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Short Communication

Susceptibility of Insect Pests Infesting Fruit and Vegetables to Methyl Iodide Fumigation.

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Abstract: To consider the methyl iodide (MI) fumigation condition against insect pests which infest fruit and vegetables, susceptibility tests on each developmental stage of 5 species were conducted. The results of MI fumigation at dosages of 9.3, 19.3 and 29.4 g/m^3 for 2 hours at 15°C revealed 100% mortalities were obtained at 9.3 g/m^3 against 2 species of leafroller moth, 19.3 g/m^3 against 2 species of noctuid moth, and 29.4 g/m^3 against a species of mealybug. Treatment schedules of MI fumigation against insect pests infesting fruit and vegetables were proposed in prior study, where it was thought that leafroller moths could be fumigated under the same conditions as the group of aphids, spider mites and thrips, while noctuid moths could be fumigated under the same conditions as the group of mealybugs. However, the noctuid species is now known to be much less susceptible than, for example, aphids and much more susceptible than the mealybugs mentioned above. If there are other species whose susceptibilities to MI are similar to noctuid species, then proposing new MI fumigation schedules against the insects' pest groups including noctuid species between the schedules against groups, including aphids and mealybugs seem appropriate.

Key Words: methyl iodide, fumigation, susceptibility, insect pest, fruit and vegetables

Introduction

Toward reducing the use and emission of methyl bromide (MB) in Japanese quarantine fumigation, our laboratory has been conducting basic tests toward developing alternatives to MB. We conducted methyl iodide (MI) fumigation tests against forest insect pests (Naito *et al.,* 2003, Soma *et al.,* 2007). After confirming its efficacy against the insect pests, procedures and safety in the practical MI fumigation survey, the Ministry of Agriculture, Forestry and Fisheries in Japan revised its import plant quarantine regulation in 2023. In the regulation, MI fumigation can be applied as a disinfestation treatment for imported logs when quarantine pests such as bark beetles are intercepted at a port of entry. On the other hand, MI fumigation against quarantine pest which infest fruit and vegetables was also studied toward the development of the MI fumigation conditions (Naito *et al.,* 2014, 2015, Kawai *et al.,* 2023). However, there are other insect pests that infest fruit and vegetables. Therefore, we conducted further MI susceptibility tests in a study using 5 species.

Materials and Methods

1. Test insect

(1) Noctuid moth

(a) armyworms, *Mythimna separata* (Walker): Larvae of *M. separata* were provided by the University of Tsukuba in 2019. The insect pests were reared for successive generations on an artificial diet (Silkmate 2S, Nosan Corporation). 0-1-day-old eggs, 3rd instar larvae (12 days after oviposition) and 6th instar larvae (36-38 days after oviposition) were provided for fumigation. To prepare the insect pests for fumigation, cut copy paper pieces (30×7 cm) folded into pleated shapes were placed in a plastic container (W28×D21×H10cm) as ovipositing media for adult females for 24-hours. After oviposition, 150-300 eggs on the paper medium were placed into a cylindrical container (15 cm in diameter×9 cm height) along with 50 g of the artificial diet. For 3rd and 6th instar larvae, 100-120 individuals were placed into the container with the artificial diet in the way of preparing eggs for fumigation.

(b) corn earworm, *Helicoverpa armigera* (Hübner): *H. armigera,*

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Due to the pupation of both species occurring underground and not on host plants, no pupal stage for either species were used for this test.

(2) Mealybug

(a) Japanese mealybug, *Planococcus kraunhiae* (Kuwana): Adult females with eggs of *P. kraunhiae* were provided by the Shimane Prefectural Agricultural Technology Center in 2021. The insect pests were reared for successive generations on a Japanese squash. For oviposition, 10-15 gravid adult females were placed in a glass petri dish (9 cm in diameter × 1.8 cm height) in which a sheet of filter paper was laid on the bottom for 2 days. After that, pieces of squash coated with paraffin were inoculated by batches of eggs and were placed into plastic cylindrical containers (15 cm in diameter × 9 cm height) and kept until the target developmental stages were reached. 0-2-day-old eggs, 3rd instar nymphs (28-30 days after oviposition) and adult females (36-38 days after oviposition) were provided for fumigation.

