Res. Bull. Pl. Prot. Japan No.60: 9 ～ 13 (2024)

Short Communication

Study on Susceptibility of Apple-Infesting Oriental Fruit Moths(*Grapholita molesta*) to Methyl Iodide Fumigation

Eriko Horiba, Yoshiaki Ozeki[1)], Kazutaka Omura[2)], Yusuke Hoshikawa[3)], Toshiyuki Akagawa and Takashi Kawai

Research Division, Yokohama Plant Protection Station

Abstract: Susceptibility tests of apple-infesting oriental fruit moths (*Grapholita molesta*) were conducted with Methyl Iodide (MI) fumigation. In addition, the susceptibility of this species to MI was compared with that of peach fruit moth (*Carposina sasakii*) whose susceptibility to MI had already been reported. As a result of fumigation at doses of 1.6-20.1 g/m^3 for 2 hours at 15°C with 0.12 t/m^3 loading, 100% mortality among one-day-old eggs, three-day-old eggs, first instar larvae, third instar larvae and aged instar larvae of oriental fruit moths and aged instar larvae of peach fruit moths were obtained at doses of 1.6, 1.6, 10.8, 10.8, 20.1 and 20.1 g/m^3, respectively. The probit analysis data showed that LC99 (95% confidence interval) for aged instar larvae of peach fruit moths and oriental fruit moths were 30.75 (27.50-35.62) mg·h/l and 19.71 (17.91-22.32) mg·h/l, respectively with 95% confidence of intervals with no overlap between the species. The results of this test indicate that the oriental fruit moth seems more susceptible to MI than the peach fruit moth, and it is assumed the MI fumigation schedules reported by Soma *et al.* (2023) can be applied against the apple-infesting oriental fruit moth.

Key Words: *Grapholita molesta,* methyl iodide, fumigation, susceptibility, apple

Introduction

In order to export Japanese agricultural products, establishing phytosanitary measures toward sufficient quarantine security is necessary. There are countries that prohibit apples imported from Japan due to the presence of oriental fruit moths and peach fruit moths in Japan. Therefore, developing phytosanitary measures are necessary for exporting plant products which host those species to those countries. Regarding peach fruit moth infestations of apples, Soma *et al.* (2023) conducted mortality tests with MI fumigation and proposed fumigation standards. On the other hand, testing against the oriental fruit moth has not been conducted. Therefore, we conducted the susceptibility tests with MI fumigation at each life stage of the apple-infesting oriental fruit moth. In addition, aged instar larvae of the peach fruit moth, which were the least susceptible to MI among each stage of the species (Soma et al., 2023), were also used in the susceptibility comparisons with the oriental fruit moth.

Materials and Methods

1. Test insect

Both the oriental fruit moth (*Grapholita molesta*) and the peach fruit moth (*Carposina sasakii*) used in the test were obtained from the Apple Research Institute, Aomori Prefectural Industrial Technology Research Center (Kuroishi city, Aomori Prefecture) in May 2017, and then reared for successive generations at 23°C, 60%RH under a photoperiod of 16L：8D in the constant temperature and humidity room (Nikke panel system Co. Ltd.) at the research division, Yokohama Plant Protection Station. The adult stages were fed 10% sucrose water and water, and larval stages fed with immature apples (Fuji and Toki varieties).

2. Test fruits

Fuji variety, *Malus* × *domestica* Borkh. bag-cultivated samples were obtained from JA Tsugaru Hirosaki in December 2021 and June 2022. Infested fruit were prepared as follows:

(1) Oriental fruit moth

[1)] Tokyo Sub-station, Yokohama Plant Protection Station

[2)] Yokohama Plant Protection Station

[3)] Haneda Airport Sub-station, Yokohama Plant Protection Station

To obtain the eggs, 50 or more adult males and females were placed into cylindrical plastic containers (5 cm diameter × 4.5 cm height) , covered by paraffin papers (10 × 10 cm, 31 μm thickness) and stored overnight in the above-mentioned room. The papers were cut so that each piece contained 70 eggs and were each attached to the surface of an apple with glue or sticky tape. After that, each infested fruit was placed into a plastic container (12.9 cm diameter × 9.7 cm height).

(2) Peach fruit moth

To obtain the eggs, 150 or more adult males and females were placed in plastic containers (13.5 cm length × 8.5 cm width × 4.5 cm height), in which paraffin papers folded into pleated shapes were placed and stored overnight in the above-mentioned room. The papers were cut so that each piece contained 30 eggs and were each attached to the surface of an apple with glue or sticky tape. Infested fruit were placed in a mold pack on a plastic tray (length 36 × width 48 × height 4 cm).

