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Susceptibility of Four Stored Grain Insects to Methyl Iodide

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Abstract: Susceptibility for developmental stages of *Sitophilus granarius* L., *Sitophilus zeamais* MOTSCHULSKY, *Tribolium confusum* JAQUELIN DU VAL, and *Plodia interpunctella* HUBNER to methyl iodide (MI) was investigated. Fumigation tests were conducted with less than 0.004 (w/v) loading for 6 hours at 15℃. The egg was the most susceptible among the stages of each species. The least susceptible stages for each of *S. granarius, S. zeamais, T. confusum, and P. interpunctella*, at 50% mortality, were the adult, the adult, the pupa, and the larva, and their LD_{50}s were 3.4 g/m^3, 2.5 g/m^3, 4.8 g/m^3, and 2.8 g/m^3, respectively. Among all four species, the least susceptible stage, at 50% mortality, was the pupa of *T. confusum*, and same result was obtained at 95% mortality. These results indicate that the most tolerant stage, among the stages of the four species, to MI is the pupa of *T. confusum*, and that a fumigation standard obtaining complete mortality for the stage would be applicable for many stored grain insects.
Key words: methyl iodide, stored grain insect, susceptibility

Introduction

To develop an alternative treatment to fumigation with methyl bromide (MB), which is an ozone-depleting substance, tests for grains using carbon dioxide and phosphine were conducted in Japan (SOMA *et al*., 1995; KISHINO *et al*., 1996; GOTO *et al*., 1996). Susceptibility tests with Methyl iodide (MI) were conducted by MUTHU and SRINATH (1974) on adults of stored products insects, and by ZETTLER *et al*. (1999) on diapausing larva of *Cydia pomonella*. However, few tests have compared susceptibility to MI among the developmental stages of stored grain insect. Therefore, we investigated the susceptibility to MI, regarding each stage of four species: *Sitophilus granarius* L., *Sitophilus zeamais* MOTSCHULSKY, *Tribolium confusum* JAQUELIN DU VAL, and *Plodia interpunctella* HUBNER, in fumigation with less than 0.004 (w/v) loading for 6 hours at 15℃.

Materials and Methods

Test insects

Test insects, except for *S. granarius*, have been reared in the Research Division of Yokohama Plant Protection Station for more than ten years. *S. granarius* was introduced from the United Kingdom in 2000 (Import permit No.12Y336).

They were reared in a breeding room at 25℃ and 60%RH, with a 16L : 8D photoperiod, and were prepared for the tests as follows.

S. granarius

A culture was reared using wheat grain. Adults were allowed to oviposite on wheat grain for 2–3 days. Eggs (1–4 days old), larvae (20–23 days after oviposition start), pupae (31–35 days after oviposition start), and adults (45–48 days after oviposition start) were prepared. Twelve or 15 grams of the grain, infested with eggs, larvae, or pupae, was placed into a plastic case (8 cm in diameter, 4 cm in height). Adults, with 10 g of wheat grain, were placed into a film case (3 cm in diameter, 5 cm in height) with holes (1.5 cm in diameter) covered with meshes (23×23 squares per cm^2) at the cap and at the bottom. These cases, including test insects, were set in the fumigation box.

S. zeamais

A culture was reared using wheat grain. Adults were allowed to oviposite on the grain for 2–3 days. Eggs (1–4 days old), larvae (20–23 days after oviposition start), pupae (30 days after oviposition start), and adults (45–48 days after oviposition start) were prepared. The preparation of fumigation was conducted as for *S. granarius.*

T. confusum

A culture was reared using wheat flour of whole grain supplemented with dry yeast. Adults were allowed to oviposite on the feed for 2–3 days. Eggs (1–4 days old), larvae (25–27 days after oviposition start), pupae (39–43 days after oviposition start), and adults (60–62 days after oviposition start) were prepared. These stages, with 10 or 15 g of feed, were placed into respective plastic cases mentioned above, and they were set in the fumigation box.