(3) Leafroller moth

(a) smaller tea tortricid, *Adoxophyes honmai* Yasuda: Pupae of *A. honmai* were introduced from Sumika Technoservice Corporation in 2022 and reared for successive generations on an artificial diet (Silkmate 2S, Nosan Corporation). To collect the eggs, 15 adult stage of males along with 15 females were placed into a plastic cylindrical container (11cm in diameter×6.5cm height) in which cut absorbent cotton containing water was placed. A paraffin-coated sheet of paper was placed between the container and the lid as an ovipositing medium upon which the adult stage pests were allowed to lay eggs for 24 hours. The paraffin paper was cut into 1-3 batches of eggs and placed on glass petri dishes (9 cm in diameter×1.8 cm height) on which a filter paper was laid and kept until just before hatching. The eggs on the cut paraffin paper were placed along with shredded artificial diet in a plastic container (W28×D21×H10 cm) and kept until the target developmental stages were reached. 1-2-day-old eggs, 1-2nd instar larvae (7-10 days after oviposition), 3-4th instar larvae (12-16 days after oviposition) and 5-6th instar larvae (17-20 days after oviposition) and pupae (25 days after oviposition) were provided for fumigation.

(b) summer fruit tortrix, *Adoxophyes orana fasciata* Walsingham: Pupae of *A. orana fasciata* were introduced from the Sumika Technoservice Corporation in 2022 and reared under the same conditions as *A. honmai*. 1-2 day-old eggs, 1-2nd instar larvae (8-14 days after oviposition), 3-4th instar larvae (17-18 days after oviposition), 5-6th instar larvae (19-21 days after oviposition) and pupae (25 days after oviposition) were provided for fumigation.

All 5 species of pests were reared at a constant temperature of 25°C, 50-70% RH under a photoperiod of 16L: 8D in rearing rooms. Each developmental stage for the test was moved to a controlled temperature fumigation room and acclimation periods at 15°C were 1 night or at least 3 hours prior to fumigation.

2. Fumigation

Fumigation was performed through the same methodology with devices described in Naito *et al.* (2014). Purity of liquid MI was 99.5 % (FUJIFILM Wako Pure Chemical Corporation). Test insects were fumigated with MI at dosages of 9.3, 19.3 and 29.4 g/m^3 for 2 hours at 15°C. This fumigation condition was set to compare the susceptibilities to MI fumigation between the insect pests and other insect pests reported by Naito *et al.* (2015). Gas concentrations of MI and temperatures in fumigation boxes during fumigation were monitored by gas chromatograph (GC-2014 with FID: Shimadzu) and a temperature recorder (Graphic logger CR-1016-A: Chino) at 15, 30, 60 and 120 minutes after dosing, respectively. After the fumigation was completed, air-fumigant mixture gas was exhausted with an aeration system for 1 hour, and tested insects and untreated controls were moved to the rearing room. The CT value (gas concentration and time product) was calculated similarly to the method described by Monro (1969).

These tests were replicated 3 or 4 times.

3. Evaluation of the mortality

Mortalities of egg stages of *A. honmai, A. orana fasciata* and *H. armigera* were evaluated by hatching at 7-9 days after fumigation, while those of *M. separata* and *P. kraunhiae,* were evaluated by counting the number of larvae at 7 and 12 days after fumigation, respectively. Mortalities at the larval stages of *A. honmai, A. orana fasciata, H. armigera* and *M. separata* and the nymphal and adult stages of *P. kraunhiae* were determined by counting the numbers of live and dead insects at 2-5 days after fumigation. Mortalities of pupae of *A. honmai* and *A. orana fasciata* were evaluated by counting the number of the adult stage at 10 days after fumigation. The numbers of tested insects in the treatment plot were estimated from the survival rates of the untreated control and the mortalities of 2 species of noctuid moth and *P. kraunhiae* were corrected by Abbott's formula (Abott, 1925).

Results and Discussion

The average temperatures and CT values in the susceptibility

Table 1. The average temperature in the fumigation box and CT value of 5 insect pest species fumigated with MI for 2 hours at 15°C.

Dosage (g/m^3)	*M. separata*		*H. armigera*		*P. kraunhiae*		*A. honmai*		*A. orana fasciata*	
	Temp. (°C)	CT value (mg·h/l)[1)]	Temp. (°C)	CT value (mg·h/l)[1)]	Temp. (°C)	CT value (mg·h/l)[1)]	Temp. (°C)	CT value (mg·h/l)[1)]	Temp. (°C)	CT value (mg·h/l)[1)]
9.3	15.7	15.4	15.1	15.9	15.1	15.6	15.5	15.4	15.6	15.5
19.3	15.8	32.2	16.0	28.0	15.2	32.5	15.3	30.9	15.3	31.0
29.4	15.6	48.6	14.8	50.6	15.1	49.2	15.2	46.5	15.2	46.6

[1)]CT value (mg·h/l) = $(7.5\ C_{15} + 22.5\ C_{30} + 45\ C_{60} + 30\ C_{120}) / 60$

C_{15}, C_{30}, C_{60} and C_{120} indicate the gas concentrations at 15, 30, 60 and 120 min. after dosing, respectively.

test are shown in Table1.

