Res. Bull. Pl. Prot. Japan No. 46: 1～7 (2010)

Synergistic and Suffocative Effects of Fumigation with a Lower-concentration Phosphine and Sulfuryl Fluoride Gas Mixture on Mortality of Sitophilus Species (Coleoptera: Dryophthoridae), a Stored Product Pest
Part 1: Dose-response Test on *Sitophilus granarius* and *Sitophilus zeamais* for Fumigation with Sulfuryl Fluoride

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Abstract: Dose-response tests were conducted on both *Sitophilus granarius* and *Sitophilus zeamais*. The results revealed that the adult stage of *S. granarius* was less tolerant to SF than that of *S. zeamais*. The various ages of *S. zeamais* adults fumigated with SF for 3 hours at 15°C indicated unique mortalities, which became more tolerant according to adult age. The LD_{50} and LD_{95} of 7-day-old adults were estimated at 12.6–13.1 mg/l and 22.5 mg/l, whereas those of 24-day-old adults were estimated at 25.0–25.2 mg/l and 32.2–33.7 mg/l, respectively. For larval and pupal stages of *S. zeamais* fumigated with SF for 24 hours at 15°C, LD_{50} and LD_{95} were estimated at 0.7–0.8 mg/l and 2.4–3.6 mg/l for larvae, and were estimated at 0.9–1.0 mg/l and 1.5–1.6 mg/l for pupae. The higher LD_{95} values were estimated for the larval stage than the pupal stage, although LD_{50}s for the larval stage were slightly lower.
Key words: sulfuryl fluoride, quarantine fumigation, mortality, *Sitophilus granarius*, *Sitophilus zeamais*

Introduction

Japan imports many kind of agricultural products from abroad, in particular a total of ca. 30 million tonnes of grain (including, e.g., wheat, maize, and soybeans) were imported and inspected by the plant quarantine authority of Japan in 2007. In cases where quarantine pests are detected at inspection, methyl bromide or aluminum phosphide (phosphine [PH_3]) fumigation is executed on the imported grains (MAFF, 1971). The category of grain has the largest quantities in plant quarantine statistics (PPS, 2007), so the amount of MB used for grain is therefore larger than for other categories, except whole logs.

The Montreal Protocol's decisions urge parties to refrain from using methyl bromide (MB) for quarantine and pre-shipment (QPS) purposes wherever possible (UNEP, 1995 and 1999). Moreover, the recommendation of the International Plant Protection Convention (IPPC) titled "Replacement or reduction of the use of methyl bromide as a phytosanitary measure" was adopted in the third section of the Commission on Phytosanitary Measures as guidance on the replacement or reduction of the use of QPS MB (IPPC, 2009).

PH_3, however, is not adopted for treating primary grain pest Sitophilus species, such as *Sitophilus granarius*, because egg and adult stages of *S. granarius* or *Sitophilus zeamais* can be effectively killed by PH_3, but larval and pupal stages did not show satisfactory mortality in the actual range of dosage and fumigation conditions in commercial (MORI and KAWAMOTO, 1966).

Sulfuryl fluoride (SF) is registered for nonfood commodities but not for foods in Japan, although it is widely used for many food commodities worldwide such as grains in mills (DRINKALL *et al*., 2005; PRABHAKARAN and WILLIAMS,

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2007). It has higher efficacy on the larval and pupal stages of several stored-product insects, although the egg stage is the most tolerant and a higher dose of SF and/or higher temperature is required to achieve sufficient efficacy (FURUKI *et al*., 2005; BELL *et al*., 2003; NAITO *et al*., 2006).

Based on the results stated above, it was considered that fumigation with a mixture gas of PH_3 and SF would kill all stages of Sitophilus species because SF kills the pupal stage that cannot be killed by PH_3, and on the contrary, PH_3 kills the egg stage that cannot be killed by SF. Fumigation with a mixture gas (PH_3+SF) was expected to provide sufficient efficacy as quarantine treatment.

Data related to susceptibility of Sitophilus species to SF should be necessary to investigate the efficacy of mixture gas fumigation. Here, we firstly report the results and estimated LD values of dose-response tests of SF fumigation on *S. granarius* and *S. zeamais* in the series of our studies.

