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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 2: Susceptibility Test on *Sitophilus zeamais* for Fumigation with a Gas Mixture, and Verification Test of a Sequential Fumigation with Two Fumigants

Takashi MISUMI, Mieko AOKI* and Hisashi KITAMURA**

Quarantine Disinfestation Technology Laboratory, Research Division, Yokohama Plant Protection Station, 1-16-10 Shin-Yamashita, Naka-ku, Yokohama 231-0801, Japan.

Abstract: Susceptibility tests of *Sitophilus zeamais* were conducted at 15°C using a gas mixture (low concentration of phosphine [PH_3]+sulfuryl fluoride [SF]) as well as PH_3 gas and SF gas. Insect mortalities were drastically increased at a gas mixture of SF 20 mg/l+PH_3 0.05–0.1 mg/l for 3 hours to adults, and that of SF 0.7 mg/l+PH_3 0.1–1.0 mg/l for 24 hours to larvae, compared to the single gas fumigations of PH_3 and SF in equivalent dosage, time, and temperature for each stage. The insect body could sufficiently take up both fumigants through respiration under the condition that respiration is not impeded, such as fumigation with lower PH_3 dosage, and mortalities was increased by the synergistic effect. On the contrary, distinctive lower mortalities were shown at a gas mixture of SF 0.9 mg/l+PH_3 0.1–1.0 mg/l for 24 hours to pupae. From the investigation of paralytic status on pupae that underwent lower dosages (0.025, 0.05, and 0.1 mg/l) of PH_3 fumigation for 20 hours, it was supposed that inhibition of SF uptake would possibly happen because the respiration of pupae could be suppressed by PH_3 more sensibly than adults and larvae. Fumigation tests for adults, which were sequentially fumigated with SF 20 mg/l for 3 hours and PH_3 0.05–0.1 mg/l for 4 hours in order and *vice versa*, showed increasing mortality regardless of the fumigation order of the two gases, despite a 1-hour aeration carried out between the two fumigations. A synergistic effect could be achieved without mixing both gases at fumigation. Mortalities of pupae at a sequential fumigation that were conducted by fumigation with SF 0.9 mg/l for 24 hours initially, and then sequentially dosed with PH_3 1.0, 1.5, or 2.0 mg/l and maintained for 48 hours, were significantly increased to 84.5% from the result of 16.5% on gas mixture fumigation. Pupal mortalities were increased to 92.0% according to PH_3 dosage rates, and those mortalities were significantly different among PH_3 dosage rate tested (α=0.05). Verification tests of sequential fumigation that were conducted by SF 2.2 mg/l for 24 hrs and PH_3 1.0, 1.5, or 2.0 mg/l for 48 hrs at 15°C achieved 100% mortality of more than *ca.* 1,000 individuals for each egg, larva, and pupa of *S. zeamais*. The sequential fumigation with SF and PH_3 was decided to be effective to eliminate all stages of two Sitophilus species.

Key words: Synergistic/suffocative effect, phosphine, sulfuryl fluoride, sequential quarantine fumigation, *Sitophilus zeamais*

Introduction

Phosphine (PH_3) and sulfuryl fluoride (SF) are fumigants used widely in various countries, and different toxic actions are known between PH_3 and SF. SF is thought to inhibit the glycolysis and fatty acid cycles via the release of fluoride ions, thereby depriving the insect energy necessary for survival (FAO, 2005). The toxicology and biochemistry of PH_3 appear to have similarities to those of hydrogen cyanide (PRICE, 1985). It has also been revealed that PH_3 inhibited the activity of cytochrome oxidase in mitochondria, and PH_3 penetrated into the insect body is dissolved into several kinds of phosphates (NAKAKITA, 1987). NAKAKITA and KURODA (1986) investigated that most PH_3 in the body of the

* Retired.

** Miyako Branch, Yokohama Plant Protection Station.

rust-red flour beetle, *Tribolium castaneum*, is irreversibly decomposed and could not be recaptured from bodies after fumigation. The inhibition of glycolysis pathways by SF fumigation means that the generation of ATP should also be interrupted, which possibly influences indirectly the activity of mitochondria, which are the point of action for PH_3 fumigation. Contrary to this, supposing PH_3 function at fumigation, energy loss should incidentally occur in the decomposition of PH_3 in the insect body, and phosphates should be generated from free phosphorus. This energy loss and increasing phosphates might causes degradation of the activity of glycolysis pathways. According to these suppositions of the activities of both SF and PH_3, a synergistic effect of SF and PH_3 gas may arise due to the different of toxic points for SF and PH_3, and their mutual enhanced toxicity is envisaged.

