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Original Papers

Quarantine Treatment by Methyl Iodide Fumigation to Apple Fruit Infested by the Peach Fruit Moth, *Carposina sasakii*

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Abstract: Tests were conducted to establish methyl iodide quarantine fumigation standards against the peach fruit moth, *Carposina sasakii* infesting apple. The susceptibilities of the egg and larval stages were tested at 2-16 g/m³ for 2 hours at 15°C with 0.12 t/m³ loading. The results showed that the aged instar larvae were the most tolerant stage. Aged instar larvae infesting the 'Fuji' variety packed in export cartons were fumigated at 20 and 23 g/m³ for 2 hours at 15°C with 0.12 t/m³ loading. A total of 37,002 larvae was completely killed in 52 replications. The minimum CT value was 32.8 mg·h/l at 23 g/m³ in 25 replications and this value could be defined as the standard indicator in the CT product monitoring method. In the CT product comparison method, the average CT values of 7 varieties were 36.4-38.4mg·h/l at 20 g/m³. Since these values were close to that of 'Fuji', it was considered that the fumigation standard established for 'Fuji' was equally effective on the peach fruit moth having infested the other 7 varieties. No chemical injury was observed in 6 major varieties. The residual methyl iodide in the fruit was less than the quantification limit of 0.01ppm in 3 days after fumigation.

Key Words: quarantine treatment, *Carposina sasakii*, apples, methyl iodide, fumigation

Introduction

Pest control technology against the peach fruit moth infesting apple fruit was developed by combining the treatment of methyl bromide (MB) fumigation and low temperature in Japan in 1990 (Kawakami *et al.*, 1994). Those apples are currently exported to the United States and other countries. However, each country has been requested to reduce the amount of MB used for phytosanitary measures, given that it is an ozone-depleting substance and develop alternative technologies under the Montreal Protocol (IPPC, 2008). Ahead of the world trend, Japan developed technology of methyl iodide (MI) fumigation treatment against the chestnut weevil, *Curculio sikkimensis* infesting chestnut (Soma *et al.*, 2005). Japan had completely phased out MB when using a critical use exemption in 2014 (Kawakami, 2016) as well as performing various trials to introduce MI fumigation technology as quarantine treatment for import and export treatment measures for various items like timber, fruit and vegetable. The results showed that MI fumigation was far more effective in terms of mortality, with no difference in the impact of injury to fruit and vegetables between MI and MB fumigation (Soma *et al*., 2007, Naito *et al*., 2011). Accordingly, if MI fumigation technology were developed as a means of controlling the peach fruit moth, *Carposina sasakii* infesting apple fruit, apples could remain a major export fruit to many countries for many years to come.

To export apples overseas, quarantine requirements for the respective countries should be met. In recent years, countries are supposed to accept treatment technologies that have been newly developed in accordance with international standards. These treatment technologies are also reviewed by an expert committee of the International Plant Protection Convention (IPPC) to determine whether they meet the treatment criteria and introduced as part of the international standard for phytosanitary measures (FAO, 2007).

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This study was conducted to establish quarantine treatment technology by MI fumigation against the peach fruit moth infesting apple fruit. The present paper aims to report the results of the successful quarantine fumigation technology development trial.

Test I. Susceptibility of each stage of the peach fruit moth by methyl iodide fumigation

Materials and Methods

1. Test fruit

A Fuji variety, *Malus* × *domestica* Borkh. cultivated in bags, was harvested over the period 2017-2019 in Aomori and Iwate Prefectures, stored at 0-2°C in an exclusive warehouse and medium-class fruits weighing around 250-280 g were prepared.

2. Peach fruit moth

The eggs of the peach fruit moth are laid during the season between early June and mid-September. The 'Fuji' apple variety, meanwhile, ripens late and is usually harvested in Aomori prefecture in early to mid-November, during which periods 4th and 5th instar larvae are found in the fruit (Ishiguri and Toyoshima, 2006). Given the relatively long harvest season for major apple varieties between September and November, there is ample scope for eggs and larvae to infest the early- and medium-ripening varieties. Accordingly, egg and larval stages were chosen as the objects of the test and the aged instar larvae would be those developing under the diapause-inducing condition.

The peach fruit moth used in this test originated from aged instar larvae coming from fruit collected from an abandoned orchard in 1999 in Hirosaki, Aomori Prefecture. They were then reared for successive generations in immature apples under a 16L:8D photoperiod at a temperature of 23°C at the Apple Research Institute in the Aomori Prefectural Industrial Technology Research Center. The peach fruit moth derived from the above rearing colony was also reared in immature apples under the same rearing conditions, with each stage prepared for the test at the Iwate Prefectural Agriculture Research Center and the Research Division, Yokohama Plant Protection Station in addition to the Aomori Apple Research Institute.

3. Preparation of test fruit

Eggs and larvae for the susceptibility test comprised 3 parts during the growing period (Kawashima, 2008).

(1) Preparation of fruit infested with eggs

The stem bowl and calyx bowl of the top of the mature 'Fuji' fruit were filled with paraffin. The fruit were infested by allowing eggs to be laid on the borderline between fruit and paraffin via either method.

a. One fruit was placed in a plastic cylindrical container (11.2 cm in diameter and 15.1 cm high). For each fruit, 2 mature male and female species were placed together under a 16L:8D photoperiod at 23°C for overnight to lay eggs. Eggs were divided into the 3 categories: those 1-2 days old (freshly laid), 4-5 days old (4 days after the eggs were laid) and 7-8 days old (7 days after the eggs were laid).
b. Six fruits were placed in a plastic container (30 × 40 cm and 13 cm high) and 15 mated mature females were placed under a 16L:8D photoperiod at 25°C for overnight to lay eggs. The eggs were then divided into the 3 stages as categorized under 'a' above.

