areas.

2. Large-Scale Mortality Test

Table 3 shows MB gas concentrations and temperatures during fumigation. The average of residual gas concentrations in 2 hours after fumigation were 40.7 - 46.5 mg/ℓ and fruit temperatures were 15.0℃. Table 4 shows results of the mortality test with six replicates conducted in 1992 through 1994 against the mature larvae of the PFM. A total number of 13,163 larvae, which were the most resistant stage to MB fumigation, were killed completely at 48 g/m^3 of MB

for 2 hours at 15℃ with 49.1 - 51.9% loading. The fumigation standard established with consideration of fumigation elements, such as LD_{95}'s (26.1 g/m^3), gas absorption ratio to fumigation items to be fumigated and an additional dose for a guarantee of quarantine seculity (MONRO, 1969) would be sufficient to attain 100% mortality of the PFM and the YPM on/in Japanese persimmons.

Table 3. Methyl bromide gas concentrations and fruit temperatures recorded in a 1 m^3 fumigation chamber. Fumigation at a dose of 48 g/m^3 for 2 hours at 15℃ with 49.1 - 51.9% loading.

Year	Replicate	No. fruit tested	loading (v/v: %)	Gas concentration (mg/ℓ)				Fruit Temp. (℃)
				10	30	60	120 min.	
1992	1	2,847	50.5	58.3	56.2	52.8	45.6	15
1993	1	2,997	50.4	55.7	53.0	49.1	46.1	15
	2	3,045	50.3	59.8	53.1	50.7	46.5	15
1994	1	3,387	50.2	53.1	49.1	46.3	41.6	15
	2	3,577	51.9	55.9	50.2	47.9	46.5	15
	3	2,687	49.1	51.8	47.9	46.3	40.7	15

Table 4. Mortality of mature larvae of the persimmon fruit moth, *Stathmopoda masinissa* in Japanese persimmons by methyl bromide fumigation at a dose of 48 g/m^3 for 2 hours at 15℃ with 49.1 - 51.9% loading.

Year	Replicate	Treatment	No. fruit infested	No. larvae tested*	No. larvae survived	Percent mortality
1992	1	Fumigated	2,847	2,377	0	100.0
		Cont.	150	142	138	2.8
1993	1	Fumigated	2,997	1,551	0	100.0
		Cont.	172	161	155	3.7
	2	Fumigated	3,045	1,041	0	100.0
		Cont.	182	108	107	0.9
1994	1	Fumigated	3,387	2,637	0	100.0
		Cont.	375	302	292	3.1
	2	Fumigated	3,577	3,003	0	100.0
		Cont.	411	348	345	0.9
	3	Fumigated	2,687	2,554	0	100.0
		Cont.	301	293	286	2.4
Total	6	Fumigated	18,540	13,163	0	100.0
		Cont.	1,591	1,354	1,323	2.3

* The number of test insects in fumigated lots was estimated on the base of survival in untreated control lots.

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和 文 摘 要

柿果実に寄生するカキノヘタムシガ*Stathmopoda masinissa* MEYRICK及びモモノゴマダラノメイガ*Conogethes punctiferalis*（GUNÉE）の臭化メチルくん蒸による検疫処理

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カキノヘタムシガ*Stathmopoda masinissa*の老齢幼虫及びモモノゴマダラノメイガ*Conogethes punctiferalis*の卵及び幼虫の臭化メチルくん蒸に対する感受性を調査し，感受性が最も低い態が確立した消毒基準で完全殺虫されるか大規模殺虫試験で確認した。臭化メチルくん蒸（2時間，15℃)に対する感受性は,カキノヘタムシガ3～5齢幼虫（LD_{50}：15.4-16.4g/m^3，LD_{95}：23.9-29.1g/m^3)がモモノゴマダラノメイガの2日齢卵（LD_{50}：13.5g/m^3,LD_{95}：19.4g/m^3），5日齢卵（LD_{50}：4.1g/m^3，LD_{95}：8.4g/m^3)及び1～5齢幼虫（LD_{50}：5.0-7.7g/m^3，LD_{95}：8.8-12.3g/m^3）に比較して最も低かった。柿“富有”に寄生したカキノヘタムシガの3～5齢幼虫は，臭化メチル48g/m^3，2時間，15℃，収容率49.1-51.9%の基準で完全殺虫された。

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