MORTALITY OF VARIOUS STORED PRODUCT INSECTS IN LOW OXYGEN ATMOSPHERES PRODUCED BY AN EXOTHERMIC INERT ATMOSPHERE GENERATOR

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ABSTRACT

Atmospheres produced by an exothermic inert atmosphere generator and composed of less than 1% oxygen, about 9-9.5% carbon dioxide, and the balance principally nitrogen were found to be lethal to all stages of both internally and externally developing stored grain insects. Time periods required for control varied between species and between life stages within the same species. Pupae and mature larvae of internally developing species were generally the most tolerant stages and immature larvae and adults of externally developing species the most susceptible. Eggs of most species were not especially tolerant of exposure to the low oxygen atmosphere, but did exhibit a tendency to become increasingly tolerant after the first day of development. Tolerance during larval-pupal development of most species also increased with each successive period of growth after hatching. The maximum period of treatment at 27°C required to give control of the most tolerant insect species was about 10 days; however, most species were either killed or suffered severe physiological damage after 3 to 5 days of exposure. As treatment temperature decreased the length of exposure time required to obtain effective control significantly increased.

INTRODUCTION

Insect infestations are one of the principal factors that adversely affect the quality of stored commodities. The food storage, transportation and processing industries, and the consumer suffer large monetary losses from stored product insects that cause damage, lower nutritional value, and make products unfit for human consumption. The presence of insects and the damage they cause also discredits the quality of agriculture products in highly competitive foreign markets. Present chemical methods used to control insects may leave objectionable residues in the treated commodity and are generally hazardous to handle and apply; also, some stored product insects are developing resistance to chemical treatment. There is therefore urgent need for acceptable and effective methods of preventing insect damage and contamination of agriculture products that would reduce or eliminate dependence on chemical pesticides during storage, handling, processing, packaging, transportation, and retail.

The insecticidal activity of modified atmospheres of oxygen, carbon dioxide and nitrogen has long been recognized and research programs to develop the use of these atmospheres for insect control are in progress in several locations throughout the world.

Four factors are essential to the use of modified atmospheres for control of insects in stored commodities; 1) the atmosphere must be easily obtained in sufficient volume to displace existing atmosphere in large bulk storage; 2) the atmosphere must be lethal to storage pests within a reasonable time; 3) the atmosphere must have no harmful effect on the quality of the treated commodity, and 4) the storage structure must have an adequate gas holding capability (Storey 1979).

One technique for producing high volumes of an oxygen-deficient atmosphere is the ignition of air and a fuel gas in an exothermic inert atmosphere generator. Such generators are used for metallurgical and chemical processes that require low oxygen environments and also by the food industry to retard metabolism and preserve freshness of fruits and vegetables. The feed industry has used generated atmospheres for the preservation of vitamin content, color, weight and palatability of alfalfa pellets (Kruger 1960). Composition of the generated atmosphere varies with the fuel-to-air ratio and composition of fuel gas burned, but usually consists of less than 1% oxygen, 9-9.5% carbon dioxide, 86-89% nitrogen, 1% argon, and 1.5% or less each of hydrogen and carbon monoxide.

Research was undertaken at the Science and Education Administration-Agricultural Research, U.S.D.A. Grain Marketing Research Laboratory to explore the use of inert atmosphere generators in regulating insect populations in stored products. Data presented here were obtained from the following references: Storey 1975a, b, c; Storey and Soderstrom 1977, Storey 1977, and Storey 1978.

MATERIALS AND METHODS

Tests to determine the toxicity of the generated atmosphere to various life stage of the major insect pests infesting stored products were conducted in a pilot inert atmosphere treatment system consisting of a laboratory-scale generator, a series of cylindrical metal silos 45 cm in diameter by 4.27 m high, a series of incubators modified to permit treatment of the insects at selected temperatures, and pneumatic grain handling equipment for loading and unloading the silos. The small scale generator was built by Gas Atmospheres, Inc., Port Washington, Wisconsin and produce about 2.83 m^3/hr (100 cu. ft/h) of combustion atmosphere in which the concentration of oxygen measured by a Seromex® paramagmetic oxygen analyzer was generally between 0.1 and 0.25% and rarely exceeded 0.5%.

During operation, natural gas and air are ignited under controlled pressure at a ratio of ca. 1 part gas to 10 parts air. Combustion takes place in a water-cooled, refractory-lined combusion chamber. The exhaust gas from the chamber passes through a water-cooled plate-coil and into a condensate separator. Water removed from the gas leaves the separator through a seal leg located beneath the separator (Fig. 1).

