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Mortality tests for Kanzawa Spider Mite, Six-Spotted Mite, Tropical Citrus Aphid and Citrus Psylla on Satsuma Mandarins by Methyl Bromide Fumigation

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Abstract: Each stage of Kanzawa spider mite, *Tetranychus kanzawai*, six-spotted mite, *Eotetranychus sexmanaculatus*, tropical citrus aphid *Toxoptera citricidus* and citrus psylla, *Diaphorina citri* on Satsuma mandarins was fumigated with methyl bromide at 48g/m^3 for 2 hours at 15℃ with 32% loading (v/v). All test insects and Arthropods were killed completely with high numbers of Kanzawa spider mite (20,724 eggs, 987 larvae, 2,929 nymphs and10,561 adults), six-spotted mite (19,083 eggs, 450 larvae, 3,686 nymphs and 6,109 adults), tropical citrus aphid (792 larvae and 188 adults) and citrus psylla (3,556 eggs, 4,596 nymphs and 3,375 adults), respectively. These results showed that the methyl bromide fumigation schedule established for exporting Satsuma mandarins to the US would provide for sufficient quarantine security against 4 species of quarantine pests.
Key words: *Toxoptera citricidus*, *Tetranychus kanzawai*, *Eotetranychus sexmanaculatus*, *Diaphorina citri*, methyl bromide, quarantine treatment, Satsuma mandarins

Introduction

On the extension of lifting import ban on Japanese Satsuma mandarins to 5 citrus production States (Florida, California, Texas, Arizona and Louisiana) in the USA, in 1995 the US quarantine authority required Japan to take quarantine measures for the following 11 species of quarantine pests, such as Japanese mealybug, *Planococcus kraunhiae*, citrus mealybug, *Pseudococcus citriculus*, arrowhead scale, *Unaspis yanonensis*, *Eotetranychus kankitus*, citrus psylla, *Diaphorina citri*, tropical citrus aphid, *Toxoptera citricidus*, citrus fruit fly, *Dacus tsuneonis*, *Parlatoria cinerea*, *Planococcus lilacinus*, Kanzawa spider mite, *Tetranychus kanzawai*, six-spotted mite, *Eotetranychus sexmanaculatus* in addition to citrus canker (*Xanthomonas campestris* pv. *citri*).

Japan mainly proposed the combined quarantine measures with methyl bromide (MB) fumigation and inspection at the orchard as the quarantine measures for those pests. In

MB fumigation, the treatment schedule (48g/m^3 for 2 hours at 15℃ with 32% loading or below) for attaining complete mortality for Japanese mealybug, citrus mealybug (MISUMI *et al.*, 1994), arrowhead scale and *Eotetranychus kankitus* (MIZOBUCHI *et al.*, 1995) had been established and applied for exporting Satsuma mandarins from 1993 to 1998.

Additional mortality tests were conducted to confirm if complete mortality of Kanzawa spider mite, six-spotted mite, tropical citrus aphid and citrus psylla could be achieved by the established MB schedule.

Materials and Methods

Test Fruits

Satsuma mandarins harvested at Fujieda-city in Shizuoka Prefecture in 1999 season were used for the mortality test as pest infesting fruit.

Test insects and Arthropods

Kanzawa spider mite: The mites were obtained from the Tottori Horticultural Experiment Station (Daiei-Cho, Tottori Prefecture) in June 1999. They were reared on kidney bean leaves in the pots at 25℃, 70% R.H. with a 16L: 8D photoperiod at the Kobe Plant Protection Station (MATSUNAGA *et al.*, 1972). The eggs, larvae, nymphs and adults on fruits were used for the test.

Six-spotted mite: The mites were obtained from the National Institute of Agro-Environmental Sciences, the Ministry of Agriculture, Forestry and Fisheries (Tsukuba-city in Ibaraki Prefecture) in October 1999. They were reared on leaves of *Mallotus japonicus* in the petri dish at the same conditions of the Kanzawa spider mite at the Hiroshima branch office of Kobe Plant Protection Station. The eggs, larvae, nymphs and adults on fruit were used for the test.

Tropical citrus aphid: The aphids were obtained from the Shizuoka Prefectural Citrus Experiment Station (Fujieda-city, Shizuoka Prefecture) in August 1998. They were reared on potted Satsuma mandarin trees at the Research Division, Yokohama Plant Protection Station. The larvae and adults were used for the test.

Citrus psylla: The adults were collected from jasmine orange trees at Urazoe-city, Okinawa Prefecture in August 1999 because the pests are not distributed in the mainland of Japan. They were reared on potted jasmine orange trees at 27℃, 70-80% R.H. with a 16 L: 8D photoperiod at the Naha Plant Protection Station. The eggs, nymphs and adults on fruits were used for the test.