P. interpunctella

A culture was reared using feed consisting of a mixture of 88 g of wheat bran, 12 g of glycerin, and 5 g of dry yeast. Adults were placed into an oviposition jar (13 cm in diameter, 28 cm in height) with mesh (7×7 squares per cm^2), and allowed to oviposite for 1 day. Eggs laid within the jar fell through the mesh into a cantainer, and then they were kept for the test of egg stage, or inoculated on the feed for that of other stages. Eggs (0–1 day old), larvae (21–22 days after oviposition start), pupae (26–29 days after oviposition start), and adults (about 2 days after emergence) were prepared. Corrugated cardboard pieces were placed into the breeding case for the pupal stage, and larvae pupated in the holes in the pieces. Pupae in the pieces of corrugated cardboard, and larvae and adults with 15 g of feed, were put in respective plastic cases (11cm in diameter, 6 cm in height), each of which has a hole (6 cm in diameter) covered with a mesh (23×23 squares per cm^2) in the lid. Eggs were put in a petri dish. These containers, including test insects, were set in the fumigation box.

Procedure of fumigation

Fumigation was conducted in a 29.5-litter fiber-glass fumigation box (26 cm×28 cm×41 cm). The fumigation box was equipped with a circulation fan, a ventilation apparatus, ports for gas application and sampling, a manometer, and temperature probes. Gas concentrations were measured with a gas chromatograph (FID; GC14B, SHIMADZU) at time intervals of 0.5, 1, 2, 3, and 6 hours after the start of fumigation. Temperatures in the box were monitored with a multi-channel automatic temperature recorder (Hybrid Recorder: AH, Chino) during fumigation. Fumigation was followed by one hour of exhausting using a ventilation apparatus.

Determination of mortality

After fumigation, insects in the containers were removed from the fumigation box and then stored in rearing condition.

S. granarius and *S. zeamais*

The adult stage was examined on the day following fumigation. Percentages of mortality for the adult stage were corrected based on survivors in untreated control. The number of adult emergence for each of pupal, larval, and egg stages was counted weekly until emergence ended; percentages of mortality for the three stages were estimated from the total numbers of adult emergence in each of untreated control and the treated plot.

T. confusum

The adult stage was examined on the 7th day after fumigation. The other stages were held until adult emergence ended. As for the pupal and larval stages, the numbers of emerged adults and dead pupae or larvae were counted. Percentages of mortality for the adult, pupal, and larval stages were corrected based on survivors in untreated control. For the egg stage, percentages of mortality were estimated from the numbers of adult emergence in untreated control and in the treated plot.

P. interpunctella

The adult stage was examined on the day following fumigation. The pupal and larval stages were held until adult emergence ended, and then the numbers of emerged adults and dead pupae or larvae were counted. Eggs on the petri dish were examined under a stereoscopic microscope on the 7th day following fumigation; the mortality was determined by counting the number of hatched and unhatched eggs. Percentages of mortality for all stages were corrected based on survivors in untreated control.

Probit analysis was done using the program of POLO-PC (LeOra software, 1987).

Results and Discussion

Temperature and residual gas

Temperatures in the fumigation box ranged from 13.5 to 15.8℃, and the average (±SD) was 15.0 (±0.4)℃. Ratios of residual gas (100×gas concentration at the end of fumigation/applied dose) of each dose was 98.5% (±7.8)% on average.

Susceptibility of test insects

S. granarius

Table 1 shows the mortality percentages of each stage, and the most susceptible stage was the egg. Table 5 shows that susceptibility at LD_{50} for the other stages decreased in the order of larva, pupa, and adult, and the LD_{50} for the adult was 3.4 g/m^3. The highest value of LD_{95} was 5.0 g/m^3, for the adult.

S. zeamais

Table 2 shows the mortality percentages of each stage, and the most susceptible stage was the egg. Table 5 shows that susceptibility at LD_{50} for the other stages decreased in the order of larva, pupa, and adult, and the LD_{50} for the adult was 2.5 g/m^3. The highest value of LD_{95} was 4.1 g/m^3, for the pupa. No significant difference was observed in 95% confidennce limit (C.L.) of LD_{95} among the three stages.

Table 1. Effects of MI on mortality of each stage of *S. granarius* in fumigation for 6 hours at 15℃.