Materials and Methods

(a) Test insects

Sitophilus granarius (granary weevil)

This species was derived from Great Britain (permit No. 12Y-336) with permission from the Ministry of Agriculture, Forestry, and Fisheries of Japan and reared for successive generation on brown rice at the National Food Research Institute (Tsukuba, Ibaraki Prefecture). Part of the rearing cultures was moved to the Research Division of the Plant Protection Station, Yokohama, Japan, with permission as a derivation of an approved culture described above (permit No. 13Y-894), and the colony, maintained for almost 7 years, was used.

Sitophilus zeamais (maize weevil)

This insect was collected at appropriate places and reared at another research institute in Japan. More than 15 years ago, part of the rearing colony was introduced to the Research Division of the Plant Protection Station in Yokohama, Japan, and was reared for successive generations on brown rice was used.

Both species were reared at a constant temperature (25°C), at 60% RH, under a photoperiod of 16 h : 8 h (light : dark) in the rearing room.

(b) The pupal development test of *S. zeamais* on brown rice

In order to investigate pupal development in grain, almost 2,000 adults (4.5 g) of *S. zeamais* were released to lay eggs on brown rice (100 g) in cylindrical plastic container (D 150×H 90 mm) for 2 days, and then the adults were removed from the container. The egg-infested brown rice was maintained in a rearing room. Part of the brown rice—30 grains—was dissected daily, and the pupal development of insects in the grain was checked from 15 to 30 days after the adults were removed from the container. This investigation was repeated twice.

(c) Methods to obtain the stages tested

Adults of *S. granarius*, ca. 15 days old, were used after they emerged from grains of brown rice. The larvae and pupae of *S. zeamais* tested were obtained by storing them for 12 days and 25 days after oviposition, respectively. The storage period of 25 days for pupae was decided from the results of a stage development test on *S. zeamais*.

Several different ages (7, 10, 20, and 24 days) of *S. zeamais* adults after emergence from grain were used for the test.

(d) Fumigation

Oviposited brown rice was obtained in almost same procedure as the test for pupal stage development described above, except for the size of the container (L 300×W 225×H 100 mm), the amount of brown rice (200 g), and the number of adults released (9.0 g, equivalent to ca. 4,000 individuals).

A mass of 0.5 g of adults with 5 g of brown rice, and each 9 g set of brown rice infested with larvae or pupae of test insects were placed into small plastic cases (D 11×H 6.5 cm) in order to set the test insect numbers for each plot to ranges of ca. 100–200 individuals. The top of the case was covered with a plastic mesh sheet using a rubber band, and then they were moved into an acrylic fumigation box (internal volume: 29.5 liters; equipped with gas injection and sampling ports, a temperature probe, a circulation fan, and air-inlet and exhaust valves) and placed in a temperature-con-

trolled fumigation room at 15°C the day before fumigation. The actual number of larvae and pupae for each plot tested were calculated by counting numbers of adults that emerged from grains on untreated control plots after fumigation.

Fumigation was carried out with a following procedure. SF gas from a gas cylinder (Vikene®, Dow AgroScience LLC) was collected and introduced into the fumigation box using a gastight syringe. Dosages of SF applied in the test were 3.5, 4.0, 4.5, 5.0, and 5.5 mg/l for *S. granarius* adults, 14, 17, 20, 23, and 26 mg/l for *S. zeamais* adults, and 0.4, 0.6, 0.8, 1.0, 1.2, 1.4, and 1.8 mg/l for larvae and pupae of *S. zeamais*. The exposure times were fixed in this study at 3 hours for adults of both Sitophilus species, and at 24 hours for larvae and pupae of *S. zeamais*. One hour of forced aeration was done after fumigation. Temperatures and gas concentrations in fumigation boxes during fumigation were periodically monitored by a temperature recorder (Chino graphic logger CR 1016-A) and a gas chromatograph (Shimadzu GC-2014 with FPD). These dose-response tests were replicated two or three times for each stage of the insects.

(e) Evaluation of mortality

After fumigation was completed, all stages of the insects treated were restored to the rearing room (25°C, 60% RH) and held until the day of evaluation.

Increase of insect mortality according to the passage of the days after exposure to PH_3 up to 12 days (*S. granarius*) and 10 days (*S. zeamais* to achieve 90% kills) are reported (QURESHI *et al*., 1965; NAKAKITA *et al*., 1974). In the preliminary test for our study, similar phenomenon of mortality increase was observed in SF fumigation. Therefore, we decided to evaluate mortality of the adult stage of species tested at 10 days after SF fumigation.