The mortalities in the susceptibility test of *M. separata* and *H. armigera* are shown in Table 2. The 100% mortality rate of eggs and 3-4 instar larvae of 2 species were obtained at a dosage of 9.3 g/m^3. The 100% mortality rate of 6th instar larvae of *M. separata* and 5-6th instar larvae of *H. armigera* were obtained at a dosage of 19.3 g/m^3. Therefore, mature instar larvae of noctuid species seem less susceptible than eggs and younger instar larvae. The mortalities of *A. honmai* and *A. orana fasciata* of the test are shown in Table 3. 100% mortalities of all tested stages were obtained at the dosage of 9.3 g/m^3.

Table 2. Corrected mortalities of each stage of noctuid moths fumigated with MI at 9.3 - 29.4g/m^3 for 2 hours at 15°C.

Dosage (g/m^3)	*Mythimna separata*									*Helicoverpa armigera*								
	egg			3-4th instar larva			6th instar larva			egg			3-4th instar larva			5-6th instar larva		
	n[1)]	mean %[3)]	SD	n[1)]	mean %[3)]	SD	n[1)]	mean %[3)]	SD	n[1)]	mean %[3)]	SD	n[1)]	mean %[3)]	SD	n[2)]	mean %[3)]	SD
0(control)	756	32.0 (23.9)	34.6	343	6.2 (6.4)	5.0	360	0.0 (0.0)	0.0	483	70.2 (67.1)	9.0	158	0.0 (0.0)	0.0	398	6.0 (3.8)	3.9
9.3	577	100 (100)		321	100 (100)		360	93.9 (93.9)	7.1	152	100 (100)		158	100 (100)	0.0	196	69.2 (71.1)	44.0
19.3	578	100 (100)		321	100 (100)		360	100 (100)		-	- -		-	- -		187	100 (100)	
29.4	582	100 (100)		321	100 (100)		360	100 (100)		163	100 (100)		158	100 (100)		196	100 (100)	

[1)]The estimated number of insects tested in a total of 3 replications.
[2)]The estimated number of insects tested at dosages of 9.3, 19.3 and 29.4g/m^3 in a total of 4, 3 and 4 replications, respectively.
[3)]The upper row is the average, and the lower row in parentheses is the weighted average calculated from each replication.

Table 3. Mortalities of each stage of leafroll moths fumigated with MI at 9.3 - 29.4g/m^3 for 2 hours at 15°C.

Dosage (g/m^3)	*Adoxophyes honmai*														
	egg			1-2nd instar larva			3-4th instar larva			5-6th instar larva			Pupa		
	n[1)]	mean %[3)]	SD	n[1)]	mean %[3)]	SD	n[2)]	mean %[3)]	SD	n[2)]	mean %[3)]	SD	n[2)]	mean %[3)]	SD
0(control)	1745	66.9 (63.0)	12.0	902	66.6 (67.7)	29.2	310	6.6 (6.1)	4.7	240	4.2 (4.2)	4.2	329	6.5 (6.7)	3.8
9.3	563	100 (100)		222	100 (100)		291	100 (100)		240	100 (100)		287	100 (100)	
19.3	513	100 (100)		183	100 (100)		291	100 (100)		240	100 (100)		295	100 (100)	
29.4	538	100 (100)		233	100 (100)		291	100 (100)		240	100 (100)		305	100 (100)	

Dosage (g/m^3)	*Adoxophyes orana fasciata*														
	egg			1-2nd instar larva			3-4th instar larva			5-6th instar larva			Pupa		
	n[1)]	mean %[3)]	SD	n[1)]	mean %[3)]	SD	n[2)]	mean %[3)]	SD	n[2)]	mean %[3)]	SD	n[2)]	mean %[3)]	SD
0(control)	1364	51.4 (52.6)	38.7	1017	74.6 (75.4)	7.1	317	1.3 (1.3)	2.1	270	5.3 (5.2)	1.1	360	11.9 (11.9)	4.7
9.3	273	100 (100)		194	100 (100)		339	100 (100)		256	100 (100)		317	100 (100)	
19.3	297	100 (100)		214	100 (100)		402	100 (100)		256	100 (100)		313	100 (100)	
29.4	430	100 (100)		253	100 (100)		364	100 (100)		256	100 (100)		317	100 (100)	

[1)]The estimated number of insects tested in a total of 4 replications.
[2)]The estimated number of insects tested in a total of 3 replications.
[3)]The upper row is the average, and the lower row in parentheses is the weighted average from each replication.