Dose (g/m³)	Egg		Larva		Pupa		Adult	
	*n**	Mortality (%±SD)	*n**	Mortality (%±SD)	*n**	Mortality (%±SD)	*n**	Mortality (%±SD)
0.5	423	45.7±15.7	739	7.0± 6.1	—	—	—	—
1.0	423	99.4± 0.6	739	13.1±12.1	—	—	—	—
1.5	423	100	739	37.9±15.0	—	—	—	—
2.0	423	100	739	77.0± 9.8	1,004	15.9±8.5	349	2.4±1.4
2.5	423	100	739	91.3± 3.8	—	—	—	—
3.0	423	100	739	97.4± 1.7	1,004	69.6±4.6	305	25.5±5.0
4.0	—	—	—	—	1,004	97.3±1.3	322	77.3±4.9
5.0	—	—	—	—	1,004	99.2±0.2	373	96.4±3.5
6.0	—	—	—	—	1,004	99.9±0.2	345	99.8±0.4

* Total number of insects in 3 replicates.

Table 2. Effects of MI on mortality of each stage *S. zeamais* in fumigation for 6 hours at 15℃.

Dose (g/m³)	Egg		Larva		Pupa		Adult	
	*n**	Mortality (%±SD)	*n**	Mortality (%±SD)	*n**	Mortality (%±SD)	*n**	Mortality (%±SD)
1.5	791	94.0±10.4	872	41.2±9.5	890	23.3± 3.1	377	3.4± 4.1
2.0	791	93.1±12.0	872	52.2±5.6	890	40.7± 2.9	439	17.6±13.1
2.5	791	95.6± 7.5	872	70.7±7.6	890	66.9±12.3	442	43.9± 5.7
3.0	791	97.4± 4.5	872	88.4±6.0	890	84.5± 8.9	430	78.7± 4.0
3.5	791	97.1± 4.9	872	94.5±1.8	890	90.6± 5.8	478	92.8± 7.5
4.0	791	99.5± 0.9	872	96.4±1.0	890	96.3± 2.6	422	98.7± 2.3

* Total number of insects in 3 replicates.

Table 3. Effects of MI on mortality of each stage of *T. confusum* in fumigation for 6 hours at 15℃.

Dose (g/m³)	Egg		Larva		Pupa		Adult	
	*n**	Mortality (%±SD)	*n**	Mortality (%±SD)	*n**	Mortality (%±SD)	*n**	Mortality (%±SD)
3.0	1,008	100	405	76.6±5.9	355	6.6±3.1	462	6.6± 4.4
4.0	1,008	100	405	92.8±3.9	355	33.3±3.0	446	33.9±14.5
5.0	1,008	100	405	94.6±2.8	355	55.8±4.6	584	71.3±16.8
6.0	1,008	100	405	99.1±0.8	355	66.2±2.2	475	94.0± 1.7
7.0	1,008	100	405	99.5±0.6	355	87.5±5.4	446	98.3± 0.4

* Total number of insects in 3 replicates.

T. confusum

Table 3 shows the mortality percentages of each stage, and the most susceptible stage was the egg. Table 5 shows that susceptibility at LD_{50} for the other stages decreased in the order of larva, adult, and pupa, and the LD_{50} for the pupa was 4.8 g/m³. The highest value of LD_{95} was 8.7 g/m³, for the pupa.

P. interpunctella

Table 4 shows the mortality percentages of each stage, and 100% mortality was obtained at 2.0 g/m³ for the adult, and at 0.5 g/m³ for the egg. Table 5 shows that the LD_{50} of the larva and pupa were 2.8 g/m³ and 2.3 g/m³, respectively, and that the highest value

Table 4. Effects of MI on mortality of each stage of *P. interpunctella* in fumigation for 6 hours at 15℃.

Dose (g/m^3)	Egg		Larva		Pupa		Adult	
	*n**	Mortality (%±SD)	*n**	Mortality (%±SD)	*n**	Mortality (%±SD)	*n**	Mortality (%±SD)
0.5	200	100	–	–	56	7.9±11.2	105	13.9±14.8
2.0	200	100	123	14.1± 6.9	56	39.2±11.5	102	100
3.0	200	100	181	54.8±37.2	–	–	–	–
3.5	200	100	–	–	56	83.6±15.8	102	100
4.0	–	–	106	96.2± 5.4	160	97.4± 1.5	–	–
5.0	–	–	125	98.0± 2.8	–	–	–	–
6.0	–	–	179	100	160	100	–	–

* Total number of insects in 2 replicates.