(2) Preparation of fruit infested with larvae

The mature 'Fuji' variety of fruit was used and the eggs were inoculated by either method.

a. Filter paper was scratched into the line by a pin and placed in a plastic cylindrical container (12 cm in diameter and 8 cm high). For each, 10 mature males and females were placed together to lay eggs under a 16L:8D photoperiod at 25°C. The filter paper was cut into sections, each containing 20-30 eggs, then placed on the stem bowl for inoculation. Eggs were reared under a 12L:12D photoperiod at 20°C to obtain diapause-inducing larvae. Larvae were then subdivided into the 3 stages of young instar larvae (3-5 days after infestation), middle instar larvae (11 days after infestation) and aged instar larvae (17-18 days after infestation) respectively.
b. Paraffin paper folding bellows were set and placed in a plastic container (30 × 21 cm, 6 cm high). Within each container, 15 mature males and females were placed together to lay eggs under a 16L:8D photoperiod at 25°C. Paraffin paper was cut into sections, each containing 30-40 eggs. It was placed on the stem bowl for inoculation and left for rearing under a 12L:12D photoperiod at 20°C to obtain diapause-inducing larvae. The larvae were divided into the 3 stages as in 'a' above.

4. Fumigation

MI fumigation conditions were set up at 2-6 g/m³ for eggs and 2-16 g/m³ for larvae for 2 hours at 15°C with 0.12 t/m³ loading.

A fumigation box with an inner volume of 30 liters (28 × 26 × 41cm ≒ 30 liters) and made of acryl was placed in the room at a constant temperature. It was installed with an air circulator (San Ace 80, Sanyo Electric Co.), dosing hole, pressure measurement hole, temperature measurement holes and air and exhaust ducts. To adjust the fruit temperature, fruit infested with eggs and larvae was placed in the fumigation box at 15 ± 0.5°C for overnight before fumigation. Liquid MI at more than 99% purity (Izutsuya Chemical Industry Co., Ltd.) was drawn into a syringe and drips allowed to fall onto the paper sheet (Kimwipe S-200, Nippon Paper Crecia Co., Ltd.). A gas circulation fan was operated during the fumigation.

The gas concentration was monitored 15, 30, 60 and 120 min respectively after dosing, by the same method as Akagawa *et al*. (1995) with gas chromatography (Flame ionization detector: FID GC-14B, Shimadzu Co.). The temperatures in the fumigation box and fruit were monitored by auto-temperature recording apparatus (KR2S00, Chino Co., Ltd.). The fumigation gas was then exhausted for 1 hour by gas-exhausting apparatus. The CT value (gas concentration and

time product) was calculated by the following formula: 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.

The CT value for the initial 15 min after dosing was excluded because this period was considered necessary for gas uniformity.

5. Method used to confirm effectiveness of mortality

After fumigation, the infested fruit were placed in a washing basket (33 × 44 × 13cm), covered in nylon gauze and stored in a rearing room under an adjusted temperature of 25°C. Fruit inoculated with eggs was stored until the end of hatch of the non-treatment and the number of eggs having hatched was counted with a microscope. Fruit infested with larvae was stored until the end of larval emergence of non-treatment and the number of survived larvae was counted by cutting those fruit open. The number of tested larvae was estimated from the number of survived larvae in treatment by the following formula: Number of tested larvae = number of survived larvae in non-treatment × number of fruit in treatment / number of fruit in non-treatment.

Results and Discussion

1. Susceptibility of eggs to methyl iodide fumigation

The temperatures in the fumigation box and fruit, remaining gas concentrations at the end of fumigation and CT values are shown in Table 1. The average temperature in the fumigation box was 15.2°C and the average temperature of the fruit was 14.8°C. The average remaining gas concentration for the application dose was 87.2%. The higher application dose reflected the tendency of a higher remaining gas concentration.

The mortality of peach fruit moth eggs is shown in Table 2. Eggs 1 or 2 days old (323) and those 4 or 5 days old (818) were completely killed at 2 g/m³. Only 2 eggs that were 7 to 8 days old (710) hatched at 2 g/m³. With these results in mind, eggs that were 7 to 8 days old egg showed a greater ability to tolerate MI fumigation than those up to 5 days old.

2. Susceptibility of larvae to methyl iodide fumigation

The temperatures in the fumigation box and fruit, the remaining gas concentrations at the end of fumigation and CT values are shown in Table 3. The average temperature in the fumigation box was 15.2°C and the average temperature of the fruit was 14.8°C. The average remaining gas concentration for the application dose was 94.8%. The higher application dose reflected a tendency toward a higher remaining gas concentration, like the test with eggs.

The mortality of peach fruit moth larvae is shown in Table 4. Young and middle instar larvae (323 and 331 respectively) were completely killed at 8 and 10 g/m³, respectively. The mortality of aged instar larvae (341) was 99.7% at 14 g/m³, but all aged instar larvae (377) were killed at a 16 g/m³.

Aged instar larvae were the most tolerant to MI fumigation among all larval stages. Accordingly, MI showed a much higher mortality at the egg stage of the peach fruit moth rather than the larval stage. This insecticidal property was completely opposite to that of MB on the same pest (Kawakami *et al.*, 1994). The insecticidal action of MI also had demonstrably similar tendencies against pests infesting timber and stored grain (Goto *et al.*, 2004, Naito *et al.*, 2003).

The Probit analysis of MI dose-response data showed that the dose required to achieve mortality of 99.99% among aged instar larvae was calculated at 16.1 g/m³ with an upper one-sided 95% confidence limit of 17.6 g/m³.

Table 1. Temperatures in the fumigation box and fruit, gas concentrations and CT values during methyl iodide fumigation of peach fruit moth eggs.[1)]

Fumigation Time	Loading	Dose	Replication	Temperature in fumigation box		Fruit temperature		Remaining gas concentration		Remaining gas[2)]	CT value	
(h)	(t/m³)	(g/m³)	(No.)	(°C)	(±SD)	(°C)	(±SD)	(mg/l)	(±SD)	(%)	(mg·h/l)	(±SD)
2	0.12	0	11	15.1	0.17	14.7	0.24	-	-	-	-	-
		2	9	15.2	0.06	14.8	0.12	1.7	0.16	85.0	3.3	0.32
		4	9	15.2	0.07	14.7	0.16	3.4	0.22	85.0	6.5	0.32
		6	9	15.2	0.04	14.8	0.18	5.5	0.21	91.7	10.5	0.38
	Average	-	-	15.2	0.10	14.8	0.19	-	-	87.2	-	-

[1)] Fumigated apple fruit (Fuji) infested with peach fruit moth eggs.