Table 4. Corrected mortalities of each stage of P. kraunhiae fumigated with MI at 9.3 - 29.4g/m^3 for 2 hours at 15°C.

Dosage (g/m^3)	*P. kraunhiae*								
	egg			3-4th instar nymph			6th instar nymph		
	n[1)]	mean%[3)]	SD	n[2)]	mean%[3)]	SD	n[2)]	mean%[3)]	SD
0(control)	1278	4.7 (5.0)	3.6	530	3.9 (4.7)	4.7	391	1.2 (1.0)	1.2
9.3	1445	96.8 (96.7)	2.2	557	75.2 (72.9)	12.9	456	65.3 (66.2)	6.8
19.3	1354	100 (100)		643	99.2 (99.2)	0.6	510	96.9 (97.1)	1.5
29.4	1397	100 (100)		602	100 (100)		467	100 (100)	

[1)]The estimated number of insects tested in a total of 3 replications.
[2)]The estimated number of insects tested in a total of 4 replications.
[3)]The upper row is the average, and the lower row in parentheses is the weighted average from each replication.

The mortalities of the *P. kraunhiae* of the test are shown in Table 4. The 100% mortality rate of egg stage was obtained at the dosage of 19.3 g/m^3 while those of 3rd instar nymphs and adult females were obtained at the dosage of 29.4 g/m^3. Therefore, 3rd instar nymphs and adult females seem less susceptible than egg-stage. Naito *et al.* (2015) reported another pest species of mealybug and 100% mortality of *Planococcus citri* obtained at a dosage of 29.4 g/m^3 for 2 hours at 15°C.

The test results mentioned above indicate that the insect pests belonging to the same family have similar tendencies on their susceptibilities to MI fumigation as 100% mortalities of 2 species belonging to the same family, noctuidae, tortricidae and pseudococcidae resulted from the same dosage.

Naito *et al.* (2014, 2015) reported that 100% mortalities of *Myzus persicae,* Tetranychus *kanzawai, Tetranychus urticae, Flankiliniella intonsa* and *Thrips tabaci* resulted at the dosage of 9.3 g/m^3 or less and that of *Planococcus citri* resulted at the dosage of 29.4 g/m^3 with MI fumigation for 2 hours at 15°C. Naito *et al.* (2015) also proposed MI fumigation schedules against insect pest-infested fruit and vegetables. For example, the dosages in the schedules of 2 hours fumigation, estimated dosages for aphids, spider mites and thrips (group A) were 20-30 g/m^3 at 10°C or higher, 13-20 g/m^3 at 15°C or higher while estimated dosages for mealybug (group B) were at 62-95 g/m^3 at 10°C or higher, 41-64g/m^3 at 15°C or higher. Considering the susceptibilities of insect pests to MI, the same fumigation schedules against leafroller moths can be applied to group A and the schedules against noctuid moths can be applied to those of group B. However, the noctuid species is much less susceptible than the insect pests of group A and much more susceptible than that of group B. There are other species which infest fruit and vegetables, and susceptibility tests with MI fumigation should be conducted against those species. If there are other species whose susceptibilities are similar to noctuid species, proposing new MI fumigation schedules against an insect pest group, including noctuid species, between the schedules of group A and group B seems appropriate for reducing the quantity of fumigant usage and injury to fruit and vegetables.

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和 文 摘 要

ヨウ化メチルくん蒸に対する青果物害虫の感受性（英文）

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青果物に寄生する害虫に対するヨウ化メチルくん蒸条件を検討するため、ヤガ類２種（アワヨトウ、オオタバコガ）、ハマキガ類２種（チャノコカクモンハマキ、リンゴコカクモンハマキ）及びフジコナカイガラムシの各発育ステージを対象に感受性試験を実施した。ヨウ化メチル単位薬量（以下、「薬量」という。）9.3、19.3 及び 29.4g/m^3、15℃、2 時間の条件でくん蒸を行った結果、100% 殺虫された薬量は、ハマキガ類２種で 9.3g/m^3、ヤガ類２種の 5-6 齢幼虫で 19.3g/m^3、フジコナカイガラムシの 3 齢幼虫及び成虫で 29.4g/m3 であった。これまで青果物害虫に対して提案されていたヨウ化メチルくん蒸による消毒基準案において、ハマキガ類はアブラムシ類、ハダニ類及びアザミウマ類と、ヤガ類はコナカイガラムシ類と同じくん蒸条件を適用できると考えられた。しかし、ヤガ類はコナカイガラムシと比較して感受性が明らかに高いことから、ヤガ類と同等の感受性の害虫が存在することが確認されれば、アブラムシ類等とコナカイガラムシ類に対する消毒条件の中間の条件を提案することが適当と考えられた。

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