To obtain diapause inducing larvae, infested fruit were stored in an incubator (MLR-352-PJ: PHC Co. Ltd.) set at 23°C, 50%RH and a photoperiod of 12L:12D until test insects reached the target developmental stages. One day-old-eggs, three-day-old eggs, first instar larvae (6-7 days after oviposition), third instar larvae (13-14 days after oviposition) and aged instar larvae (26-28 days after oviposition) of the oriental fruit moth and aged instar larvae (26-28 days after oviposition) of the peach fruit moth were provided for fumigation. Those stages were determined based on the results of the developmental test that measures the head width of larvae every number of days after oviposition (Narita et al., 1987, Roberts *et al.,* 1978). Infested fruit and filler fruit were moved to a fumigation room set at 14.5-14.8°C on the day before fumigation.

3. Fumigation treatment and evaluation of the mortality

Fumigation was performed using the same methods and 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 1.6-20.1g/m^3 for 2 hours at 15°C with 0.12 t/m^3 loading. The gas concentration was measured by gas chromatograph (GC-2014 with FID: Shimazu Co. Ltd.) at 15, 30, 60, 90 and 120 minutes after the dose application. The temperatures in the fumigation box were recorded by temperature recorder (Graphic logger CR-1016-A: Chino Co. Ltd.), and the fruit core temperatures were monitored by data logger with sensor probes (Ondotori RTR-52: T & D Co. Ltd.) at the start of fumigation and at the time of measuring the gas concentration. After fumigation, forced aeration (3 *l*/min) for 1 hour was applied. After aeration, treated test fruits were placed into mold pack on a plastic tray (length 36 × width 48 × height 4 cm) and the tray was put in a nylon gauze bag. The fumigated and untreated control fruit were kept at 25°C, 60% RH under a photoperiod of 12L:12D for 7 - 21 days until the mortality was evaluated. In the evaluation, the number of surviving larvae were counted similarly to the method described by Soma *et al.* (2023). The test was replicated three times.

Results and Discussion

The CT values (gas concentration and time product) and the fruit core temperatures during the fumigation are shown in Tables 1 and 2. The CT value was calculated similarly to the method described by Monro *et al.* (1969).

The calculation was made from after15min after dosing due to the MI not spreading uniformly for the first 15 minutes. CT values of aged instar larvae of the oriental fruit moths were lower than those of the peach fruit moths at every dosage. The surface of the fruit infested by larvae of the oriental fruit moths became rough due to the feces discharged by larvae, and this roughness may have contributed to the lower CT value. The fruit core temperatures ranged from 14.6 to 15.7°C.

Table 1. Average CT values fumigated with methyl iodide at 15°C for 2 hours with 0.12 t/m^3 loading.

Dosage (g/m^3)	Oriental fruit moth										Peach fruit moth	
	One day old egg		Three days old egg		First instar larva		Third insta larva		Aged instar larva		Aged instar larva	
	mg·h/l[1)]	SE	mg·h/l	SE	mg·h/l	SE	mg·h/l	SE	mg·h/l	SE	mg·h/l	SE
1.6	2.9	0.0	2.9	0.0	3.1	0.0	3.2	0.0	2.4	0.0	2.9	0.0
4.6	8.2	0.0	8.3	0.0	8.2	0.0	8.3	0.0	7.5	0.0	9.4	0.1
7.7	-		-		13.6	0.1	14.5	0.0	11.8	0.1	13.7	0.0
10.8	-		-		18.9	0.1	19.1	0.0	15.3	0.2	23.0	0.3
13.9	-		-		22.9	0.0	24.0	0.0	22.1	0.1	25.1	0.1
17	-		-		-		-		24.3	0.1	30.5	0.0
20.1	-		-		-		-		30.7	0.1	35.4	0.1

[1)]CT value (mg·h/l) = (7.5 C_{15} + 22.5 C_{30} + 30 C_{45} + 30 C_{60} + 15 C_{120})/60.
C_{15}, C_{30}, C_{45}, C_{60} and C_{120} mean the gas concentration at 15, 30, 45, 60 and 120 minutes after dose application, respectively.

Table 2. Average fruit core temperature of test fruit methyl iodide fumigation at 15°C for 2 hours with 0.12 t/m^3 loading.

Dosage (g/m^3)	Oriental fruit moth					Peach fruit moth
	One day old egg	Three days old egg	First instar larva	Third instar larva	Aged instar larva	Aged instar larva
	°C	°C	°C	°C	°C	°C
1.6	14.9	14.9	14.9	14.6	14.8	15.2
4.6	14.9	15.0	14.8	15.1	14.9	15.7
7.7	-	-	14.8	14.8	14.7	14.8
10.8	-	-	14.9	14.8	15.0	15.4
13.9	-	-	14.9	14.6	14.9	15.3
17	-	-	-	-	15.0	15.3
20.1	-	-	-	-	14.9	14.8

Mortalities at each stage at each dose are shown in Table 3. Regarding the oriental fruit moth, at all stages except for aged instar larva, 100% mortalities were confirmed at 10.8g/m^3 or less while 20.1g/m^3 for aged instar larvae resulted in 100% mortality. The test results suggested that aged instar larvae were the least susceptible among all life stages of the oriental fruit moth.