All the pests and Satsuma mandarin fruit were stored for 24 hours at fumigation temperature of 15℃ before fumigation.

Fumigation

A 31.5 liter fiber-glass fumigation box (26.0cm × 28.0cm × 43.0cm in size) equipped with gas circulation and ventilation apparatus, ports for gas application, gas sampling, a manometer and temperature probes was used in the fumigation room. All the pests on Satsuma mandarin were placed in the fumigation boxes and fumigated with MB at 48g/m^3

for 2 hours at 15℃ with 32% loading(v/v). MB enclosed in the vacuum vial was sampled by a syringe and then injected a certain amount of gas into the fumigation box. The circulation fan was operated throughout fumigation. After fumigation, gas-air mixture was exhausted for one hour by the ventilation apparatus. The interference refractometer (Riken type Models 18) was used to monitor gas concentrations at time intervals of 10, 30, 60, 90 and 120 minutes after the commencement of fumigation. The automatic temperature recorder (Hybrid Recorder; LE, Chino) was used to monitor the temperature in the fumigation box. The tests were replicated three times.

Evaluation of Mortality

After fumigation, each stage of 4 species of the pests was stored at the same conditions of the rearing of the pests. After 24 hours, the mortality of larvae, nymphs and adults was determined under the microscope by stimulating with a needle, while the mortality of eggs was determined by counting the number of hatched eggs every day for 5 days under the microscope. The number of the pests in the fumigated lot was determined based on survival rate of the untreated control lot.

Results and Discussion

Fumigation Temperatures and CT Products

Temperatures and CT products obtained from the fumigation with 48g/m^3 of MB for 2 hours at 15℃ with 32% (v/v) loading are shown in Table 1. Fruit temperatures and air temperatures were at 14.9-15.5℃ and at 14.7-15.3℃, respectively. The CT products ob-

Table 1. Fumigation temperatures and CT products obtained from mortality tests for 4 species of the pests with methyl bromide fumigation at 48g/m^3 for 2 hours at 15℃ with 32% loading (v/v).

Pests	Stage	Replicate	CT product		Temperature(℃)	
			mg·h/*l*	Average(±SD)	Fruit pulp	Air in box
Kanzawa spider mite	all stages	1	97.3	94.8±2.2	15.5	15.1
		2	93.6		15.3	15.2
		3	93.5		15.2	15.3
Six-spotted mite	all stages	1	93.2	95.1±1.6	15.0	15.2
		2	95.7		14.9	15.1
		3	96.3		15.1	15.2
Tropical citrus aphid	all stages	1	90.3	90.4±0.6	15.0	14.7
		2	89.9		15.0	15.3
		3	91.1		15.0	15.1
Citrus psylla	all stages	1	87.2	86.2±1.3	15.0	15.3
		2	84.7		15.0	14.9
		3	86.8		15.0	15.0

Note. Mortality tests for kanzawa spider mite, six-spotted mite and citrus psylla were conducted in 1999, while the test for tropical citrus aphoid was conducted in 1998.

tained from the fumigation for the tropical citrus aphid and the citrus psylla were lower than those for two species of the pests. The reason was considered that gas was absorbed by a piece of wetted sponge or a sheet of wetted paper used for maintained moderate moisture to be avoided natural mortality of the pests during storage before fumigation and during fumigation.

Evaluation of Mortalities

Test results of the mortality for 4 species of the pests in 3 replicated tests are shown in Table 2 (Kanzawa spider mite), Table 3 (six-spotted spider mites), Table 4 (tropical citrus aphid) and Table 5 (citrus psylla), respectively.

All the pests were killed completely with the fumigation schedule of 48g/m^3 for 2hours at 15℃ with 32% loading (v/v) with high numbers of Kanzawa spider mite: a total of 35,201 (eggs; 20,724, larvae; 987, nymphs; 2,929 and adults; 10,561), six-spotted mite: a total of 29,328 (eggs; 19,083, larvae; 450, nymphs; 3,686 and adults; 6,109), tropical citrus aphid: a total of 980 (larvae; 792 and adults; 188) and citrus psylla: a total of 11,527 (eggs; 3,556, nymphs; 4,596 and adults; 3,375), respectively.

These results showed that Kanzawa spider mite, six-spotted mite, tropical citrus aphid and citrus psylla would be killed completely by the fumigation schedule established for

Table 2. Mortality of the Kanzawa spider mite, *Tetranychus kanzawai* fumigated with methyl bromide at 48g/m^3 for 2 hours at 15℃ with 32% loading (v/v).

