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Lethal effect of ozone gas on the adults of *Sitophilus oryzae* (Coleoptera: Curculionidae) and *Oryzaephilus surinamensis* (Coleoptera: Cucujidae)

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Introduction

For many years alternation of atomospheric gas concentrations or purging the storage facility with CO_2 or N_2 was considered as a possible means for protecting commodities against damage by stored-product insects (PRESS and HAREIN 1967, HAREIN and PRESS 1968). Interest has been stimulated in this method of control as a result of the increased concern with toxic residues which may result from the present chemical methods used.

On the other hand, some investigations were carried in lethal effects of inert gas, helium (COOK 1950, FRANKEL and SCHNEIDERMAN 1958, ALINIAZEE 1971, 1972) and argon (COOK 1950, FRANKEL and SCHNEIDERMAN 1958) on insects. A study of oxygen poisoning in insects also has been made by several authors (GOLDSMITH and SCHNEIDERMAN 1956, 1960, CLARK 1958, 1959, CARRIDEN 1960, CLARK and CRISTOFALO 1961, BOND 1965, ALINIAZEE 1973). In addition, a little is known about lethal effect of ozone on insects (BEARD 1965, LEVY, CHIU and CROMROY, 1972, LEVY, JOUVENAZ and CROMROY, 1674).

In the present paper investigation was undertaken to study the effect of ozone on the adult mortalities in the rice weevil, *Sitophilus oryzae* (L.) and the saw-toothed grain beetle, *Oryzaephilus surinamensis* (L.).

Materials and Method

The vessel used in the experiment was round open-topped plastic, inverted frustum in shape, 12 cm in diameter at the base, 15 cm at the top and 14 cm in height. The open side of the vessel was closed tightly with plastic lid having a central hole, 2.8 cm in diameter, covered by a vinyl cloth through which the air in the vessel and the excess ozone escaped all through the experiment. Ozone generator, "Fuji Neo Ozonator (YS-1)", manufactured by Yokoyama Electric Works Ltd., Kawasaki, Kanagawa, was used. The regulator of amount of ozone produced was set a scale at the degree of 10 (maximum). The gas was introduced continuously during the exposure period through this vinyl tube, 5 mm in diameter, passing through the lid from the generator at the bottom of the vessel. Flow rate in the test vessel was 3.5 l/min and amount of ozone produced ranged from 0.66 to 0.83 mg/min.

The adults of the rice weevil and the saw-toothed grain beetle were obtained from laboratory cultures maintained at about 28°C and about 70 % R. H., breeding in wheat for the rice weevil and 'oshimugi', barley pressed and flattened out after cleaning, for the saw-toothed grain beetle. No attempt was made to sex the adults. One hundred individuals of adults of unknown age of each species were placed in the vessel containing 50 g of wheat for the rice weevil and 30 g of 'oshimugi' for the saw-toothed grain beetle, after 5 minutes of replacing the air by stream of ozone.

The exposure periods were 10, 40, 50, 90, 120, 150, 180, 210, 256 and 300 minutes for the rice weevil and 1, 2, 3, 4, 6 and 18 hours for the saw-toothed grain beetle. Immediately after the exposure the numbers of inactive adults were recorded and then the insects were left in the vessel. Mortality counts were conducted each day up to ten days. Temperature in the experimental room during the treatment was about 24°C in average for the rice weevil and about 28°C in average for the saw-toothed grain beetle. As a control one hundred adults of each species were placed in the same condition with the experiments, except for the exposure to ozone and the mortality was recorded daily till ten days after the start.

Results and Discussion

Tables 1 and 2 show the number of inactive adults in per cent immediately after

Table 1. Comparison between the proportion of inactive adults immediately after the exposure and the mortality at one-day count for the rice weevil.

Exposure-time (min.)	10	40	50	90	120	150	180	210	256	300
Inactive adults immediatcly after exposure (%)	6	9	15	67	89	90	95	89	95	93
Mortality at one-day count (%)	6	20	21	18	41	76	73	61	80	87

Table 2. Comparison between the proportion of inactive adults immediately after the exposure and the mortality at one-day count for the saw-toothed grain beetle.