[2)] 100 × remaining gas concentration / dose.

Table 2. Mortality of peach fruit moth eggs infesting apple fruit by methyl iodide fumigation at 2-6 g/m³ for 2 hours at 15°C with 0.12 t/m³ loading.

Dose	1-2 days old egg						4-5 days old egg						7-8 days old egg					
	Replication	Fruit	Eggs tested	Eggs survived	Mortality	Corrected mortality*	Replication	Fruit	Eggs tested	Eggs survived	Mortality	Corrected mortality*	Replication	Fruit	Eggs tested	Eggs survived	Mortality	Corrected mortality*
(g/m³)	(No.)	(No.)	(No.)	(No.)	(%)	(%)	(No.)	(No.)	(No.)	(No.)	(%)	(%)	(No.)	(No.)	(No.)	(No.)	(%)	(%)
0	5	17	277	249	10.1	-	5	14	482	404	16.2	-	6	21	565	457	19.1	-
2	4	14	323	0	100	100	5	22	818	0	100	100	5	19	710	2	99.7	99.6
4	4	17	284	0	100	100	5	15	639	0	100	100	5	16	688	0	100	100
6	3	19	286	0	100	100	4	15	485	0	100	100	6	17	667	0	100	100

* 100 × (% Mortality in Treated - % Mortality in Control) / (100 - % Mortality in Control).

Table 3. Temperatures in the fumigation box and fruit, gas concentrations and CT values during methyl iodide fumigation of peach fruit moth larvae.[1)]

Fumigation time	Loading	Dose	Replication	Temperature in fumigation box		Fruit temperature		Remaining gas concentration		Remaining gas[2)]	CT value	
(h)	(t/m³)	(g/m³)	(No.)	(°C)	(±SD)	(°C)	(±SD)	(mg/l)	(±SD)	(%)	(mg·h/l)	(±SD)
2	0.12	0	17	15.1	0.11	14.8	0.14	-	-	-	-	-
		2	2	15.3	0.07	15.0	0.35	1.9	0.07	95.0	3.6	0.14
		4	5	15.2	0.07	14.9	0.20	3.4	0.05	85.0	6.4	0.08
		6	5	15.2	0.05	14.8	0.21	5.6	0.19	93.3	10.7	0.35
		8	14	15.2	0.09	14.9	0.22	7.6	0.39	95.0	14.5	0.79
		10	14	15.2	0.07	14.8	0.18	9.5	0.37	95.0	18.1	0.62
		12	10	15.2	0.08	14.8	0.15	11.7	0.57	97.5	22.2	0.97
		14	8	15.1	0.07	14.8	0.21	13.8	0.54	98.6	26.4	1.09
		16	4	15.2	0.05	14.8	0.06	15.9	0.52	99.4	30.5	1.01
	Average	-	-	15.2	0.11	14.8	0.14	-	-	94.8	-	-

[1)] Fumigated apple fruit (Fuji) infested with peach fruit moth larvae.

[2)] 100 × remaining gas concentration /dose.

Table 4. Mortality of peach fruit moth larvae infesting apple fruit by methyl iodide fumigation at 2-16 g/m³ for 2 hours at 15°C with 0.12 t/m³ loading.

Dose	Young instar larvae						Middle instar larvae						Aged instar larvae					
	Replication	fruit	Eggs inoculated	Larvae tested *	Larvae survived	Mortality	Replication	fruit	Eggs inoculated	Larvae tested *	Larvae survived	Mortality	Replication	fruit	Eggs inoculated	Larvae tested *	Larvae survived	Mortality
(g/m³)	(No.)	(No.)	(No.)	(No.)	(No.)	(%)	(No.)	(No.)	(No.)	(No.)	(No.)	(%)	(No.)	(No.)	(No.)	(No.)	(No.)	(%)
0	5	38	1,010	350	350	0	5	38	1,034	355	355	0	7	64	1,708	449	449	0
2	1	9	150	25	11	56.0	1	12	189	45	10	77.8	-	-	-	-	-	-
4	2	16	362	89	1	98.9	2	15	360	72	2	97.2	2	21	421	47	14	70.2
6	2	16	375	96	1	99.0	2	16	354	75	1	98.7	2	21	383	47	6	87.2
8	5	35	920	323	0	100	5	36	955	324	4	98.8	5	47	1,231	288	23	92.0
10	5	36	920	333	0	100	5	37	958	331	0	100	5	48	1,241	290	16	94.5
12	3	20	575	237	0	100	3	20	609	249	0	100	4	36	1,052	354	4	98.9
14	2	14	369	166	0	100	2	14	428	181	0	100	4	35	1,127	341	1	99.7
16	-	-	-	-	-	-	-	-	-	-	-	-	4	42	1,177	377	0	100

* Total number of larvae for all replications at each dose. The number of insects per replication was calculated by the following formula:
Number of tested larvae = number of survived larvae in non-treatment × number of fruit in treatment / number of fruit in non-treatment

Test II. Test on gas penetration to export cartons and sorption

Materials and Methods

1. Test fruit

'Fuji' apple fruit were harvested in 2019 in Aomori and Iwate Prefectures, stored at 0-2°C in an exclusive warehouse and medium-sized fruits were prepared, weighing around 250-280 g.

2. Packing for exporting fruit

The packing carton is 38 × 25 × 44 cm in size with 2 openings for grip measuring 2 × 4 cm. Ten kilograms of fruit was packed in 2 layers, with a mold tray, mesh sheet and paper sheet.