It was considered that fumigation with a gas mixture of PH_3 and SF would kill all stages of Sitophilus species because SF kills the pupal stage, a stage tolerant to PH_3, and on the contrary, PH_3 kills the egg stage which requires a tremendously high dosage rate for SF to kill. Fumigation with a gas mixture (PH_3+SF) was expected to provide sufficient efficacy as quarantine treatment and mortality tests with higher dosages of the gas mixture had been carried out. That result, however, showed incomplete mortality for both pupal stages of *S. granarius* and *S. zeamais*, and it was supposed that the earlier dosing of PH_3 than SF possibly interrupted SF uptake to pupa bodies. It is reported that dosing SF first and then dosing PH_3 with a time lag was effective to kill pupae because complete mortality was obtained in the fumigation in which SF was dosed 1 hour before PH_3 dosing (NAITO *et al.*, 2006). On the other hand, NAKAKITA *et al.* (1974) investigated the relationship between PH_3 concentration and mortality. They identified that a lower concentration of PH_3 provided higher mortality. Phosphine has the action of restraining the respiration of *S. zeamais* adults according to the increase of PH_3 concentration; specifically, PH_3 of 5,000 ppm nearly inhibited respiration completely after 6 hours of exposure, while PH_3 of 25 ppm and 500 ppm enabled respiration over 24 hours. It was explained that different toxic actions existed between low and high concentration of PH_3 (NAKAKITA, 1987). Therefore, it is possible that PH_3 may not inhibit SF uptake and its toxic effect on Sitophilus species when a lower concentration of PH_3 is mixed with SF and be applied.

Here, we report the synergistic and suffocative effects on mortality of *S. zeamais* after conducting a comparison test using gas mixture (lower concentration PH_3+SF) fumigation and single gas fumigation of PH_3 or SF. We analyzed the different concentration of PH_3 that causes paralysis of bodies and decreases respiration activity for pupae, which showed the suffocative effect of the gas mixture fumigation. We also report the test to ascertain the appropriate fumigation order of the two gases, the mortality test of fumigation that doses PH_3 24 hours after the initial dosing of SF to avoid the action of PH_3 that restrains the respiration activity for pupae, and the verification test to confirm the effectiveness of the sequential fumigation of SF and PH_3 as quarantine treatment for imported grain.

Materials and Methods

(a) Test insects and stages

Each of the stages—egg, larval, pupal, and adult—of *S. zeamais* were prepared and conditioned for fumigation. The ages of each egg, larval, and pupal stage used in tests were 1, 12, and 25 days after oviposition, respectively. Adults that were used were only those aged 24 days after emergence from grains of brown rice. Each stage of test insects or insect-infested grains was weighed and confined in plastic cases (D 11×6.5 cm) with brown rice (Adults [0.5 g] with brown rice [5 g] and each 9 g of brown rice infested with egg, larva or pupa), and then placed in a 29.5-liter fumigation box in a fumigation room at 15°C. All test insects were derived from a same *S. zeamais* rearing colony used in the Dose-Response test, and the procedures, including the method of obtaining eggs oviposited on brown rice, were done similarly to our former study (MISUMI *et al.*, 2010).

(b) Fumigation

In this study, a series of five fumigation tests using SF, PH_3, or a gas mixture of both gases were carried out as follows.

Dosage rates of SF used in the tests were decided based on the estimated LD_{50} values for adults, larvae, and pupae acquired in the former study (MISUMI *et al.*, 2010) for precise comparison of mortalities.

Table 1. Fumigation conditions of test plots fumigated with gas mixtures, sulfuryl fluoride (SF), and phosphine (PH_3) at 15°C.