Exposure-time (hr.)	1	2	3	4	6	18
Inactive adults immediately after exposure (%)	18	14	10	15	19	31
Mortality at one-day count (%)	25	20	29	38	22	46

the exposure and the mortality at one-day count for the two species. For the rice weevil the proportion of adult knocked down was larger than that of death adults at one-day count in the exposure-times of more than 90 minutes, although for the saw-toothed grain beetle the mortality was higher than the proportion of inactive adults without exception. From the result it seems that the rice weevil is highly sensitive to ozone and temporarily fall into a state of coma after relatively long time exposure.

All mortality counts at each of the ten days after the exposure were corrected hereafter for the natural mortality using Abbott's formula and data were analyzed according to the method of probits. Figures 1 and 2 show the relative sensitivity of adults of the two species on each day. Log time-probit regression formula and median lethal exposure-time corresponding to 50 and 95 per cent mortality are shown in Tables 3 and 4.

For the rice weevil 100 per cent mortality occured at 4-day count in the 300 minutes, at 5-day count in the more than 150 minutes, and at 9-day count in the more than 120 minutes exposure, while for the saw-toothed grain beetle 100 per cent mortality did not occur in all the experiments. The values of LT_{50} and LT_{95} of the saw-toothed grain beetle do not vary practically after the sixth day. This means that the exposure to ozone has produced all the lethal effects on the beetles by the sixth day and

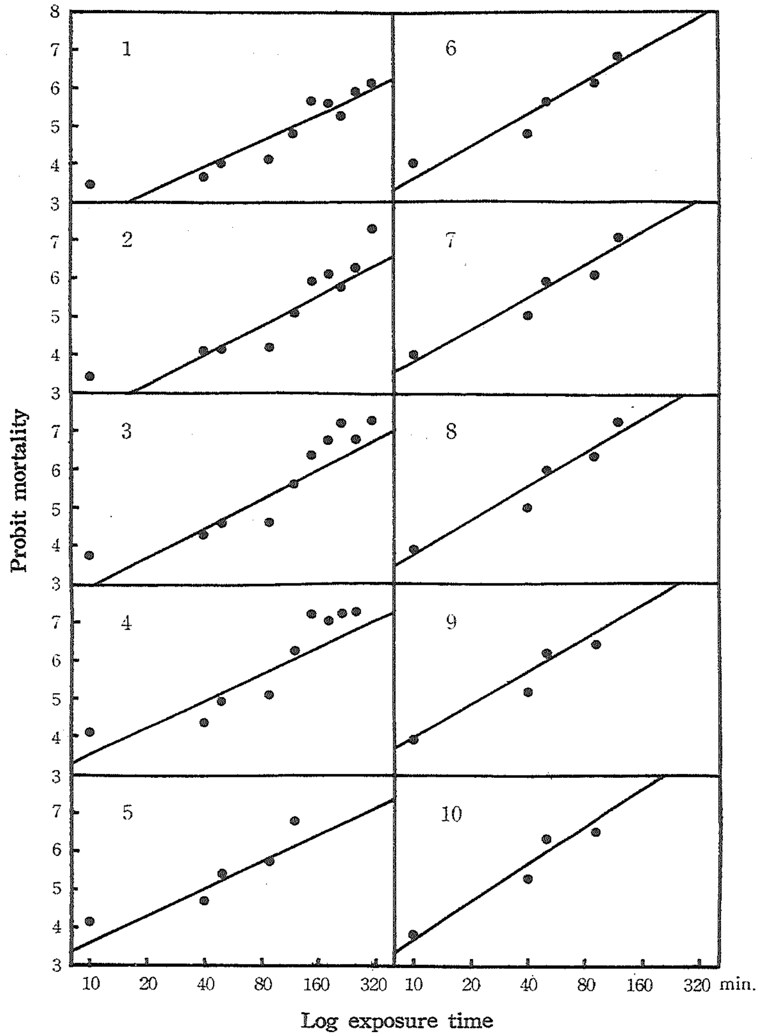

Fig. 1. Log exposure-time — probit mortality regression lines for the rice weevil exposed to ozone on each day after treatment. Numerals, 1 to 10, in the figure indicate days after exposure.

do not bring about additional effect after that.