3. Fumigation

Fumigation treatment was conducted at 20 and 30 g/m³ for 2 hours at 15°C with 0.12 t/m³ loading. A fumigation box with an inner volume of 250 liters (100 × 50 × 50 cm) and made of PVC resin was placed in a room at constant temperature. It was installed with 2 air circulators (San Ace 80, Sanyo Electric Co.), a dosing hole, pressure measurement hole, temperature measurement sensors and an air exhausting hole. The packed and unpacked fruit were placed separately in the fumigation box.

Same as the susceptibility test, liquid MI at more than 99% purity was dripped by syringe onto a paper sheet (Kim wipe S-200, Nippon Paper Crecia Co., Ltd.) measuring 12 × 21.5 cm and evaporated by an air circulator. Gas concentrations were monitored at a single spot within the fumigation box and 2 spots in the carton at 15, 30, 60 and 120 min after dosing application. Thirty milliliters of gas was sampled with the syringe through a Teflon tube with an internal diameter of 2 mm. The gas concentration was determined by the same method as for the susceptibility test. Temperatures were monitored at 1 spot of the space in the fumigation box and 2 spots in the carton with the auto-temperature recording apparatus (KR2S00, Chino Co., Ltd.). After fumigation, gas was exhausted for 1 hour by gas-exhausting apparatus.

Results and Discussion

Gas concentrations and CT values during fumigation are shown in Table 5. At 15 min after the start of fumigation, the gas concentration in the packing carton was more than 90% of that of the space in the fumigation box. It showed that the gas had effectively penetrated and diffused into the carton. The average CT value in the carton was 77.5% compared to that of the space in the fumigation box. This was attributed to gas sorption into the carton, fruit and packing materials.

Based on the result of the susceptibility test, the dose required to achieve mortality of 99.99% for peach fruit moth aged larvae was calculated at 16.1 g/m³ with an upper one-sided 95% confidence limit of 17.6g/m³. And as fruit were packed by materials, as much as

Table 5. Gas concentration change and CT values in packed and unpacked fruit by methyl iodide fumigation at 20 and 30g/ m³ for 2 hours at 15°C with 0.12 t/m³ loading.

Replication No.	Dose (g/m³)	Packing condition [1]	Gas concentration measured point	Gas concentration (mg/l) 15min	30min	60min	120min	Remaining gas[2] (%)	CT value (mg·h/l)	CT value[3] (%)
1	20	Fruit	Chamber space	23.4	22.9	22.2	20.1	100.5	38.2	100
		Fruit in the carton	Chamber space	20.5	19.5	18.4	16.4	82.0	31.9	83.4
			Carton	19.2	18.6	17.5	15.9	79.5	30.5	79.7
			Carton/Chamber (%)	93.7	95.4	95.1	97.0	-	-	95.5
2	20	Fruit	Chamber space	24.1	23.7	23.0	21.5	107.5	39.9	100
		Fruit in the carton	Chamber space	20.3	19.2	17.9	16.1	80.5	31.2	78.2
			Carton	19.4	18.7	17.4	15.8	79.0	30.4	76.2
			Carton/Chamber (%)	95.6	97.4	97.2	98.1	-	-	97.4
3	30	Fruit	Chamber space	36.0	34.8	33.6	30.9	103.0	58.2	100
		Fruit in the carton	Chamber space	30.3	28.8	27.0	24.3	80.8	47.0	80.7
			Carton	27.8	27.3	25.8	23.1	77.0	44.6	76.7
			Carton/Chamber (%)	91.6	94.8	95.6	95.3	-	-	95.0
Average		Fruit	Chamber space	-	-	-	-	-	-	100
		Fruit in the carton	Chamber space	-	-	-	-	-	-	80.8
			Carton	-	-	-		-	-	77.5
			Carton/Chamber (%)	93.6	95.9	96.0	96.8	-	-	96.0

[1] The carton was exclusively for apples, measuring 38 × 25 × 44 cm and with holes for lifting. The box included 2 sheets of mold tray made of paper, mesh sheets and paper sheets, with apple fruit stacked in 2 layers.

[2] 100 × remaining gas concentration /dose.

[3] 100 × CT value for fruit in carton / CT value for fruit only.

22.5% gas sorption occurred and it emerged that a dose of 77.5% could be regarded as substantially effective.

Taking those views into account, the amount of 16.1g/m³ for $LD_{99.99}$ should be corrected to take the degree of gas sorption in packing materials into account. Correcting the $LD_{99.99}$ of 16.1 g/m³, estimated dose for $LD_{99.99}$ was 16.1/0.775 ≒ 20.8 g/m³ and correcting the upper limit of $LD_{99.99}$ of 17.6 g/m³, estimated dose for $LD_{99.99}$ was 17.6/0.775 ≒ 22.7 g/m³. From these perspectives, 20 g/m³ for expected complete mortality and 23 g/m³ for a more reliable quarantine fumigation standard were set up for the large-scale mortality test.

Test III. Large-scale mortality test of the peach fruit moth infesting apple fruit

Materials and Methods

1. Test fruit

'Fuji' apple fruit cultivated in bags were harvested in early November 2019-2022 in Aomori and Iwate Prefectures, stored at 0-2°C in an exclusive warehouse and prepared with medium or large-sized fruit weighing around 250-330 g.

2. Preparation for infested fruit

The origin of the peach fruit moth and preparation of the infested fruit at 3 institutions were the same as the susceptibility test.

Mature fruit of the 'Fuji' variety was used and the eggs were inoculated by either method.

a. Filter paper was scratched into the line by a pin and placed in a plastic cylindrical container (12 cm in diameter and 8 cm high). Within each, 10 mature males and females were placed together to lay eggs. Filter paper with eggs was cut into a section with 20-30 eggs was placed on the stem bowl for inoculation. They were reared under a 12L:12D photoperiod at 20°C for 17-18 days to obtain aged instar larvae.

b. Paraffin paper was set folding of bellows and placed in a plastic container (13.5 × 8.5 × 4.5 cm high or 30 × 21 × 6 cm high). Within each, 15 or 75 mature males and females were placed together to lay eggs. Paraffin paper with eggs was cut apart into sections each containing 30-40 eggs. They were placed on the part of stem bowl for inoculation and reared under a 12L:12D photoperiod at 20°C for 17-18 days.