Species and stage	Dosage (mg/l)		Exposure time (hours)
	SF	PH_3	
S. zeamais (adult)	20	–	3
	20	0.05	3
	20	0.10	3
	–	0.05	3
	–	0.10	3
S. zeamais (larva)	0.7	–	24
	0.7	0.1	24
	0.7	0.5	24
	0.7	1.0	24
S. zeamais (pupa)	0.9	–	24
	0.9	0.1	24
	0.9	0.5	24
	0.9	1.0	24

(i) Comparison test of efficacy mixing two gases in fumigation

Fumigation with a gas mixture (PH_3+SF) was performed as follows. First, PH_3 gas was collected from a gas cylinder (Nippon Chemical Industrial Co. Ltd., PH_3 13.22% and N_2 balance) and introduced to the fumigation box using a gas-tight syringe, and then SF gas collected from a gas cylinder (Vikene, Dow AgroScience LLC) was immediately injected into the fumigation box with same handling as the PH_3 injection. Details, such as dosage rate, exposure time, and other fumigation conditions of tested plots are shown in Table 1. Plots of independent fumigation of SF or PH_3 were set for comparing the efficacy to that of the gas mixture. A forced aeration was done for 1 hour after fumigation. For the test plots with the larval and pupal stages, a plot of PH_3-only fumigation was not provided because the efficacy of PH_3 must not be strong against larval and pupal stages of *S. zeamais* (MORI and KAWAMOTO, 1966).

(ii) Test to investigate the influence of PH_3 concentration on respiration activity and paralysis

In order to investigate the paralytic status of the pupal stage of *S. zeamais*, pupae were carefully taken out of grains by dissection and were fumigated with three levels of lower-concentration PH_3 at dosages of 0.025, 0.05, and 0.1 mg/l for 20 hours at 15°C. A forced aeration was done for 10 minutes after fumigation. Gases, handlings, and other conditions of fumigation were the same as described in paragraph (i).

(iii) Test to investigate efficacy according to fumigation order of two gases

The adult stage of *S. zeamais* was fumigated with SF for 3 hours at 15°C and was aerated for 1 hour at first; then, PH_3 fumigation was done for 4 hours at 15°C, followed by 1 hour aeration, and *vice versa*.

Plots of independent fumigation of SF or PH_3 were also made for comparing efficacy. Dosage rates were 20 mg/l for SF, 0.05 and 0.1 mg/l for PH_3 at 15°C. Under these conditions, the two fumigants were never mixed, and the two fumigations were executed sequentially. Therefore, each of the fumigants exerted their toxicities independently and were not directly influenced by each other during the SF and PH_3 fumigation. Gases, handlings, and other conditions of fumigation were the same as described in paragraph (i).

(iv) Sequential fumigation test to identify the method without suffocative effect on efficacy

The pupal stage was sequentially fumigated with SF and PH_3 as follows. At first, SF gas at a dosage of 0.9 mg/l was introduced into the fumigation box, and 24 hours later, PH_3 gas at a dosage of 1.0, 1.5, or 2.0 mg/l was added without conducting an exhaust of SF gas, which means fumigation with a gas mixture was conducted for 48 hours. A forced aeration was done for 1 hour after 72 hours fumigation in total. Test plots for both single fumigation of SF for 24 and 72 hours were made to compare the efficacy. Gases, handlings, and other conditions of fumigation were the same as described in paragraph (i).

(v) Verification test of sequential fumigation with SF and PH_3

All fumigation verification tests were carried out the same as described in paragraph (iv), except for the dosage rates of SF. In order to kill all stages of *S. zeamais*, a 2.2 mg/l of SF dosage rate was determined from estimated LD_{95} for the pupal stage of *S. zeamais*, although LD_{95} values for the larval stage were estimated higher than the pupal stage (MISUMI *et al*., 2010). It is expected that the synergistic effect with two fumigants acted against larvae, and they could be killed completely.

Plots for single fumigation of SF for 24 hours and PH_3 for 48 hours were made to compare efficacy. Gases, handlings, and other conditions of fumigation were the same as described in paragraph (i).

For all types of fumigation, temperatures in the fumigation boxes were monitored periodically using a temperature recorder (Chino graphic logger CR 1016-A), and SF and PH_3 gas concentrations in fumigation boxes were also measured periodically using a gas chromatograph (SF: Shimadzu GC-2014 with FPD; PH_3; Shimadzu GC-2014 with TCD) to confirm all fumigations would be conducted properly. These tests were replicated three times each except for the test in (iii) that was two times.