The saw-toothed grain beetle requires very long time of exposure, about 10 or 100 hours, to reach 50 or 95 per cent mortality in contrast to short time of 25 or 70 minutes for the rice weevil at the tenth day after exposure. The saw-toothed grain beetle is about 86-fold more tolerable to ozone as compared with the rice weevil on the base of LT_{95} values.

Summary

Effect of ozone on the adult mortalities was investigated in the rice weevil, *Sitophilus oryzae* (L.) and the saw-toothed grain beetle, *Oryzaephilus surinamensis* (L.). The insects were exposed to flow of ozone of amount ranged from 0.66 to 0.83

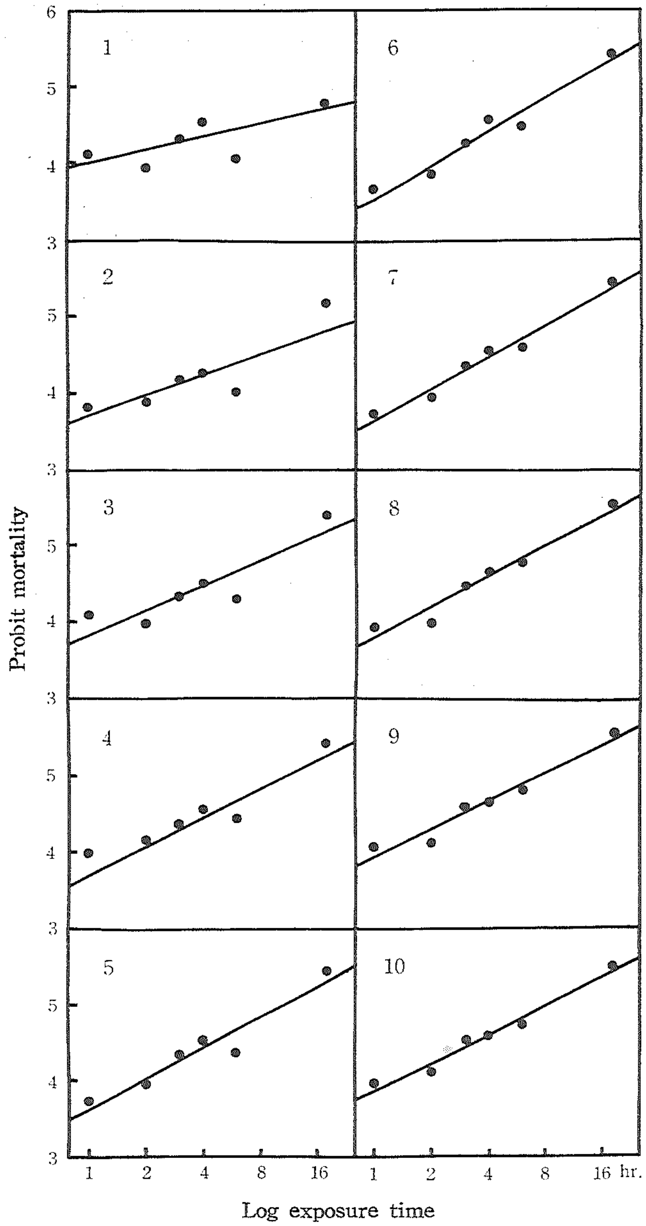

Fig. 2. Log exposure-time — probit mortality regression lines for the saw-toothed grain beetle exposed to ozone on each day after treatment. Numerals, 1 to 10, in the figure indicate days after exposure.

mg/min. The exposure periods were 10 to 300 minutes for the rice weevil and 1 to 18 hours for the saw-toothed grain beetle. Mortality counts were made at each of the ten days after exposure.