3. Fumigation

Fumigation was conducted at the Japan Fumigation Technology Association using a fumigation box with a volume of 250 liters and at the Research Division, Yokohama Plant Protection Station using a fumigation box with a volume of 520 liters.

Fumigation was applied at 20 and 23 g/m³ for 2 hours at 15°C with 0.12 t/m³ loading. Fumigation was conducted with either method as follows:

(1) The same fumigation box (250 liters) as used in the test on gas penetration to export cartons and sorption was set in the room under constant temperature. Ten kilograms of infested fruit in the mold tray was packed in the carton and 3 cartons were placed in the fumigation box. Test fruit, including fumigated and untreated ones, were kept at 14.5 ± 0.5°C for the fruit temperature acclimation.

Liquid MI at more than 99% purity was taken into a syringe and dripped onto a paper sheet measuring 12 × 21.5 cm (Kimwipe S-200, Nippon Paper Crecia Co., Ltd.). Two gas circulation fans were operated during the fumigation. The gas concentrations in the 2 cartons

and within the fumigation box were monitored through a Teflon-lined tube with an inner diameter of 2 mm. A gas sample was taken by a syringe with volume of 30 ml at 15, 30, 60 and 120 min after dosing respectively. The gas concentration was determined by the same method as for the susceptibility test. The temperatures in the fruit of 2 cartons and within the fumigation box were monitored with auto-temperature recording apparatus (KR2S00, Chino Co., Ltd.). After fumigation, gas was exhausted for 1 hour by gas-exhausting apparatus.

(2) Fumigation was conducted in a 520 liter stainless steel fumigation box equipped with circulation and ventilation apparatus and ports for gas application and sampling and to measure the pressure and temperature. After cartons filled with 10.4 kg each of infested fruit and filler fruit were placed in a fumigation room overnight at a fixed temperature of 14.5°C, 6 cartons were placed in the fumigation box and 1 non-treated carton was kept at 14.5°C in the room.

Liquid MI with purity of more than 99.5% was applied (Fujifilm Wako Pure Chemical Co.). Liquid MI was sampled with the syringe and allowed to drip onto the crumpled sheet of paper measuring 22.5 × 21.5 cm (JK Wiper of 150-S, Nihon Paper Crecia Co., Ltd.) in the fumigation box. Five auxiliary small fans (Silky Wind S, Rhythm Co., Ltd.) were operated during fumigation. The fixed gas circulation apparatus was also operated at intervals of on for 30 sec and off for 4 min and 30 sec up until 10 min after the gas was applied and operated on for 30 sec and off for 9 min and 30 sec until the end of fumigation. A Teflon tube with 4 mm diameter was placed for gas sampling by a syringe from 1 point of the space in the fumigation box and from 2 points in the carton. Gas concentrations were monitored 15, 30, 60 and 120 min after gas application with a gas chromatograph (GC-2014 with FID, Shimadzu Co.). The temperatures in the fruit of 2 cartons and within the fumigation box were also monitored by a data logger with sensor probes (RTR-52, T&D Co., Ltd.). After fumigation, gas was exhausted for 1 hour by gas-exhausting apparatus.

4. Evaluation of mortality effectiveness

After fumigation, the test fruit in the mold tray were covered with a nylon gauze and placed in the carton. This fruit were kept in the rearing room at 25°C and 70% R.H. During the evaluation, survived larvae were counted by cutting open the fruit 7–14 days after fumigation. Moribund larvae were kept further to examine whether they were alive or dead.

The number of tested larvae was estimated from the number of survived larvae in non-treatment by the following formula: Number of tested larvae = number of survived larvae in non-treatment × number of fruit in treatment / number of fruit in non-treatment.

Results and Discussion

The temperatures in the fumigation box and fruit are shown in Table 6 and the gas concentrations in the fumigation box and carton are shown in Table 7. The average temperature in the fumigation box was 14.9 ± 0.32°C and peaked at 15.9°C. The temperature in the fruit was 14.7 ± 0.11°C, peaking at 14.9°C. The fruit temperature never exceeded a set temperature of 15°C.

Average and minimum CT values at 20 g/m³ were 33.2 and 30.7 mg·h/l in the fumigation box and 32.1 and 29.8 mg·h/l in the carton. The average and minimum CT values at 23 g/m³ were 36.1 and 33.6 mg·h/l in the fumigation box and 35.3 and 32.8 mg·h/l in the carton.

The mortality of peach fruit moth aged instar larvae by methyl iodide fumigation at 20 and 23 g/m³ for 2 hours at 15°C with 0.12 t/m³ loading is shown in Table 8.

During the tests at 20 g/m³, an estimated 16,306 aged instar larvae were killed in a total of 27 replications and 6 larvae were found in moribund in a total of 4 replications. At 23 g/m³, 20,696 insects were killed in a total of 25 replications and 3 larvae were found in moribund in a total of 2 replications. All 9 moribund larvae were found within the fruit and unable to move. They were reared with fruit on an ongoing basis, but all died within 1-7 days. As results of the large-

Table 6. Temperatures in the fumigation box and fruit in the large-scale mortality test.

Dose (g/m³)	Volume of fumigation box (*l*)	Replication (No.)	Temperature measured point		Temperature (°C) 15min	30min	60min	120min	Mean (°C)	(±SD)
20	250	27	Chamber space	Mean	14.9	14.8	14.8	14.9	14.9	0.38
				Max	15.5	15.4	15.5	15.5	-	-
			Fruit	Mean	14.7	14.7	14.7	14.7	14.7	0.12
				Max	14.9	14.9	14.9	14.9	-	-
23	250	15	Chamber space	Mean	14.8	14.8	14.8	14.8	14.8	0.26
				Max	15.9	15.7	15.5	15.3	-	-
			Fruit	Mean	14.6	14.6	14.6	14.6	14.6	0.10
				Max	14.8	14.8	14.8	14.8	-	-
	520	10	Chamber space	Mean	14.8	14.9	15.0	15.1	15.0	0.25
				Max	15.1	15.3	15.3	15.4	-	-
			Fruit	Mean	14.7	14.8	14.8	14.8	14.8	0.10
				Max	14.9	14.9	14.9	14.9	-	-
Total		52	Chamber space	Mean	14.8	14.8	14.9	14.9	14.9	0.32
			Fruit	Mean	14.7	14.7	14.7	14.7	14.7	0.11

Table 7. Gas concentrations and CT values by methyl iodide fumigation at 20 and 23g/m³ for 2 hours at 15°C with 0.12 t/m³ loading in the large-scale mortality test.