(c) Evaluation of fumigation efficacy and of paralytic status

Fumigation efficacy was evaluated in the same ways as in the former study (MISUMI *et al*., 2010). Adults were investigated under a microscope, and eggs, larvae, and pupae were evaluated by emerging from grains. Increasing mortality according to the passage of days after exposure to PH_3, up to 12 days for *S. granarius* and 10 days for *S. zeamais*, are reported (QURESHI *et al*., 1965; NAKAKITA *et al*., 1974). We evaluated mortality of the adult stage of species tested at 10 days after fumigation.

To investigate the paralytic status of the *S. zeamais* pupal stage by PH_3 fumigation with 3 levels of lower dosages, we applied a method similar to that of NAKAKITA (1987), which investigated paralysis of *S. zeamais* adults. Paralysis status was judged by carefully stimulating pupal bodies a few times using the side parts of the tip of an insect needle and distinguishing paralysis status whether a pupa wriggled its abdomen a few times or there was no movement. Pupae were categorized as paralyzed if there was no movement. That evaluation was continued periodically after forced exhaustion was finished.

The statistical software JMP 8.0 (SAS Institute Inc., 2008) was used for statistical analysis of the data. Arcsine transformation was applied for the data on mortality rates before statistical analysis.

Results and Discussion

1. Comparison test of efficacy mixing two gases for fumigation

Higher mortalities of *S. zeamais* adults fumigated with the gas mixture of a low concentration of PH_3 and SF for 3 hours at 15°C were acquired (Table 2).

When those adults were fumigated with SF alone, the mortality rate was nearly 30%, and mortality rates for fumigation of only PH_3 at 0.05 mg/l and 0.10 mg/l were 2.3% and 5.9%, respectively. However, mortality rates soared to 70.9%

Table 2. Mortality of *S. zeamais* adult fumigated with SF, PH_3, and gas mixture (SF+PH_3) for 3 hours at 15°C.

Test Stage	Dosage (mg/l)		Insect tested (N)	Mortality[1)] (%±SD)
	SF	PH_3		
Adult	20	–	513	29.2±3.8 a
	–	0.05	614	2.3±0.6 b
	–	0.10	579	5.9±1.9 b
	20	0.05	511	70.9±7.2 c
	20	0.10	517	82.7±5.9 c
	Control		517	1.1±1.0 –

[1)] Different letters following the mortality rate in the column indicate it is significant (α=0.05, Tukey-Kramer HSD).

and 82.7% with fumigation of the SF and PH_3 gas mixture. There are significant differences in sole fumigation of SF or PH_3 and fumigation with the gas mixture SF+PH_3 (α=0.05, Tukey-Kramer HSD). Adding mortality rates that gained in sole fumigation with SF and PH_3, it becomes only 32.3% or 35.9% (=30%+[2.3% or 5.9%]). These values are far below the mortalities actually achieved in gas mixture fumigation. A synergistic effect must act to provide those elevated mortalities.

The larval and pupal susceptibilities in the comparison test fumigated with the gas mixture of a low concentration of PH_3 and SF for 24 hours at 15°C are shown in Table 3.

Larval mortalities are similar to that of adults; however, a contrary result was obtained for pupae: 30.2% of larvae were killed in fumigation with only SF at a dosage rate of 0.7 mg/l for 24 hour at 15°C. While they were treated with SF and PH_3 as a gas mixture, mortality rates were significantly increased to 83.2–96.0%, and those differences in mortality rates are significant (α=0.05, Tukey-Kramer HSD). Increasing the phosphine dosage rate from 0.1 mg/l to 1.0 mg/l accelerated mortality rates in gas mixture fumigation. 43.2% of pupae were killed in sole fumigation with SF at a dosage of 0.9 mg/l. Pupal mortality rates for the gas mixture, however, decreased to less than half for SF individual fumigation, although those differences are nonsignificant (P>0.05, Tukey-Kramer HSD). Increasing the phosphine dosage rate in gas mixture plots from 0.1 mg/l to 1.0 mg/l slightly affected the pupal mortality rates positively.