Stage	Replicate	Control		Fumigation		
		No. of mite tested	Survivor (%)	No. of mite treated*	No. of survivor	Mortality (%)
Eggs	1	2,307	94.2	7,109	0	100
	2	1,368	80.5	7,152	0	100
	3	2,229	92.7	6,463	0	100
	Total	5,904	90.4	20,724	0	100
Larvae	1	1,335	99.3	400	0	100
	2	137	99.3	361	0	100
	3	327	99.4	226	0	100
	Total	1,799	99.3	987	0	100
Nymphs	1	152	92.9	1,348	0	100
	2	44	81.8	1,027	0	100
	3	98	74.5	554	0	100
	Total	294	85.0	2,929	0	100
Adults	1	544	90.1	4,671	0	100
	2	532	90.6	3,780	0	100
	3	297	89.2	2,110	0	100
	Total	1,373	90.1	10,561	0	100
	Grand total	9,370	—	35,201	0	100

* Based on survivor in control.

Table 3. Mortality of the six-spotted mite, *Eotetranychus sexmanaculatus* fumigated with methyl bromide at 48g/m^3 for 2 hours at 15℃ with 32% loading (v/v).

Stage	Replicate	Control		Fumigation		
		No. of mite tested	Survivor (%)	No. of mite treated*	No. of survivor	Mortality (%)
Eggs	1	2,463	94.0	5,712	0	100
	2	740	79.3	1,453	0	100
	3	2,477	98.6	11,918	0	100
	Total	5,680	94.1	19,083	0	100
Larvae	1	20	50.0	29	0	100
	2	0	–	0	–	–
	3	6	100	421	0	100
	Total	26	61.5	450	0	100
Nymphs	1	851	89.1	2,248	0	100
	2	1	100	2	0	100
	3	487	100	1,436	0	100
	Total	1,339	93.1	3,686	0	100
Adults	1	422	87.2	1,692	0	100
	2	130	95.4	816	0	100
	3	425	99.5	3,601	0	100
	Total	977	93.7	6,109	0	100
	Grand total	8,022	–	29,328	0	100

* Based on survivor in control.

Table 4. Mortality of the tropical citrus aphid, *Toxoptera cutricidus* fumigated with methyl bromide at 48g/m^3 for 2 hours at 15℃ with 32% loading (v/v).

Stage	Replicate	Control*		Fumigation		
		No. of insect tested	Survivor (%)	No. of insect treated**	No. of survivor	Mortality (%)
Larvae	1	423	41.6	253	0	100
	2	423	41.6	314	0	100
	3	423	41.6	225	0	100
	Total	423	41.6	792	0	100
Adults	1	84	70.2	70	0	100
	2	84	70.2	73	0	100
	3	84	70.2	45	0	100
	Total	84	70.2	188	0	100
	Grand total	507	–	29,328	0	100

* One untreated control lot was prepared in 3 replicated tests.

** Based on survivor in control.

Table 5. Mortality of the citrus psylla, *Diaphorina citri* fumigated with methyl bromide at 48g/m^3 for 2 hours at 15℃ with 32% loading (v/v).

Stage	Replicate	Control		Fumigation		
		No. of insect tested	Survivor (%)	No. of insect treated*	No. of survivor	Mortality (%)
Eggs	1	246	80.1	220	0	100
	2	280	81.4	732	0	100
	3	270	83.0	2,604	0	100
	Total	796	81.5	3,556	0	100
Larvae	1	298	93.0	1,562	0	100
	2	614	94.5	1,373	0	100
	3	152	88.8	1,661	0	100
	Total	1,064	93.2	4,596	0	100
Adults	1	379	92.9	1,154	0	100
	2	230	93.0	1,104	0	100
	3	352	97.4	1,117	0	100
	Total	961	94.6	3,375	0	100
	Grand total	2,821	—	11,527	0	100

* Based on survivor in control.

exporting Satsuma mandarins to the US, and that the fumigation schedule would provide for sufficient quarantine security.

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和 文 摘 要

温州みかん果実に寄生したカンザワハダニ，コウノシロハダニ，ミカンクロアブラムシ及びミカンキジラミの臭化メチルくん蒸殺虫試験

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温州みかんに寄生させたカンザワハダニ，コウノシロハダニ，ミカンクロアブラムシ及びミカンキジラミの各態を臭化メチル48g/m^3，15℃，2時間，収容率32%の条件で3回反復くん蒸した。その結果，カンザワハダニの卵が20,724卵，幼虫987頭，若虫2,929頭，成虫10,561頭，コウノシロハダニの卵が19,083卵，幼虫450頭，若虫3,686頭，成虫6,109頭，ミカンクロアブラムシの幼虫が792頭，成虫188頭，ミカンキジラミの卵が3,556頭，若虫4,596頭，成虫3,375頭がそれぞれ有効虫数として供試され，いずれも完全殺虫された。これらの結果は，対米輸出温州みかん用として既に確立された臭化メチルくん蒸基準により4種害虫が完全殺虫されることを示している。