Dose (g/m³)	Volume of fumigation box (*l*)	Replication (No.)	Average number of fruit (No.)	Average fruit weight (kg)	Gas concentration measured point		Gas concentration (mg/l) 15min	30min	60min	120min	CT value (mg·h/l)
20	250	27	112	30.2	Fumigation space	Mean	21.6	20.6	18.9	17.2	33.2
						Min.	19.7	18.8	17.5	16.1	30.7
					Carton	Mean	20.5	19.7	18.4	16.7	32.1
						Min.	18.9	18.3	17.0	15.6	29.8
23	250	15	116	30.1	Fumigation space	Mean	23.7	22.7	21.1	19.1	36.9
						Min.	21.8	21.1	19.3	17.3	33.7
					Carton	Mean	22.6	21.9	20.5	18.7	35.8
						Min.	20.8	20.3	18.8	16.9	32.8
	520	10	215	62.3	Fumigation space	Mean	22.4	21.4	20.0	18.3	35.0
						Min.	21.8	20.7	19.3	16.7	33.6
					Carton	Mean	21.9	21.1	19.8	18.3	34.7
						Min.	21.0	20.3	19.0	17.3	33.1
	Average and Min.				Fumigation space	Mean	23.2	22.2	20.7	18.8	36.1
						Min.	21.8	20.7	19.3	16.7	33.6
					Carton	Mean	22.3	21.6	20.2	18.5	35.3
						Min.	20.8	20.3	18.8	16.9	32.8

Table 8. Mortality of peach fruit moth aged instar larvae by methyl iodide fumigation at 20 and 23 g/m³ for 2 hours at 15°C with 0.12 t/m³ loading in the large-scale mortality test.

Dose (g/m³)	Volume of fumigation box (*l*)	Replication (No.)	Treatment	Fruit (No.)	Eggs inoculated (No.)	Larvae survived (No.)	Larvae tested[1] (No.)	Mortality (%)
20	250	27	Non-treated	697	18,773	4,402	4,402	-
			Treated	2,550	69,948	0	16,306	100[2]
23	250	15	Non-treated	367	9,854	2,344	2,344	-
			Treated	1,525	40,224	0	9,825	100
	520	10	Non-treated	126	3,787	1,420	1,420	-
			Treated	593	17,795	0	10,871	100[3]
Total	-	52	Non-treated	1,190	32,414	8,166	8,166	-
			Treated	4,668	127,967	0	37,002	100

[1] Total number of larvae for all replications at each dose. The number of insects per replication was calculated by the following formula: Number of tested larvae = number of survived larvae in non-treatment × number of fruit in treatment / number of fruit in non-treatment

[2] All 6 moribund larvae found in the total of 4 replications died within 1-7 days.

[3] All 3 moribund larvae found in the total of 2 replications died within 2-6 days.

scale mortality test, it was confirmed that a total of 37,002 aged instar larvae of the peach fruit moth were completely killed in 52 replications.

With the test results in mind, a higher dose of 23g/m³ should be applied as a more reliable quarantine fumigation standard. Accordingly, the MI fumigation standard to ensure the complete mortality of the peach fruit moth was at 23 g/m³ for 2 hours at 15°C or above with 0.12 t/m³ or less loading.

Test IV. Survey on the CT value difference among apple varieties to methyl iodide fumigation

Materials and Methods

1. Survey by CT product comparison method and CT product monitoring method

The CT product comparison method is defined as follows:

The 'Fuji' used in the large-scale mortality test was set as a standard variety, while fumigations for other varieties were conducted under equivalent conditions as for 'Fuji' and CT values of other varieties were compared with 'Fuji'.

If no significant difference was observed between 'Fuji' and other varieties, the mortality effectiveness is considered the same among them.

The CT product monitoring method is defined as follows:

Fumigations are conducted under the same conditions as for 'Fuji'. If all CT values of other varieties are found to be the same or greater than the minimum CT value of 'Fuji' obtained in the large-scale mortality test, the mortality effectiveness for other varieties is guaranteed to be equivalent to that of 'Fuji'.

2. Test fruit

Eight varieties of 'Sekaiichi', 'Mutsu', 'Shinano Gold', 'Orin', 'Shinano sweet', 'Jonagold', 'Kinsei' and 'Fuji' apples were harvest-

ed over the period 2019-2022 in Aomori and Iwate Prefectures and prepared with freshly harvested fruit and storage fruit for 30 and 60 days at 0-2°C with the weight of 314-485g.

3. Fumigation

In the CT product comparison method, fumigation was conducted using a 30 liter box and the same methods and devices as the susceptibility test. The fumigation was conducted at 20 g/m³, which was expected to achieve complete mortality of the peach fruit moth, for 2 hours at 15°C with 0.12 t/m³ loading. This test was conducted in 12-33 replications for 2 to 3 years.

In the CT product monitoring method, the same criteria were used for the large-scale mortality test.

Results and Discussion

1. Mortality effectiveness among apple varieties with the CT product comparison method

CT values for all varieties from the result of the fumigation at 20 g/m³ are shown in Table 9.