High concentration of PH_3 causes paralysis and reduces oxygen consumption for adult of *S. zeamais*, and it is likely that PH_3 did not reach the toxic site in the cell (NAKAKITA *et al.*, 1974). It is reported that a decreasing rate of PH_3 uptake is observed according with increasing PH_3 concentration at higher dosage, and *T. castaneum* adults may be plunged into narcosis (WINKS, 1985). In the case of our study, for adults, dosages of 0.05 and 0.1 mg/l at 15°C are equivalent to 35 and 70 ppm. It is considered that those dosages were sufficiently low and would not provide paralysis of adults. In the case of larvae, higher mortalities were also obtained in the gas mixture with SF+PH_3, even when the dosage rate was PH_3 1.0 mg/l (equivalent to *ca.* 700 ppm). It implied that the larval stage may be insensitive to a concentration of this level and it possibly does not affect larval respiration. PH_3 toxicity to insect cells would not be pulled out without the existence of oxygen (NAKAKITA, 1987). SF fumigation showed a definite increase in the rate of oxygen uptake in fumigated insects (MEIKLE *et al.*, 1963). Consequently, in cases where the PH_3 gas concentration is sufficiently lower and does not inhibit respiration, SF could be taken into the insect body and exercise its characteristic of increasing oxygen uptake for the insect. Higher oxygen levels in cells possibly strengthen PH_3 toxicity action, and such gas mixture fumigation should provide higher mortality to adults and larvae of *S. zeamai*s.

Contrary to the results for adults and larvae, pupal mortalities were decreased drastically in cases of gas mixture (SF+PH_3) fumigation. It appears that the toxic action of SF must be interrupted when mixed with PH_3. Accordingly, in the case of pupal stage, even a lower concentration of PH_3 (0.1 mg/l) could have a negative influence. Research on the toxic action of PH_3 to *S. zeamais* related to the inhabitation of respiration was carried out and analyzed using only the adult stage (NAKAKITA *et al.*, 1974; NAKAKITA, 1976a, 1976b, 1987), and not the pupal stage. Although it is theoreti-

Table 3. Mortality of *S. zeamais* larvae and pupae fumigated with SF and gas mixture (SF+PH_3) for 24 hours at 15°C.

Test Stage	Dosage (mg/l)		Insect tested[2] (N)	Mortality[3] (%±SD)
	SF	PH_3		
Larva	0.7	–	524	30.2±3.4 a
	0.7	0.1	524	83.2±5.3 b
	0.7	0.5	524	94.7±1.1 c
	0.7	1.0	524	96.0±0.1 c
Pupa[1]	0.9	–	626	43.2±18.5 a
	0.9	0.1	595	9.4± 6.5 a
	0.9	0.5	707	14.2±13.0 a
	0.9	1.0	676	16.5±12.6 a

[1] Actual pupation rate on control plot was 88.1%.
[2] Numbers of larva and pupa insects tested are estimated from emerged adults (larvae=524, pupae=692) on untreated control plots as well as infestation rates (pupae).
[3] Different letters following the mortality rate in the columns indicate it is significant (α=0.05, Tukey-Kramer HSD).

cally reasonable that toxic action of PH_3 for pupae is similar to that for the adult stage, the pupal stage is motionless, whereas adult and larval stages move. The amount or mechanism of respiration for the pupal stage might be different from other stages.

2. Test to investigate the influence of PH_3 concentration on respiration activity and paralysis

A synergistic effect on adults and larvae of *S. zeamais* was recognized in gas mixture fumigation with SF and PH_3; in pupae, however, a suffocative effect was shown. The level of PH_3 concentration that would cause paralysis of pupa was investigated to identify factors blocking SF uptake into the pupal body.

The level of PH_3 concentration that would cause paralysis of pupa was investigated. Almost all pupae of *S. zeamais* were become progressively active and recover from paralysis corresponding to time after fumigation (Table 9). Other pupae not classified as paralyzed were active, and there was no dead pupae in PH_3 fumigation.

Pupae of the plot exposed with PH_3 0.1 mg/l took a significantly longer time to recover from paralysis than those of PH_3 0.025 mg/l and 0.05 mg/l, and both time and dosage are significant factors (α=0.05, analysis of covariance). Additionally, the differences of least squares means for dosages is also significant between PH_3 0.1 mg/l and below 0.05 mg/l plots (α=0.05, Tukey-Kramer HSD). That means pupal stage can induce the suppression of respiration more sensibly than adult or larval stages should be confirmed.

When PH_3 0.05 mg/l is used in gas mixture fumigation with SF, pupal stages might be killed. However, considering the adoption of gas mixture fumigation to actual quarantine fumigation, dosages less than PH_3 0.05 mg/l are too low and insufficient to kill the egg stage of Sitophilus species. The method of gas mixture fumigation with PH_3 at even lower dosage rates with SF is considered unsuitable to quarantine treatment that requires complete mortality of all stages of Sitophilus species.