Adult mortality was determined under a binocular stereo microscope, and moribund adults that could hardly walk were considered dead. Mortality for larval and pupal stages was determined by counting individuals that emerged from grains of brown rice stored in the rearing room. The counting were conducted daily and continued until recognizing that zero individuals emerged in five successive days during storage.

(f) Data analysis

Acquired data from the evaluation was analyzed using computer software for probit analysis (Polo Plus, ver. 2.0; LeOra Software 2002) and LD_{50} and LD_{95} were estimated with 95% confidence limits.

Results and Discussion

(a) The pupal stage development test of *S. zeamais* on brown rice

The pupal rate of rearing days was obtained (Fig. 1). The maximum pupation rate was observed at the 26-day point, but adult emergence from grain was recognized at the 27-day point. To avoid the risk of adult contamination, we considered that 25 days after adults were removed should be the most efficient and adequate time to provide the pupal stage for the test.

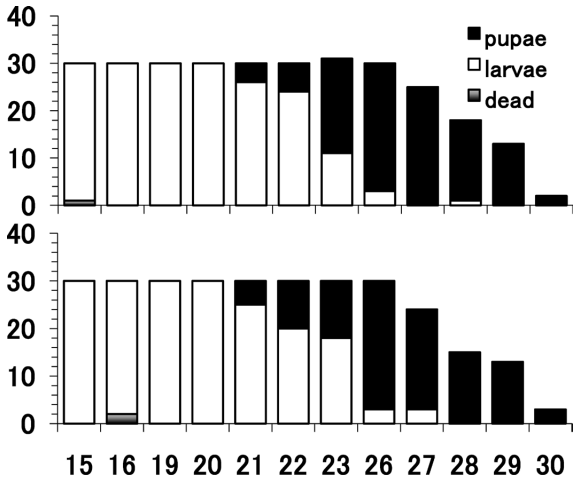

Fig. 1. Stages development of *S. zeamais* on brown rice at 25°C, 60% RH

(b) The dose-response test

Mortality of adults (15 days old) of *S. granarius* fumigated with SF 3.5–5.5 mg/l for 3 hours at 15°C provided relatively higher susceptibilities (Table 1). Those mortalities did not show a goodness of fit to probit analysis, and estimation of LD values failed.

LD_{50} and LD_{95} of *S. zeamais* adults fumigated with SF for 3 hours at 15°C were successfully estimated (Table 2). The slopes of the probit regression line are almost equivalent in various adult ages, and larger LD values were estimated according to the adult age getting older after emergence. Significant differences in LD values were observed among adult ages due to non-overlapping of 95% confidence limits. In particular, LD_{50} for 24-day-old adults showed almost twice that of the 7-day-old adults.

Comparing mortality in Table 1 and estimated LD values in Table 2, the adult stage (15 days old) of *S. granarius* showed mortality of 43.7% at a dosage of 3.5 mg/l, whereas LD_{50}s of *S. zeamais* adults (7 days old) were estimated at 12.6–13.1 mg/l over the same exposure time and temperature. It could be considered that there is more than three times the difference in dosage to attain almost half the mortality between two Sitophilus species. In the case of a higher mortality rate, *S. granarius* adults were killed 86.0% and 82.0% at dosages of SF 5.0 mg/l and 5.5 mg/l, respectively; meanwhile LD_{95}s of *S. zeamais* were estimated at 22.5 mg/l. That difference, therefore, is at least more than four times. It is revealed that the adult stage of *S. granarius* was less tolerant to SF than that of *S. zeamais*. The egg, larval, and pupal stages of *S. granarius* showed higher susceptibility to SF than those stages of *S. zeamais* (NAITO *et al*., 2006). Therefore, all stages of *S. granarius* were considered less tolerant to SF than those of *S. zeamais*.

LD_{50} and LD_{95} estimated for larvae and pupae of *S. zeamais* fumigated with SF for 24 hours at 15°C are shown in Table 3.

The slopes attained for larvae differed from that for pupae, and estimated LD values were considered rather dispersed because slopes changed in replications and wider 95% confidence limits were estimated. The larval stage required longer preservation periods until the day of evaluation after fumigation. The long rearing periods to grow into adults might cause such dispersed data.

LD_{50} and LD_{95} of pupa are estimated as 0.9–1.0 mg/l and 1.5–1.6 mg/l, respectively. These values are quite low, and it will be possible to achieve a 100% kill of the pupal stage with a lower SF dosage than the results of NAITO *et al*. (2006), which was SF 30 mg/l.

