LOW TEMPERATURES: EFFECTS ON CONTROL OF SITOPHILUS ORYZAE (L.) WITH MODIFIED ATMOSPHERES

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INTRODUCTION

A considerable amount of information has been obtained in recent years concerning the effects of modified or controlled atmospheres on stored-product insects. However, most laboratory studies have been carried out at temperatures ranging from 26° to 33° C. Bailey and Banks (1974), in a summary of 10 of the more recent laboratory studies, found only one that was conducted at temperatures as low as 15.6° C, and this one (Harein and Press, 1968) had to do only with adult Tribolium castaneum (Herbst). Again, when Banks (1978) summarized results of field studies with modified atmospheres, he noted only one low temperature large-scale field study (Banks et al., 1978). This was conducted at 13.5° C, and 77 to 79% mortality of all life stages of Sitophilus oryzae (L.) was obtained by a 10-day exposure to atmospheres containing from 47 to 68% carbon dioxide (CO_2) (balance of modified atmosphere was air).

Earlier Banks and Annis (1977) had suggested when a storage facility containing grain at 15° C is purged with nitrogen (N_2) for insect control, the exposure time must be greater than 24 wk for complete disinfestation, even when the oxygen (O_2) level is held at 0-1.2%.

Because of the lack of information on the combination of low temperature and modified atmospheres, the following study was undertaken.

METHODS AND MATERIALS

The insects used in this study were immature S. oryzae that are continuously reared at the Savannah laboratory on 12% m.c. soft red winter wheat at 26.7° C and 60% RH. The cultures for the tests were established by seeding 360 g of this wheat with ca. six hundred 1- to 3-wk-old adults for 72 hr. At the end of this period, the adults were sieved off leaving immature insects 0 to 3 days old in the cultures. S. oryzae used in these tests were therefore from 0 to 3, 7 to 10, 14 to 17, 21 to 24, or 28 to 31 days old at the beginning of the exposure. Wheat containing insects of these ages was blended together for 5 min in a ball mill, and 5 g of this blend was placed in a 16 x 14 (to cm)

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mesh wire screen cage measuring 6.4 cm high x 1.9 cm diam. An adequate number of cages was filled to provide a group of three 5-g cages for each exposure plus another cage that was used as the control for each group.

The exposure chambers were similar to those described by Harein and Press (1968) and consisted of 2.8-liter glass jars that were partly submerged in laboratory baths filled with water. These baths were equipped with refrigeration systems so the water temperature could be reduced to levels below the ambient temperature. Four such baths were used, and they were individually set to maintain the water temperature at ca. 1.6°, 4.4°, 10.0°, or 15.0° C. The jars were closed with metal screw-top lids fitted with 23- and 2.5-cm lengths of 0.6-cm o.d. copper tubing; these were used as gas inlet and gas exit tubes, respectively. The lids were also fitted with a neoprene stopper so a humidity sensor could be inserted. During exposure, the cages were suspended in the exposure chambers from a 5-cm length of steel wire hung from the underside of the neoprene stopper.

The gas mixtures were released from the cylinders through two-stage regulators and flowed through a micrometering valve and flowmeter into gas washing bottles that contained a glycerin-water mixture that adjusted the RH of the gases to ca. 56%. The gases then flowed into the exposure chambers. A flow rate of 200 cc/min was used for the first hour to purge the chambers. A rate of 30 cc/min was used for the balance of the exposure periods. The RH was monitored with an electric hydrometer (model 15-2001 humidity indicator and narrow range humidity sensors, Hygro-dynamics, Inc.), and the temperature was recorded daily.[1/]

A Fisher-Hamilton model 29 gas partitioner equipped with dual columns was used for daily analysis of ternary mixtures. A Vidar model 6300 digital integrator was used to measure the areas under the peaks. A Beckman model E-2 oxygen analyzer was used for daily analysis of binary mixtures.

Insects and grain at four temperatures were exposed to gases from cylinders containing air, 100% N_2, 100% CO_2, or a blend containing ca. 60% CO_2, 9% O_2, and 31% N_2. (Small leaks in the system reduced the actual concentrations of 100% N_2 to ca. 99% and 100% CO_2 to ca. 98%; this was expected). Exposures were for periods of 1, 2, 3, or 4 wk. Controls were held in similar cages in a room maintained at 26.7°±1°C. and 60±5% RH. Four replicates (3 cages per replicate) were tested at each of the four temperatures and four exposure periods for normal air and with the CO_2 mixture. Three replicates (3 cages per replicate) were tested similarly with the ca. 100% CO_2 flow and two with the ca. 100% N_2 flow.