3. Test to investigate efficacy according to fumigation order of two gases

To explore the method to completely kill all stages of *S. zeamais*, a test comparing adult mortalities in fumigation order with two gases was conducted, and various levels of mortality were obtained (Table 4).

For single gas fumigation, SF fumigation at a dosage of 20 mg/l provided 36.9% mortality, and PH_3 fumigation of 0.05 mg/l and 0.1 mg/l indicated mortalities of 0.8% and 37.3%, respectively. Regardless of the order of fumigation, despite the 1-hour of forced aeration between the two kinds of fumigation, mortalities were drastically increased with combined SF and PH_3 fumigation. There are significant differences among fumigation plots (α=0.05, Kruskal-Wallis test). It is known that SF and PH_3 do not chemically react with each other. This fact implies that both gases that are taken into the insect body may jointly affect toxicological points in the body. It is revealed that both PH_3 and SF gases exert synergistic effects in connected fumigation of the two gases, and they do not need to be mixed simultaneously. The plots in which SF fumigation was followed by PH_3 fumigation provided higher mortalities than the plots in which PH_3 fumigation were executed before SF fumigation.

4. Sequential fumigation test to identify the method without suffocative effect on efficacy

Mortalities of the pupal stage of *S. zeamais* in sequential fumigation of two fumigants are provided in Table 5.

It shows that mortalities for the plots of sequential fumigation of two gases were increased compared to the results of simple gas mixture fumigation. For instance, sequential fumigation with SF 0.9 mg/l and PH_3 1.0 mg/l surely

Table 4. Paralytic status of pupal stage of *S. zeamais* fumigated with PH_3 at dosages of 0.025, 0.05, and 0.1 mg/l for 20 hours at 15°C.

Dosage[1)] (mg/l)	Tested (N)	Numbers indicate paralytic status after fumigation (N)								
		10 min.	20 min.	30 min.	1 hr.	2 hrs.	3 hrs.	5 hrs.	8 hrs.	
0.025	16	6	3	1	1	1	1	0	–	a
0.050	16	7	3	1	0	–	–	–	–	a
0.100	16	11	10	7	7	5	3	2	2	b
Control	16	0	0	0	0	0	0	0	0	–

[1)] Different letters at the right side indicate the differences of least squares means of dosages are significant (α=0.05, Tukey-Kramer HSD).

improved mortality up to 84.5% from the 16.5% obtained in gas mixture fumigation. 100% mortality is achieved in the plot of SF-only fumigation for 72 hours. The plots of sequential fumigation with three concentrations of PH_3 resulted in incomplete mortalities. Each of the plots tested are judged that to show significant differences in mortality rates (α=0.05, Tukey-Kramer HSD). These results imply that PH_3 gas certainly inhibits SF uptake after PH_3 injection during 72 hours total fumigation. However, mortalities were significantly improved according to increase in the dosage of PH_3. Fumigation efficacy must be increased by the synergistic effect of two fumigants. For effective and efficient fumigation in actual plant quarantine, the sequential fumigation method to kill pupae of *S. zeamais* with SF at first, then to kill other stages with PH_3 exerting a synergistic effect, was considered appropriate.

5. Verification test of sequential fumigation with SF and PH_3

Changes in gas concentrations of SF and PH_3 in the fumigation box are described in Fig. 1. Both SF and PH_3 concentrations were correctly and stably maintained, and gas losses were not observed. That result could support that both fumigations did not react with each other. The results of the sequential fumigation for eggs, larvae, and pupae are showed in Table 7.

Test insects for a single SF fumigation of egg and larval stages could not be killed completely, and these results accorded to our expectation based on the LD_{95} values estimated for larvae (MISUMI *et al.*, 2010). Not less than *ca.* 1,000 individuals in total for each stage of *S. zeamais* were 100% killed by sequential fumigations with SF and PH_3 regardless of the PH_3 dosage rates. It was confirmed for every stage of *S. granarius*, a quarantine pest that is less tolerant to SF and PH_3 fumigation than *S. zeamais* (NAITO *et al.*, 2006; MISUMI *et al.*, 2010). Sequential fumigation using SF and PH_3 is therefore effective to eliminate all stages of two Sitophilus species. Besides, SF had good efficacy against infestations of *Tribolium confusum* and *Ephestia kuehniella* compared to that of MB in mills (SMALL, 2007). PH_3 fumigation has been introduced as quarantine treatment, and its efficacy for stored-product insects has been endorsed except for some Sitophilus species because PH_3 fumigation has been applied in quarantine for many years without any problem with its

Table 5. Mortality of *S. zeamais* adults on the sequential fumigation of SF for 3 hours followed by PH_3 for 4 hours and *vice versa* at 15°C.