Average CT values of 'Sekaiichi', 'Mutsu', 'Shinano Gold', 'Orin', 'Shinano sweet', 'Jonagold' and 'Kinsei' varieties obtained in 12-33 replicated trials for 2 to 3 years were 38.0, 38.4, 37.8, 38.2, 38.3, 37.3 and 36.4 mg·h/l, respectively. The average CT value of 'Fuji' was 37.5 mg·h/l with the standard deviation of ±2.03 (35.47-39.53 mg·h/l). The average CT values of the other 7 varieties were within the range of 'Fuji'. Misumi *et al*. (2001) reported that a variation of 4.3% was observed on 'Fuji' during a 3 year survey of the relationship between the harvest year and the MB CT value. Misumi *et al*. (2001) also reported that if the variation in the CT value of apples fumigated under the same fumigation schedules was less than 9.4%, the mortality effectiveness between varieties would be unaffected. Although the MI fumigant used in this test differed from MB, the maximum variation in 7 varieties against 'Fuji' was 2.9%, which was smaller than the above 4.3 and 9.4%. Therefore, it is considered that the fumigation standard suggested in the large-scale mortality test has mortality effectiveness to these 7 varieties infested with the peach fruit moth as well as 'Fuji'.

2. Confirmation of the standard CT value used for the monitoring method

The minimum CT value of 'Fuji' from Table 7 of the large-scale mortality test was 32.8 mg·h/l at 23 g/m³. Accordingly, the minimum CT value of 32.8 mg·h/l from the standard variety of 'Fuji' could be applied to any varieties as the standard indicator for mortality effectiveness in the practical fumigation.

Test V. Fumigation injury test on apple fruit

Materials and Methods

1. Test fruit

Eight varieties of 'Sekaiichi', 'Mutsu', 'Shinano Gold', 'Orin', 'Shinano sweet', 'Jonagold', 'Kinsei' and 'Fuji' were harvested in 2019-2022 in Aomori and Iwate Prefectures and prepared with freshly harvested fruit and storage fruit for 30 days at 0-2°C and 60 and 90 days at 0°C.

2. Fumigation

Fruit of each variety were separately fumigated at 20 g/m³ for 2 hours at 15 °C with 0.12 t/m³ loading in the same methods as the susceptibility test. After fumigation, gas was exhausted for 1 hour by gas-exhausting apparatus.

3. Evaluation of the injury test

After fumigation, test fruit were stored under different temperatures and for differing periods. They were initially stored at 15°C for 5 days or stored under different timings and temperatures for 3 days at 15°C→ 7 days at 5°C → 3 days at 15°C. Checks for any change in fruit skin color, fruit flesh decay and taste were conducted. The degree of chemical injury was assessed with 5 levels, namely – No injury, ± Not apparent, + Slight, ++ Moderate and +++ Severe.

Results and Discussion

The occurrence of chemical injury in 8 varieties is shown in Table 10. No change in taste was found in any varieties. For fruit just after harvest, no injury was observed in the 'Mutsu', 'Shinano Sweet', 'Jo-

Table 9. Average CT values in apple varieties by methyl iodide fumigation at 20 g/m³ for 2 hours at 15°C with 0.12 t/m³ loading.

Apple variety	Replication	Average fruit weight		Average CT value		Variation
	(No.)	(g)	(±SD)	(mg・h/l)	(±SD)	(%)*
Sekaiichi	12	485	38.4	38.0	1.26	-1.3
Mutsu	12	320	48.2	38.4	0.75	-2.4
Shinano Gold	21	356	78.9	37.8	2.31	-0.8
Orin	33	314	28.1	38.2	1.88	-1.9
Shinano Sweet	12	348	22.2	38.3	0.90	-2.1
Jonagold	24	331	22.5	37.3	1.31	0.5
Kinsei	15	343	27.7	36.4	2.02	2.9
Fuji	39	325	66.1	37.5	2.03	-

*Variation (%)= 100［1-average CT value of each apple variety / Fuji CT value(37.5)］

Table 10. Occurrence of chemical injury in apple varieties by methyl iodide fumigation at 20 g/m³ for 2 hours at 15°C with 0.12 t/m³ loading.[1)]

Apple variety	Storage period (Day)	Storage temperature (°C)	Replication (No.)	Surveying fruit (No.)	Degree of injury[2)]		
					Skin	Flesh	Taste
Sekaiichi	0	-	3	45	+	-	-
	30	0	3	45	-	-	-
Mutsu	0	-	3	69	-	-	-
	30	0	3	69	-	-	-
Shinano Gold	0	-	9	72	+++	-	-
	30	2	9	87	+++	-	-
	30	0	6	63	++	-	-
	60	0	4	42	+	-	-
Orin	0	-	18	204	-, +	-	-
	30	2	6	99	-	-	-
	30	0	10	186	-, ++	-	-
	60	0	3	21	-	-	-
	90	0	3	33	-	-	-
Shinano Sweet	0	-	6	74	-	-	-
	30	0	3	63	-	-	-
Jonagold	0	-	6	81	-	-	-
	30	0	6	96	-	-	-
	60	0	6	54	-	-	-
Kinsei	0	-	7	76	-	-	-
	30	0	3	30	-	-	-
	60	0	3	15	-	-	-
Fuji	0	-	18	166	-	+	-
	30	2	9	97	-	±	-
	30	0	7	103	-	-	-
	60	0	3	22	-	-	-

[1)] After fumigation, fruit were stored at either 15°C for 5 days or temperature variations of 15°C for 3 days, 15°C for 7 days and 15°C for 3 days respectively.

[2)] Marks for the degree of injury were expressed with five levels - : No injury ± : Not apparent + : Slight ++ : Moderate +++ : Severe.

nagold' and 'Kinsei' varieties. Symptoms of light brown discoloration spots were observed on the skin of 'Sekaiichi' and symptoms of watercore breakdown in the flesh of 'Fuji'. Equivalent symptoms were also reported in 'Fuji' fumigated with MB (Soma *et al*., 1994). However, the symptoms were more minor than those occurring due to MB fumigation. The symptoms affecting 'Fuji' and 'Sekaiichi' could be avoided by storing the fruit for more than 30 days at 0°C prior to fumigation. In 'Orin', chemical injury on skin was observed depending on the production area. Further tests may be necessary in a few varieties as the occurrence of chemical injury was seen to vary depending on the production area and year.