At the end of the exposure period, the contents of the cages were placed in 120-ml glass containers with filter paper lids and held at 26.7°C. for 1 wk. Emergence counts were made at 1 wk and weekly thereafter for 5 more wk.

Effectiveness of treatment was determined by dividing the total number of insects that emerged after treatment by the total number that emerged in the controls and converting this to percent reduction in emergence (RIE).

RESULTS AND DISCUSSION

Samples of atmosphere analyzed for all replicates had the following percentages (± S.D.) of carbon dioxide, oxygen, and nitrogen: normal air - 20.9±0.1% O_2, balance N_2 and rare gases; CO_2 mixture - 60.4±0.7% CO_2, 8.9±0.2% O_2, and 30.7±0.6% N_2; 100% CO_2 - 97.7±0.9% CO_2, 0.4±0.2% O_2, and 1.9±0.6% N_2; and 100% N_2 - 0.5±0.1% O_2 and 99.6±0.1% N_2. The mean (± S.D.) of the relative humitities were: natural air - 55.9±3.1%; in CO_2 mixture - 56.0±3.3%; 100% CO_2 - 56.2±3.2%; and in 100% N_2 - 55.3±2.8%. Mean temperatures (± S.D.'s) in the exposure chambers are given in Tables 1 through 4.

Tables 1 through 4 present results of exposures to the normal air and to three modified atmospheres at the four temperatures. At 1.6° C (Table 1) there was no emergence of insects exposed to normal air or to 98% CO_2 after 2 wk of exposure. Exposures of this length to the CO_2 mixture or to 99% N_2 gave a 99% or higher RIE, but increasing the exposure time to 3 or 4 wk did not produce a 100% RIE when insects were exposed to the CO_2 mixture. Those exposed 4 wk to 99% N_2 had a 100% RIE.

At 4.7° C, the only atmospheres producing 100% RIE were: normal air after a 3-wk exposure and 98% CO_2 after a 4-wk exposure (Table 2); however, these two atmospheres gave a 99% RIE after an exposure of 2 wk. S. oryzae exposed to the CO_2 mixture had RIE of over 99% after a 3-wk exposure; those exposed 4 wk to 99% N_2 had a 99.9% RIE.

At 10.4° C, the only 100% RIE occurred when insects were exposed to the CO_2 mixture for 4 wk (Table 3). However, the RIE was over 99% when insects were exposed to the CO_2 mixture for 3 wk or 98% CO_2 for 4 wk. When the insects were exposed to air or to 99% N_2, mortality gradually increased with increasing exposure time and reached about 98% after 4 wk.

At 15.7° C, the RIE was 99% after a 2-wk exposure to the CO_2 mixture and 100% after a 3-wk exposure (Table 4). Insects exposed for 3 or 4 wk to 98% CO_2 or to 98% N_2 for 4 wk had an RIE above 99%.

Low numbers of insects emerged from the 60% CO_2 and 99% N_2 atmospheres after 2- or 3-week exposures at 1.6° C. The fact that no emergence occurred from the samples exposed to only air is assumed to be the result of the cold reducing the respiration rate of the insects to a low level and thus preventing significant, but not total, venting of the abnormal atmospheres. This venting in response to atmospheres high in N_2 or in response to atmospheres high in CO_2 but with some oxygen present prevents complete anoxia which has been shown to be one of the major causes of death due to exposure to these atmospheres. This venting

TABLE 1

Percent reduction in emergence (% RIE) when immature S. oryzae were exposed to air or to one of three modified atmospheres at 1.6°±0.1° C (S.D.) for indicated periods.*

Atmosphere	% RIE after exposure of (wk)			
	1	2	3	4
Air	98.7	100.0	100.0	100.0
60% CO_2	95.6	99.4	99.8	99.9
98% CO_2	99.8	100.0	100.0	100.0
99% N_2	94.5	99.1	99.9	100.0

TABLE 2

Percent reduction in emergence (% RIE) when immature S. oryzae were exposed to air or to one of three modified atmospheres at 4.7°±0.2° C (S.D.) for indicated periods.*

Atmosphere	% RIE after exposure of (wk)			
	1	2	3	4
Air	93.3	99.8	100.0	100.0
60% CO_2	71.0	94.6	99.7	99.9
98% CO_2	90.1	99.3	99.9	100.0
99% N_2	50.3	91.0	97.6	99.9

*Mean and S.D. for adult emergence for all controls (26.7° C) were 1 wk - 63.8±15; 2 wk - 89.6±45.5; 3 wk - 172.8±41.5; and 4 wk - 239.5±35.9.