The aged instar larvae of the peach fruit moth also showed 100% mortality at 20.1g/m^3. Comparative estimates of the susceptibility of both species, lethal dose for 99% mortality (LD_{99}) and lethal CT value for 99% mortality (LC_{99}) were made by probit analysis after Box-Cox transformation of dose rates and CT value (Yamamura, 2022). Table 4 shows LD_{99} (95% confidence interval) for aged instar larvae of the peach fruit moth were 15.16(13.93-16.80) g/m^3 and that of the oriental fruit moth were 11.98(11.09-13.16) g/m^3 with no overlap in the 95% confidence intervals.

Table 3. Mortality of each stage of oriental fruit moth and aged instar larvae of peach fruit moth by methyl iodide fumigation at 15°C for 2 hours with 0.12 t/m^3 loading.

Dose rate (g/m^3)	Oriental fruit moth															Peach fruit moth		
	One day old egg			Three days old egg			First instar larva			Third instar larva			Aged instar larva			Aged instar larva		
	n[1)]	mean %[2)]	SD	n	mean %	SD	n	mean %	SD	n	mean %	SD	n	mean %	SD	n	mean %	SD
0(control)	330	0 (0)	0	318	0 (0)	0	628	0 (0)	0	1253	0 (0)	0	1410	0 (0)	0	1454	0 (0)	0
1.6	330	100 (100)	0	318	100 (100)	0	406	26.7 (31.5)	20.8	502	63.3 (62.4)	14.4	433	15.9 (15.9)	1.3	406	20.2 (22.7)	12.7
4.6	333	100 (100)	0	343	100 (100)	0	300	91.6 (92.0)	4.8	490	88.4 (88.4)	5.7	447	54.1 (53.5)	9.2	365	43.3 (45.8)	19.2
7.7	-	-		-	-		396	99.6 (99.7)	0.6	480	98.9 (99.0)	0.5	574	91.0 (91.1)	2.8	388	84.5 (85.8)	7.1
10.8	-	-		-	-		409	100 (100)	0	442	100 (100)	0	496	99.4 (99.4)	0.6	429	94.5 (94.4)	1.3
13.9	-	-		-	-		404	100 (100)	0	511	100 (100)	0	482	99.4 (99.4)	1.0	412	99.5 (99.5)	0.4
17	-	-		-	-		-	-		-	-		518	99.8 (99.8)	0.3	410	99.2 (99.3)	0.7
20.1	-	-		-	-		-	-		-	-		466	100 (100)	0	455	100 (100)	0

[1)]Number of tested incects = number of survived larvae in untreated control × number of fruit treated / number of fruit in untreated control
[2)]The upper row is the average and the lower row in parenthesis is the weighted average from each replication.

Table 4. Estimated LD_{50} and LD_{99} of the oriental fruit moth and the peach fruit moth fumigated with methyl iodide at 15 °C for 2 hours with 0.12 t/m^3 loading.

LD(g/m^3)[1)]	Oriental fruit moth			Peach fruit moth
	First instar larva	Third instar larva	Aged instar larva	Aged instar larva
LD_{50}	3.28	3.98	4.82	5.54
(95%CL)	(2.52 - 3.71)	(3.56 - 4.30)	(4.60 - 5.03)	(5.21 - 5.85)
LD_{99}	6.67	8.72	11.98	15.16
(95%CL)	(5.93 - 8.87)	(7.66 - 10.81)	(11.09 - 13.16)	(13.93 - 16.80)

[1)]LD values were calculated by probit analysis after Box-Cox transformation of dose rates (Yamamura, 2022).

Table 5. Estimated LC_{50} and LC_{99} the oriental fruit moth and the peach fruit moth fumigated with methyl iodide at 15 °C for 2 hours with 0.12t/m^3 loading.

LC(mg・h/l)[1)]	Oriental fruit moth			Peach fruit moth
	First instar larva	Third instar larva	Aged instar larva	Aged instar larva
LD_{50}	4.27	6.47	7.38	10.61
(95%CL)	(3.78 - 4.70)	(5.31 - 7.35)	(7.07 - 7.68)	(10.05 - 11.12)
LD_{99}	11.09	15.91	19.71	30.75
(95%CL)	(10.35 - 12.03)	(14.40 - 18.27)	(17.91 - 22.32)	(27.50 - 35.62)

[1)]LC values were calculated by probit analysis after Box-Cox transformation of CT value (Yamamura, 2022).