Table 5. Comparison of susceptibility to MI on each stage of four species of stored grain insects in fumigation for 6 hours at 15℃.

Species	Stage	LD_{50} g/m^3 (95% C.L.)	LD_{95} g/m^3 (95% C.L.)
S. granarius	Larva	1.5 (1.2–1.7)	3.2 (2.6–4.7)
	Pupa	2.6 (2.5–2.7)	3.9 (3.7–4.2)
	Adult	3.4 (3.3–3.5)	5.0 (4.8–5.3)
S. zeamais	Larva	1.8 (1.7–1.9)	3.9 (3.5–4.5)
	Pupa	2.1 (2.0–2.2)	4.1 (3.8–4.5)
	Adult	2.5 (2.4–2.6)	3.7 (3.4–4.0)
T. confusum	Larva	2.1 (1.7–2.4)	4.7 (4.4–5.1)
	Pupa	4.8 (4.6–5.0)	8.7 (8.0–9.6)
	Adult	4.2 (4.0–4.4)	6.2 (5.8–6.8)
P. interpunctella	Larva	2.8 (2.4–3.2)	4.4 (3.7–6.3)
	Pupa	2.3 (1.7–2.7)	3.9 (3.4–5.1)

of LD_{95} was 4.4 g/m^3, for the larva. No significant difference was observed in 95% C.L. of LD_{50} and LD_{95} between the larva and pupa.

Results of the susceptibility tests showed that the most tolerant stage for the four species to MI was the pupa of *T. confusum*, and that a fumigation standard obtaining complete mortality for this stage would be applicable for many stored grain insects.

AKIYAMA *et al*. (1980) conducted laboratory tests in fumigation with MB for 5 hours at 15℃, and indicated that CT products (concentration×time; mg・h/*l*), at 50% mortality for pupa of *S. zeamais* and *T. confusum*, were 53.5 and 141.0 mg・h/*l*, respectively. To compare the mortality efficacy between MB and MI, temporary CT products of MI were calculated with applied dose and time, because gas concentrations showed almost the same values as the doses during fumigation. The doses of MI, at 50% mortality for pupae of *S. zeamais* and *T. confusum* in this study, were 2.1 g/m^3 and 4.8 g/m^3, respectively; the temporary CT products were estimated to be 12.6 (2.1×6) and 28.8 (4.8×6) mg・h/*l*, respectively. These values were less than one-fourth of that of MB in the study by AKIYAMA *et al*. This fact suggests that MI has a larger effect than MB to disinfest many stored grain insects in fumigation at 15℃.

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和 文 摘 要

貯穀害虫 4 種のヨウ化メチル感受性

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オゾン層破壊物質に指定された臭化メチルの代替消毒技術開発のため、ヨウ化メチルに対するグラナリアコクゾウムシ、コクゾウムシ、ヒラタコクヌストモドキ及びノシメマダラメイガの感受性を調査した。くん蒸は 15℃, 6 時間、収容比 0.004 (w/v) 以下で行った。

それぞれの種において、最も感受性が高い態は卵であった。グラナリアコクゾウムシ、コクゾウムシ、ヒラタコクヌストモドキ及びノシメマダラメイガの半数致死における感受性はそれぞれ、成虫、成虫、蛹及び幼虫が最も低くなり、それらの LD_{50} 値はそれぞれ、3.4 g/m^3、2.5 g/m^3、4.8 g/m^3 及び 2.8 g/m^3 となった。よって、これら 4 種の各態のうち、半数致死における感受性が最も低いのはヒラタコクヌストモドキの蛹となり、 95％ 致死における感受性でも同様の結果が得られた。

これらのことから、貯穀害虫 4 種の各態のうち、ヨウ化メチルに対し最も耐性であるのはヒラタコクヌストモドキの蛹であり、これを完全殺虫できるくん蒸基準は、多くの貯穀害虫に適用できると考える。