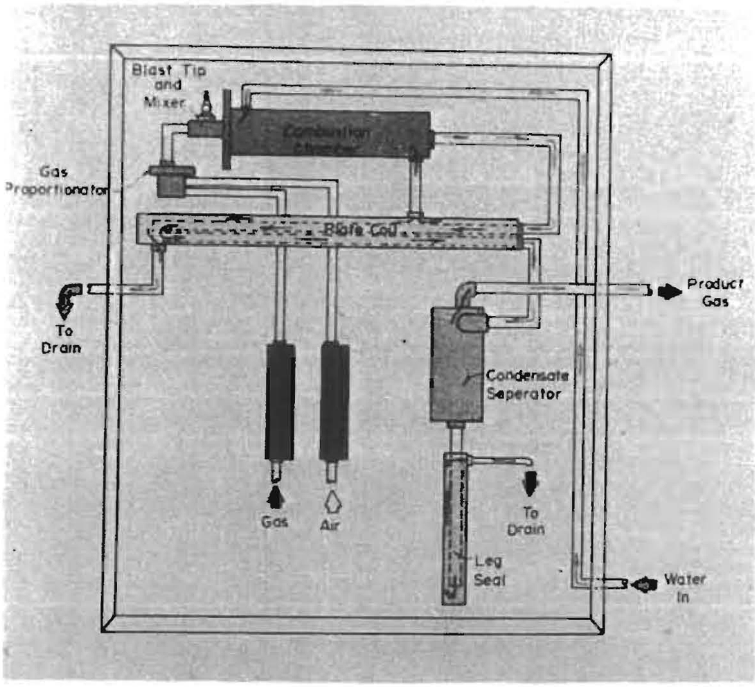

Fig. 1. Flow chart of laboratory scale inert atmosphere generator. During operation natural gas and air are ignited under controlled pressure at a ratio of about 1 part gas to 10 parts air. Combustion takes place in a watercooled refractory-lined combustion chamber. The exhaust gas from the chamber passes through a water-cooled platecoil and into a condensate separator

The RH of the inert atmosphere was maintained at 50 $\pm$ 5% by passing the inert atmosphere through a self-draining manifold immersed in a refrigerated constant temperature water bath to lower the temperature of the inert atmosphere below the dewpoint and then reheating the gas to room temperature before release into the silos or temperature-regulated incubators.

Silos used in the testing program were equipped with perforated metal tubes about 2 cm dia placed crosswise through the silos at intervals of 60 cm (Fig. 2). Threaded fittings on the end of the tubes permitted insertion of screened cages containing test insects and also served as sample points for analysis of the atmosphere within the silos. Each silo was loaded with 545 kg of hard winter wheat with a moisture content of about 12%. The inert atmosphere was released at the base of the silos and passed upward through the grain mass. Air exhausting from the top of the silos was vented to the outside. The rate of flow of inert atmosphere into each silo was 1.5 liter/min. Oxygen levels in the inert atmosphere were measured

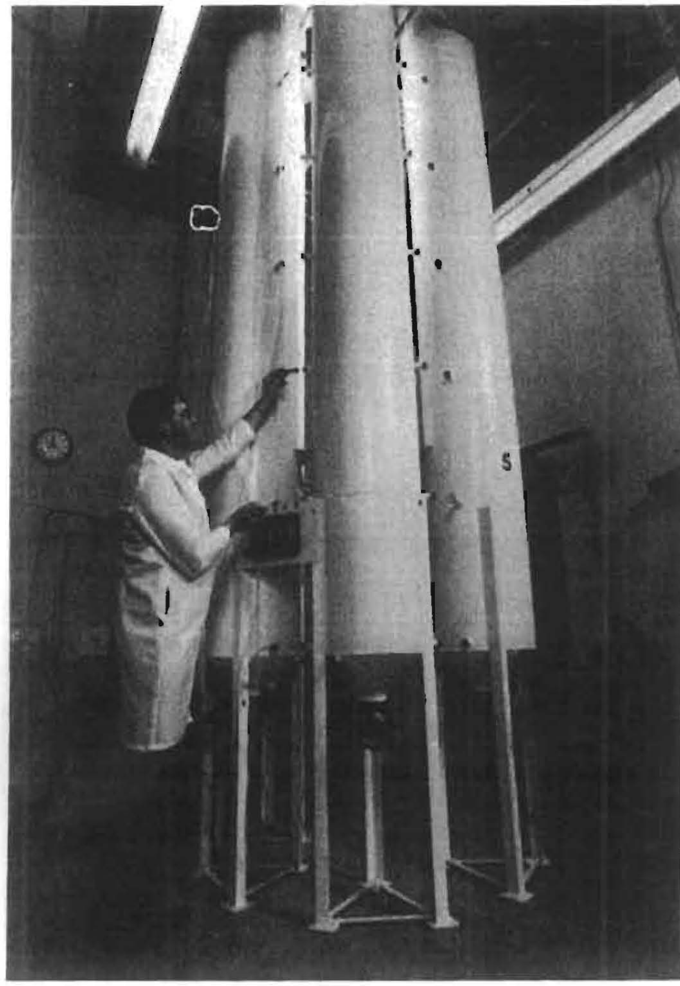

Fig. 2. Cages of test insects are inserted in metal silos (45 cm in diameter by 4.27 m high) containing 545 kg of wheat. Generated atmosphere is passed upward through the silos.

periodically throughout the testing period.