Test Stage	Dosages (mg/l) in injection order		Exposure time (hours)	Insect tested (N)	Mortality[2] (%±SD)
	1st	2nd			
Adult	SF 20	PH_3 0.05	7[1]	445	80.6±2.8
	SF 20	PH_3 0.1	7[1]	440	99.5±0.0
	PH_3 0.05	SF 20	7[1]	407	68.9±2.8
	PH_3 0.1	SF 20	7[1]	486	83.5±3.9
	SF 20	–	3	379	36.9±5.3
	–	PH_3 0.05	4	376	0.8±0.4
	–	PH_3 0.1	4	427	37.3±1.7
	Control		7	414	0.5±0.1

[1] One-hour forced aeration was conducted between SF (3 hours) and PH_3 (4 hours) fumigation.
[2] There are significant differences among treated plots (α=0.05, Kruskal-Wallis test).

Table 6. Mortality of *S. zeamais* pupae in sequential fumigation with SF and PH_3 at 15°C.

Test Stage	Dosage (mg/l)		Exposure time (hrs)	Insects tested[1] (N)	Mortality[2] (%±SD)
	SF	PH_3			
Pupa	0.9	–	24	870	44.0±0.4 a
	0.9	–	72	606	100.0±0.0 b
	0.9	1.0	72 (=24+48)	923	84.5±0.5 c
	0.9	1.5	72 (=24+48)	871	87.9±1.7 d
	0.9	2.0	72 (=24+48)	881	92.0±1.4 e

[1] Number of insects tested are estimated from emerged adults (N=909) on untreated control plots as well as infestation rate. Actual pupation rate on the control plot was 84.9%.
[2] Different letters following mortality rates in the column indicate that it is significant (α=0.05, Tukey-Kramer HSD).

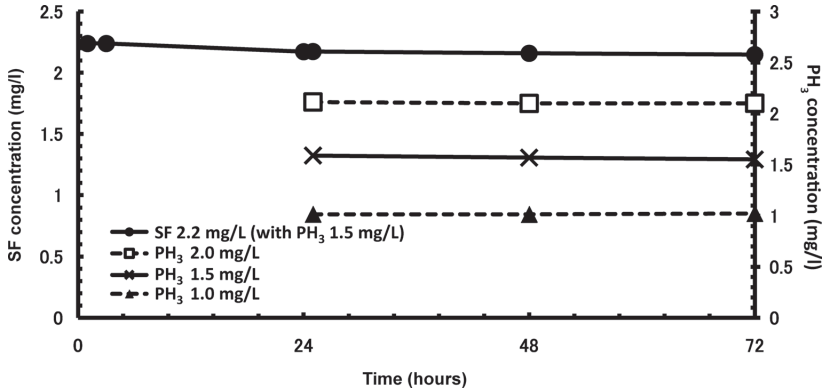

Fig. 1. Changes of gas concentrations for SF and PH_3 in sequential fumigation. PH_3 gas was injected at 24 hours after SF dosing into the fumigation box.
(Data on SF concentrations for fumigations mixed at dosages of PH_3 1.0 mg/l and 2.0 mg/l were omitted because each SF concentration curve shows the same patterns regardless of PH_3 dosage mixed with SF.)

Table 7. Mortality of *S. zeamais* eggs, larvae, and pupae in sequential fumigation with SF and PH_3 at 15°C.