The rice weevil was highly sensitive to ozone and temporarily fall into a state of coma after more than 90 minutes exposures. The LT_{50} and LT_{95} values at the tenth

Table 3. Susceptibility of the rice weevil to ozone. Log exposure time-probit regression formula, LT_{50} and LT_{95} at each of the ten days after exposure.

Days after exposure	Regression formula (Y = log exposure-times (min.); X = probit mortalities)	LT_{50} (min.)	LT_{95} (min.)
1	Y = 0.1741 + 2.3225 X	119.65	501.08
2	Y = −0.1825 + 2.6046 X	97.68	350.27
3	Y = 0.3779 + 2.5434 X	65.68	242.83
4	Y = 1.1508 + 2.3546 X	43.13	177.17
5	Y = 0.7719 + 2.7609 X	34.00	113.42
6	Y = 0.9268 + 2.7485 X	30.35	101.80
7	Y = 0.9393 + 2.8197 X	27.56	89.64
8	Y = 0.7532 + 2.9707 X	26.89	82.40
9	Y = 1.0596 + 2.8711 X*	23.58	75.09
10	Y = 0.4087 + 3.2844 X*	25.00	68.82

* Significant regression at the 5 % level.

Table 4. Susceptibility of the saw-toothed grain beetle to ozone. Log exposure time-probit regression formula, LT_{50} and LT_{95} at each of the ten days after exposure.

Days after exposure	Regression formula (Y = log exposure-times (hr.); X = probit mortalities)	LT_{50} (hr.)	LT_{95} (hr.)
1	Y = 4.0125 + 0.5417 X	66.5	30917.0
2	Y = 3.7093 + 0.8634 X*	31.3	1473.7
3	Y = 3.8358 + 1.0865 X	11.8	251.9
4	Y = 3.6847 + 1.2605 X	11.1	154.8
5	Y = 3.6203 + 1.3649 X*	10.3	117.3
6	Y = 3.5338 + 1.4827 X*	9.7	91.9
7	Y = 3.6335 + 1.4205 X*	9.2	95.3
8	Y = 3.8069 + 1.3213 X*	9.9	99.2
9	Y = 3.9172 + 1.2415 X*	7.4	108.5
10	Y = 3.8533 + 1.2655 X*	8.1	111.7

* Significant regression at the 5 % level.

day after exposure were about 25 and 70 minutes for the rice weevil and about 10 and 100 hours for the saw-toothed grain beetle. The saw-toothed grain beetle was about 86-fold more tolerable to ozone than the rice weevil.

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オゾンのココクゾウ，ノコギリコクヌスト成虫に対する致死効果

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残留農薬の毒性がひきおこす問題を回避して貯穀害虫を防除する一つの方法として，CO_2, N_2, O_2, He, Ar の致死効果が調べられている．しかし，O_3 の致死効果についての研究は，あまり行なわれていない．O_3 発生量 0.66〜0.83 mg/min，通気量 3.5 l/min の気体に，それぞれ 100 匹のココクゾウ成虫を 10〜300 分間，ノコギリコクヌスト成虫を 1〜18 時間さらし，その後の死亡率を 10 日間にわたって毎日調べた．ココクゾウはO_3に弱く，90 分以上さらすと多数が一時動かなくなる．2 時間の暴露で 9 日後には 100 %が死亡する．ノコギリコクヌストは O_3 に対し極めて強い．ここでの 18 時間までの暴露では，その致死効果は 6 日後までに出つくし，以後に効果は出ない．暴露 10 日後の LT_{50}，LT_{95} の値はココクゾウで約 25，70 分，ノコギリコクヌストで約 10，100 時間であった．LT_{95} の値を基にして計算すると，ノコギリコクヌストは O_3 に対し，ココクゾウの 86 倍も強い．

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