Test VI. Methyl iodide residue in apple fruit

Materials and Methods

1. Test fruit

'Fuji' apples of medium or large size, weighing around 250-480 g, harvested in 2021 in Aomori and Iwate Prefectures and stored for 30 days at 0°C were prepared.

2. Fumigation and fruit processing for residue analysis

The test fruit were fumigated at 23 g/m³ for 2 hours at 15°C with 0.12 t/m³ loading by the same methods as for the large-scale mortality test. After fumigation, gas was exhausted for 1 hour by exhausting apparatus. The fruit packed in the carton was delivered on the same day to the Japan Food Research Laboratories officially recognized as an analytical organization. The air temperatures during the 3 deliveries ranged between 5.1 and 9.5°C.

3. Residue analysis of methyl iodide

A portion of the fruit without the peduncle was sampled for residual analysis in accordance with the Codex general standard. Residual analysis of MI followed the officially approved method (Food Safety Division, Pharmaceutical and Food Safety Bureau, Ministry of Health, Labor and Welfare of Japan, 2005). The fruit analytical sample was kept under sealed condition, residual MI was collected into the hexane layer and the residual amount was measured by a gas chromatograph with a μ-Electron capture detector attached: μ-ECD (6890, Agilent Technologies Inc). Residue analysis was conducted 3 times, at 1, 3 and 7 days after fumigation with a quantification limit of 0.01 ppm.

Results and Discussion

1. Gas concentration and CT value

The gas concentrations in the carton and CT values are shown in Table 11. They were all found within the range of average concentration and CT value observed in the large-scale mortality test.

2. Methyl iodide residual amount

The result of the residual analysis of MI for the 'Fuji' variety is

Table 11. Gas concentrations in the carton and CT values in fumigation box and carton by methyl iodide fumigation at 23 g/m³ for 2 hours at 15°C with 0.12 t/m³ loading.

Apple variety	Production area	Fruit (No.)	Fruit weight (kg)	Gas concentration measured point	Average gas concentration (mg/l)				CT value (mg･h/l)
					15min	30min	60min	120min	
Fuji	Aomori Prefecture	78	30.0	Fumigation box*	22.8	21.6	20.1	18.4	35.2
				Carton	22.0	21.1	19.9	18.0	34.6
	Iwate Prefecture	96	30.0	Fumigation box*	22.5	21.4	20.0	18.2	34.9
				Carton	21.1	20.3	19.3	17.7	33.6

* Inner volume of 250 liter.

Table 12. Methyl iodide residue amounts in apples fumigated at a dose of 23 g/m³ for 2 hours at 15°C with 0.12 t/m³ loading.

Apple variety	Production area	Analysis	Analytical residual amount ppm (mg/kg)			
			Control	1 day after fumigation	3 day after fumigation	7 day after fumigation
Fuji	Aomori Prefecture	Methyl iodide	<0.01	0.34	<0.01	<0.01
	Iwate Prefecture	Methyl iodide	<0.01	0.21	<0.01	<0.01

Quantification limit was of 0.01 ppm.

shown in Table 12. Residues were determined at 0.21 and 0.34 ppm 1 day after fumigation. However, the residue was less than the quantification limit of 0.01 ppm in 3 days after fumigation.

Establishment of methyl iodide quarantine fumigation standards for export apple fruit infested by the peach fruit moth

In the large-scale mortality test against the peach fruit moth infesting apple fruit variety 'Fuji' with MI fumigation, 37,002 of aged larvae, at the most tolerant stage, were completely killed at 20 and 23g/m³ for 2 hours at 15°C with 0.12t/m³ loading. Considering an effective level for mortality, 23g/m³ for 2 hours at 15°C or above with 0.12t/ m³ or less for MI fumigation standard is appropriate. Concerning mortality effectiveness between 'Fuji' and 7 other varieties fumigated under the same conditions, differences in varieties would not affect the mortality effectiveness because the average CT values were close among them. Accordingly, the established fumigation standard would be applicable to many apple varieties. In addition, the CT value in the large-scale mortality test can be used to indicate the effectiveness of the mortality. To ensure the efficacy of the fumigation standard to every variety, 32.8mg·h/l, which was obtained from the large-scale mortality test, may be used as minimum required CT value in commercial fumigations.

Apple fruit for export capable of meeting these fumigation standards will be able to dispel concerns of importing countries about the risk of peach fruit moth invasion.

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和 文 摘 要

ヨウ化メチルくん蒸によるりんご果実に寄生したモモシンクイガの検疫処理

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りんご果実に寄生したモモシンクイガに対するヨウ化メチルくん蒸検疫消毒基準を確立するため各試験を実施した。卵及び幼虫の感受性試験は、2-16 g/㎥、15 ℃、2 時間、収容比 0.12 t/m^3 の条件で調査した。その結果、最も耐性の態は老齢幼虫であった。モモシンクイガ老齢幼虫が寄生した品種“ふじ”をカートンボックスに収容し、20 及び 23 g/m^3、15 ℃、2 時間、収容比 0.12t/m^3 の条件でくん蒸した。52 回の反復くん蒸の結果、合計 37,002 頭の老齢幼虫は全て殺虫された。23 g/m^3 における最低 CT 値は、32.8 mg·h/l（25 反復）であり、この値は CT モニタリング法における指標にできるものと考える。CT 比較法では、20 g/m^3 で 7 品種の平均 CT 値が 36.4～38.4 mg·h/l であった。これらの値は“ふじ”の値に近いことから、“ふじ”で設定されたくん蒸基準は、他の 7 品種に寄生しているモモシンクイガに対しても同等の効果があると考える。主要 6 品種にくん蒸障害は認められなかった。果実中のヨウ化メチル残留量は、くん蒸 3 日後で定量限界の 0.01 ppm 未満であった。

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[5)] 国立研究開発法人農業・食品産業技術総合研究機構植物防疫研究部門果樹茶病害虫防除研究領域