TABLE 3

Percent reduction in emergence (% RIE) when immature S. oryzae were exposed to air or to one of three modified atmospheres at 10.4°±0.6° C (S.D.) for indicated periods.*

Atmosphere	% RIE after exposure of (wk)			
	1	2	3	4
Air	89.3	99.4	95.0	97.9
60% CO_2	72.6	92.4	99.4	100.0
98% CO_2	76.1	89.5	95.6	99.6
99% N_2	64.3	72.5	84.6	98.1

TABLE 4

Percent reduction in emergence (% RIE) when immature S. oryzae were exposed to air or to one of three modified atmospheres at 15.7°±0.5° C (S.D.) for indicated periods.*

Atmosphere	% RIE after exposure of (wk)			
	1	2	3	4
Air	67.1	88.5	89.3	89.1
60% CO_2	80.5	99.0	100.0	100.0
98% CO_2	97.4	97.2	99.6	99.9
99% N_2	41.7	86.0	82.7	99.7

*Mean and S.D. for adult emergence for all controls (26.7° C) were 1 wk - 63.8±15; 2 wk - 89.6±45.5; 3 wk - 172.8±41.5; and 4 wk - 239.5±35.9.

is associated with spiracular control and body weight loss. The majority of the weight loss is assumed to be water, and death is partially caused by desiccation in high N_2 atmospheres (Navarro, 1978). Jay and Cuff (unpublished manuscript) confirmed this relationship in experiments that showed a high weight loss in larvae, pupae, and adults of T. castaneum when they were exposed to a ca. 60% CO_2 concentration similar to the one used in these tests. Therefore, we assumed that the S. oryzae in the present tests, even in the presence of low temperatures, reacted enough to actively engage in the venting or pumping of their respiratory systems. Their metabolic rate was thereby increased, and their body temperature was slightly increased so they were actually protected from the direct effects of the low temperatures, but, the magnitude of this activity was not enough to induce heavy water losses and desiccation causing death.

Jay and Cuff (unpublished manuscript) also showed that in very high CO_2 concentrations, similar to the 98% CO_2 used in these studies, the body weight of T. castaneum was not reduced as mortality increased. Friedlander and Navarro (1979) studied the mode of action of high CO_2 concentrations on pupae of Ephestia cautella (Walker). They found that treatments with 99% CO_2 drastically reduced ATP levels in these insects. In the tests reported here, 98% CO_2 gave 100% RIE after 2, 3, and 4 wk of exposure at 1.6° C, but cold alone did the same when the insects were exposed to normal air. I therefore believe that the insects exposed to 98% CO_2 were completely anesthetized, could not raise their body temperature by venting, and were therefore killed by a combination of low temperature and CO_2.

From a practical standpoint then, there is little need to use modified atmospheres or any other control techniques against S. oryzae when grain temperatures are below 10.4° C. The cold alone will produce a high mortality, almost 98% RIE, after 4 wk. At a temperature of 15.7° C, the mixture with 60% CO_2 will give a RIE of 99% in 2 wk and 100% in 3 wk, and an atmosphere containing 98% CO_2 will give a RIE of over 99% in 3 wk but would be more expensive to use. Moreover, at this temperature, cold alone gave an RIE of almost 90% in 2 wk, though it did not increase its effectiveness by additional exposures of up to 4 wk. The data reported here compare favorably with those of Banks (1978) who obtained 77 to 79% mortality with a 10-day exposure to 47 and 68% CO_2 at 13.5° C. For example, an exposure to the mixture contained 60% CO_2 at 10.4° C for 1 wk gave 72.6% RIE; a 2-wk exposure to this concentration and temperature gave a 92.4% RIE (Table 3). However, they cannot be compared with the estimated mortality of Banks and Annis (1977) for an exposure time of more than 24 wk at 15° C for high N_2 (low O_2) concentrations. Since Table 4 shows that a 4-wk exposure at 15.7° C to 99% N_2 will give a 99.7% RIE, a 100% RIE would probably be obtained by an additional exposure of 1 to 2 wk, not 20 wk.

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FOOTNOTE

1/Mention of a commercial product in this paper does not constitute an endorsement of this product by the USDA.