Test Stage	Dosage (mg/l)		Exposure time (hours)	Insects tested[2)] (N)	Mortality (%±SD)
	SF	PH_3			
Egg	2.2	–	24	329	17.5±9.7
	2.2	1.0	72[1)]	329	100.0±0.0
	2.2	1.5	72[1)]	329	100.0±0.0
	2.2	2.0	72[1)]	329	100.0±0.0
	–	2.0	48	329	100.0±0.0
Larva	2.2	–	24	553	84.7±3.8
	2.2	1.0	72[1)]	553	100.0±0.0
	2.2	1.5	72[1)]	553	100.0±0.0
	2.2	2.0	72[1)]	553	100.0±0.0
Pupa	2.2	–	24	581	100.0±0.0
	2.2	1.0	72[1)]	656	100.0±0.0
	2.2	1.5	72[1)]	659	100.0±0.0
	2.2	2.0	72[1)]	571	100.0±0.0

[1)] 72 hours = 24+48 hours.
[2)] Number of insects tested are estimated from emerged adults (eggs=329, larvae=553, pupae=571) and infestation rate (in the case of pupae) on untreated control plots.

efficacy (MAFF, 1971). This means that sequential fumigation with SF and PH_3 will be effective for various kinds of pest on imported grains.

It has recently been reported that SF has a higher level of GWP (global warming potential) than previously estimated (MILLET *et al.*, 2009). Lower SF dosages would be better in practical fumigation for a contribution to reduce the emission of greenhouse gases and for efficiency. With assistance from the efficacy of PH_3 and the synergistic effect, sequential fumigation can drastically reduce SF dosage while retaining fumigation efficacy. Sequential fumigation with lower dosages of both SF and PH_3 will be able to perform not only efficient fumigation but will also reduce a potential risk to the environment.

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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（英文） 2. コクゾウに対する混合ガスによる殺虫試験 及び連続くん蒸による確認試験

三角 隆・青木美愛子*・北村 寿**
横浜植物防疫所調査研究部消毒技術開発担当

コクゾウムシの混合ガス（低濃度リン化水素[PH_3]＋フッ化スルフリル[SF]）くん蒸による殺虫試験では、成虫のSF 20 mg/l+PH_3 0.05 ～ 0.1 mg/l、3時間、15℃、及び幼虫のSF 0.7 mg/l+PH_3 0.1 ～ 1.0 mg/l、24時間、15℃のいずれの処理においても、同薬量のPH_3とSFの単独くん蒸よりも、殺虫率を劇的に増加させた。PH_3ガス濃度が低い場合など、呼吸が阻害されない条件では、虫体に両くん蒸剤が十分に取り込まれ、両ガスによる相乗効果により殺虫率を上昇させたと考えられる。

しかし、蛹のSF 0.9 mg/l+PH_3 0.1 ～ 1.0 mg/l、24時間処理では、SFの単独くん蒸より殺虫率が著しく低下した。PH_3くん蒸の低薬量(0.025, 0.05及び0.1 mg/l)、20時間処理における蛹の麻痺状態の調査から、蛹では成虫・幼虫よりも、PH_3により敏感に呼吸が抑制され、SFの取り込み阻害が起こる可能性が示唆された。

コクゾウ成虫に対して、SFくん蒸、20 mg/l、3時間処理及びPH_3くん蒸、0.05 ～ 0.1 mg/l、4時間処理を、両薬剤のくん蒸順番を変えて実施した。両くん蒸の間に1時間の排気時間があるにも関わらず、くん蒸の順番に関係なく、殺虫率が向上し、PH_3とSFは混合くん蒸でない場合でも相乗効果を発揮した。

コクゾウ蛹をSF 0.9 mg/lで24時間くん蒸後、PH_3 1.0、1.5又は2.0 mg/lを追加投薬してさらに48時間くん蒸した処理では、混合ガスくん蒸の16.5%に比較して、殺虫率が84.5%まで上昇した。また、PH_3単位薬量の増加に伴って殺虫率も92.0%に上昇し、それら薬量区の殺虫率間では統計的に有意差が得られた（α=0.05）。

したがって、SFでくん蒸後、低濃度PH_3を追加投薬する「連続くん蒸」方法は、両薬剤の相乗効果を発揮させ、コクゾウムシ全態を完全殺虫する方法として適当と考えられた。

SF 2.2 mg/lで24時間くん蒸後にPH_3 1.0, 1.5又は2.0 mg/lを追加投薬して、さらに48時間くん蒸する連続くん蒸により、コクゾウムシ卵、幼虫、蛹の各態について約1,000頭が完全殺虫された。このことから、SFとPH_3の連続くん蒸はSitophilus属2種のすべての態を殺虫するのに効果的であると判断された。

* 退職
** 横浜植物防疫所宮古出張所