Table 1. Mortality of *S. granarius* adults fumigated with SF for 3 hours at 15°C

SF dosage (mg/l)	Insects tested (N)	Mortality (%± SD)
3.5	302	43.7 ± 1.9
4.0	297	72.4 ± 1.9
4.5	306	84.0 ± 0.3
5.0	250	86.0 ± 4.9
5.5	300	82.0 ± 1.9
Cont.	352	0.9 ± 0.6

Note: Results were obtained from 2 replications. The age of adults tested was 15 days after emergence.

Table 2. LD values for *S. zeamais* adults estimated from fumigation with SF at dosages of 14–26 mg/l for 3 hours at 15°C

Days after emergence	Insects tested (N)	LD_{50} mg/l (95% CL)[1)]	LD_{95} mg/l (95% CL)[1)]	Slope
7	1,248	13.1 (12.1–14.0)	22.5 (21.6–23.7)	0.18
7	1,269	12.6 (6.0–15.1)	22.5 (20.3–28.0)	0.17
10	1,206	15.1 (13.8–16.1)	23.8 (22.5–25.8)	0.20
20	2,480	23.0 (22.7–23.4)	30.9 (30.1–31.8)	0.21
24	1,457	25.0 (23.6–27.2)	32.2 (29.2–40.0)	0.23
24	1,980	25.2 (24.7–25.8)	33.7 (32.3–35.7)	0.19

[1)]Natural deaths are included for estimates.

Table 3. LD values for *S. zeamais* larvae and pupae estimated from fumigation with SF at dosages of 0.4–1.8 mg/l for 24 hours at 15°C

Stage	Insects tested[1] (N)	LD_{50} mg/l (95% CL)	LD_{95} mg/l (95% CL)	Slope
larvae	1,379	0.71 (0.24–0.94)	3.63 (2.71–6.60)	0.56
	1,274	0.81 (0.54–0.99)	2.36 (1.93–3.39)	1.06
pupae[2]	1,392	0.99 (0.84–1.15)	1.59 (1.37–2.07)	2.73
	1,693	0.89 (0.77–1.02)	1.63 (1.42–1.99)	2.24
	1,240	0.96 (0.91–1.02)	1.48 (1.39–1.61)	3.20

[1] Numbers of insects tested are calculated from the emerged adults in the control and the infesting rate for each tested plot.
[2] Actual pupation rate in pupal stage plot was 86.9 ± 3.5%.

LD_{50} for the larval stage is considered slightly lower than that of the pupal stage. In the LD_{95} cases, however, higher values for the larval stage were estimated, and significant differences were observed. That result means the verification test using the larval stage will also be necessary to appropriately establish the fumigation standard with a gas mixture of PH_3 and SF, the same as the test for the pupal stage.

All gas concentrations and temperatures in the fumigation box, which were monitored during each of fumigation, were not unusual.

Acknowledgements

We would sincerely like to thank Mr. Noboru OGAWA of the Nagoya Plant Protection Station for providing valuable suggestions.

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和 文 摘 要

低濃度リン化水素とフッ化スルフリル混合くん蒸における Sitophilus属穀類害虫（Coleoptera: Dryophthoridae）に対する殺虫相乗作用と阻害作用 1. グラナリアコクゾウとコクゾウに対するフッ化スルフリルによる薬量–反応試験

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SFくん蒸に対するグラナリアコクゾウとコクゾウの用量－反応試験を実施した。その結果、グラナリアコクゾウ成虫はコクゾウ成虫よりもSFに対して耐性がないことが明らかになった。15°C 3時間でSFくん蒸した様々な日齢のコクゾウ成虫は、成虫年齢に応じて耐性が強くなるという特徴的な殺虫効果を示し、7日齢の成虫は12.6–13.1 mg/lのLD_{50}値及び22.5 mg/lのLD_{95}値が得られたが、24日齢の成虫では、それぞれ25.0–25.2 mg/lと32.2–33.7 mg/lになった。15°C 24時間でSFくん蒸されたコクゾウの幼虫及び蛹の場合、LD_{50}とLD_{95}値は、幼虫では0.7–0.8 mg/lと2.4–3.6 mg/l、蛹では0.9–1.0 mg/lと1.5–1.6 mg/lが得られた。幼虫のLD_{50}値は蛹よりもやや低かったものの、LD_{95}値は蛹より高い値が得られた。

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