Table 5 shows that the LC_{99} (95% confidence interval) for aged instar larvae of the peach fruit moth was 30.75 (27.50-35.62) mg·h/l while that of the oriental fruit moth was 19.71 (17.91-22.32) mg·h/l with no overlap in the 95% confidence intervals. The results of this test also suggest that the oriental fruit moth is more susceptible to MI than the peach fruit moth.

From this test result, the assumption is that the MI fumigation schedules reported by Soma *et al.* (2023) can be applied against the oriental fruit moth-infesting apple in that the schedules were established to disinfest the peach fruit moth whose susceptibility to MI seems lower than that of the oriental fruit moth.

Acknowledgments

We are grateful to Mr. Yoichi Ishiguri of the Apple Research Institute, Aomori Prefectural Industrial Technology Research Center, for providing useful advice about preparing infested test fruits. We would like to thank Dr. kohji Yamamura of the National Agriculture and Food Research Organization for providing R command for probit analysis after Box-Cox transformation.

References

Narita, H (1987) Studies on the Ecology and Control of Peach fruit Moth *(Carposina niponensis Walsingam). Bulletin of the Akita fruit-tree experiment station.* **17**: 31-128.

Naito, H., H. Hayashi, H. Nishizaki and K. Yamada (2014) Effects of methyl iodide fumigation on mortality of carmine spider mite, *Tetranychus urticae* Koch, Kanzawa spider mite, *T. kanzawai* Kishida (Acari: Tetranychidae) and green peach aphid, *Myzus persicae* (Sulzer) (Hemiptera: Aphididae). *Res. Bull. Pl. Prot. Japan* **50**: 71-78.

Monro, H. A. U. (1969) Manual of fumigation for insect control. FAO Agricultural Studies: 25-30.

Roberts, W.P., J.R. Proctor and J.H.H. Phillips (1978) Effect of constant temperatures on the number of larval instars of the Oriental fruit moth, *Grapholita molesta* (Lepidptera: Tortricidae). *Can. Entmol.* **110**: 623-626.

Soma, Y., M. Takahashi, M. Machida, F. Kawakami, Y. Ishiguri, M. Kato, T. Kawai, K. Omura, M. Saito, Y. Ozeki, Y. Hoshikawa and K. Mishiro (2023) Quarantine Treatment by Methyl Iodide Fumigation to Apple Fruits Infested by Peach Fruit Moth Carposina Sasaki. *Res. Bull. Pl. Prot. Japan.* **59**: 1-12.

Yamamura, K (2022) Box-cox transformation is indispensable for estimating the required dose from dose-mortality curve. Abstracts of the 66th Annual Meeting of the Japanese Society of Applied Entomology and Zoology: **44** (In Japanese).

和　文　摘　要

ヨウ化メチルくん蒸によるりんご生果実に寄生したナシヒメシンクイの感受性試験（英文）

堀場　絵梨子・大関　喜朗[1)]・大村　和孝[2)]・星川　佑輔[3)]・赤川　敏幸・川合　崇之

横浜植物防疫所調査研究部

ナシヒメシンクイの侵入を警戒している国にりんごやなしの生果実を輸出するためには、植物検疫措置を講じる必要がある。そこで我々は、ヨウ化メチルくん蒸によるナシヒメシンクイの感受性比較試験を行った。また、同一の試験条件下での比較のため、すでに感受性比較試験が行われているモモシンクイガの老齢幼虫も供試し、同様に試験を実施した。薬量 1.6 ～ 20.1g/m^3、15℃、収容比 0.12t/m^3、2 時間の条件下でくん蒸を行った結果、ナシヒメシンクイの 1 日齢卵及び 3 日齢卵は薬量 1.6g/m^3、1 齢幼虫及び 3 齢幼虫は薬量 10.8g/m^3、両種の老齢幼虫は薬量 20.1g/m^3 で 100% 殺虫が確認された。CT 値でプロビット解析を行ったところ、両種の老齢幼虫の LC_{99} 値（95% 信頼区間）は、モモシンクイガが 30.75（27.50-35.62）mg·h/l、ナシヒメシンクイが 19.71（17.91-22.32）mg·h/l となり、95% 信頼区間は重ならなかった。この結果から、ナシヒメシンクイの方がモモシンクイガよりヨウ化メチルに対する感受性が高いと考えられ、相馬ら（2023）によって提案されたモモシンクイガの消毒基準がナシヒメシンクイにも適用できると考えられた。

[1)]横浜植物防疫所東京支所

[2)]横浜植物防疫所

[3)]横浜植物防疫所羽田空港支所