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

Takashi MISUMI, Mieko AOKI, Nobuaki TANIGAWA*, Hisashi KITAMURA** and Norihito SUZUKI
Quarantine Disinfestation Technology Laboratory, Research Division, Yokohama Plant Protection Station. 1-16-10 Shin-yamashita, Naka-ku Yokohama, 231-0801, Japan.

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 LD10 and LD90 of 7-day-old adults were estimated at 12.6-13.1 mg/l and 225 mg/l, whereas those of 24-day-old adults were estimated at 230-252 mg/l and 322-337 mg/l, respectively. For larval and pupal stages of *S. zeamais* fumigated with SF for 24 hours at 15°C, LD10 and LD90 were estimated at 0.7-0.8 mg/l and 24-36 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 LD90 values were estimated for the larval stage than the pupal stage, although LD50 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 [PH3]) 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 (UNEPI, 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).

PH3, 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 PH3 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, 2005; 2006).

* Tokyo Sub-station, Yokohama Plant Protection Station.
** Miyako Branch, Yokohama Plant Protection Station.
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 PH₃ and SF would kill all stages of Sitophilus species because SF kills the pupal stage that cannot be killed by PH₃, and on the contrary, PH₃ kills the egg stage that cannot be killed by SF. Fumigation with a mixture gas (PH₃+SFF) 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. zeama 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 zeama* (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 16:8 h (light:dark) in the rearing room.

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

In order to investigate pupal development in grain, almost 2,000 adults (4.5 g) of *S. zeama* 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. zeama* 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. zeama*.

Several different ages (7, 10, 20, and 24 days) of *S. zeama* 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, 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_{90} 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.
(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$_{95}$ 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$_{95}$s of *S. zeamais* adults (7 days old) were estimated at 126–131 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 225 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

<table>
<thead>
<tr>
<th>SF dosage (mg/l)</th>
<th>Insects tested (%)</th>
<th>Mortality (%± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.5</td>
<td>302</td>
<td>43.7 ± 19</td>
</tr>
<tr>
<td>4.0</td>
<td>297</td>
<td>72.4 ± 19</td>
</tr>
<tr>
<td>4.5</td>
<td>306</td>
<td>84.0 ± 0.3</td>
</tr>
<tr>
<td>5.0</td>
<td>250</td>
<td>86.0 ± 4.9</td>
</tr>
<tr>
<td>5.5</td>
<td>300</td>
<td>82.0 ± 19</td>
</tr>
<tr>
<td>Cont.</td>
<td>352</td>
<td>0.9 ± 0.6</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Days after emergence</th>
<th>Insects tested (%)</th>
<th>LD$_{50}$ mg/l (95% CL)$^i$</th>
<th>LD$_{95}$ mg/l (95% CL)$^i$</th>
<th>Slope</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>1,248</td>
<td>131 (121–140)</td>
<td>225 (216–237)</td>
<td>0.18</td>
</tr>
<tr>
<td>7</td>
<td>1,269</td>
<td>126 (60–151)</td>
<td>225 (203–280)</td>
<td>0.17</td>
</tr>
<tr>
<td>10</td>
<td>1,206</td>
<td>151 (138–161)</td>
<td>238 (225–258)</td>
<td>0.20</td>
</tr>
<tr>
<td>20</td>
<td>2,480</td>
<td>230 (227–234)</td>
<td>309 (301–318)</td>
<td>0.21</td>
</tr>
<tr>
<td>24</td>
<td>1,457</td>
<td>25.0 (236–27.7)</td>
<td>322 (292–400)</td>
<td>0.23</td>
</tr>
<tr>
<td>24</td>
<td>1,980</td>
<td>25.2 (247–25.8)</td>
<td>337 (323–357)</td>
<td>0.19</td>
</tr>
</tbody>
</table>

$^i$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

<table>
<thead>
<tr>
<th>Stage</th>
<th>Insects tested(^{(1)}) (N)</th>
<th>LD(_{50}) mg/l (95% CL)</th>
<th>LD(_{95}) mg/l (95% CL)</th>
<th>Slope</th>
</tr>
</thead>
<tbody>
<tr>
<td>larvae</td>
<td>1.379</td>
<td>0.71 (0.24–0.94)</td>
<td>3.63 (2.71–6.60)</td>
<td>0.56</td>
</tr>
<tr>
<td></td>
<td>1.274</td>
<td>0.81 (0.54–0.99)</td>
<td>2.36 (1.93–3.39)</td>
<td>1.06</td>
</tr>
<tr>
<td>pupae(^{(2)})</td>
<td>1.392</td>
<td>0.99 (0.84–1.15)</td>
<td>1.59 (1.37–2.07)</td>
<td>2.73</td>
</tr>
<tr>
<td></td>
<td>1.693</td>
<td>0.89 (0.77–1.02)</td>
<td>1.63 (1.42–1.99)</td>
<td>2.24</td>
</tr>
<tr>
<td></td>
<td>1.240</td>
<td>0.96 (0.91–1.02)</td>
<td>1.48 (1.39–1.61)</td>
<td>3.20</td>
</tr>
</tbody>
</table>

\(^{(1)}\) 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.

**REFERENCES**


QURESHTI, A. H., E. J. BOND and H. A. U. MONRO (1965) Toxicity of hydrogen phosphide to the granary weevil, *Sitophilus*


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

三角 隆・青木美愛子・谷川展暉*・北村 寿**・鈴木則仁
横浜植物防除研究所調査研究部消毒技術開発担当

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126-131 mg/l
のLD50値及び225 mg/lのLD95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、LD50 と LD95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LD50値は蛹よりもやや低かったものの、
LD95値は蛹よりも高い値が得られた。

* 現在、横浜植物防除研究所東京支所
** 現在、横浜植物防除研究所宮古出張所