Test insects were also exposed to the generated atmosphere in 0.47-liter jars placed in the incubators. Each jar was equipped with a lid fitted with a rubber stopper. Plastic lines inserted through the stoppers served as inlet and outlet tubes for the generated atmosphere. The rate of gas flow through the jars ranged from 25 to 50 cc/min. Airflow rates were controlled by purge meters that adjusted and monitored the air movement through each jar. Test insects together with a suitable food supply were placed in separate small screen cages in the jars or directly into food medium held in the jar and exposed to the atmosphere flowing through the jar. Tests were conducted over a temperature range of 15 to 32 $\pm$ 2°C for exposure periods of 1 to 336 hours depending on the species and stage of development. Most treatments were replicated three times. Parallel control experiments were conducted in atmospheric air for each combination of species, stage of development, exposure time and treatment temperature. Mortalities of some immature stages were based on the relative numbers of adults emerging from treated and untreated samples; other mortality data were based on direct counts of living insects from specific numbers of insects exposed to the treatment.

Times required to kill 95% of the treated insects were estimated by transforming mortality data to probits and calculating the regression of probits on time. Lethal times (LT) were then estimated by using the linear calibration technique described by Snedecor and Cochran (1967).

RESULTS

The effectiveness of the generated inert atmosphere varied between insect species and between the development stages of the same species. A comparison by species and stage of the time periods required for 95% (or 100%) mortality when the temperature was 27°C and the R.H. was 50 ± 5% is shown in Table 1. Pupae and mature larvae of internally developing species such as the weevils (Sitophilus spp.) and lesser

TABLE 1.

A comparison of time (hours) required for 95 or 100% mortality of various stages of insects exposed at 27°C and 50 ± 5% RH to an atmosphere produced by an exothermic atmosphere generator: Composition, 1.0% O_2 and 9.0-9.5% CO_2, the balance principally N_2.

Insect	Eggs1/	Larvae2/	Pupae1/	Adults
Rice weevil (Sitophilus oryzae)	70	79-246	107-241	48
Granary weevil (Sitophilus granarius)	85	38-137	120-148	55
Lesser grain borer (Rhyzopertha dominica)3/	72	72-192	144-216	36
Angoumois grain moth (Sitotroga cerealella)3/	48	72-120	120	24
Navel orangeworm (Amyelois transitella)	8-28	13-27	35-38	17
Confused flour beetle (Tribolium confusum)	30-40	7-20	24-53	17
Red flour beetle (Tribolium castaneum)	25-40	8-23	17-47	18
Indianmeal moth (Plodia interpunctella)3/	24	8	24	8
Almond moth (Ephestia cautella)3/	48	8	24	8
Cowpea weevil (Callosobruchus maculatus)3/	96	120-192	192	48

1/ Range over each day of development.
2/ Range over each week of development.
3/ Minimum exposure time for 100% mortality.

grain borer (Rhyzopertha dominica (F.)) were generally the most tolerant stages and early-instar larvae and adults of externally developing species such as the flour beetles (Tribolium spp.), the Indianmeal moth (Plodia interpunctella (Hubner)), and almond moth (Ephestia cautella (Walker)) were the most susceptible. Eggs, which often exhibit a high degree of resistance to chemical fumigation, were not especially tolerant of the generated atmosphere, but the eggs of some species such as the flour beetles tended to become more tolerant after the first day of development. For example, 1-day-old eggs of T. castaneum (Herbst) required only 25 hours of exposure to cause 95% mortality, but 4-day-old eggs required nearly 40 hours of exposure to achieve the same level of mortality. Age response to the treatment among eggs of the navel orangeworm, Amyelois transitella (Walker) showed an opposite effect with 0 to 1-day-old eggs needing only 8 hours of exposure. Tolerance during the larval-pupal period of most species tended to increase with each successive period of development after hatching. Among Sitophilus spp. the period of greatest tolerance appeared to correspond with the cessation of feeding by the mature larvae and to end midway through the pupal development period. Susceptibility of Tribolium spp. during the pupal period followed a U-shaped course that more closely paralleled the oxygen uptake and the productions of carbon dioxide common during pupal metabolism, that is, there were high uptake-production values and increased susceptibility during the early and late pupal periods and low uptake-production values and decreased susceptibility near mid-pupal life.

Temperature had a pronounced influence on the length of time it took to kill insects exposed to the oxygen deficient generated atmosphere. The time required to kill 95% of various species of adult stored-product insects was reduced by about 90% when the treatment temperature was increased from 15° to 32°C (Table 2). Within

TABLE 2.

Time (hours) required to obtain 95% mortality of adult stored-product insects exposed at 4 temperatures to a modified atmosphere produced by an exothermic inert atmosphere generator: composition, 1.0% O_2 and 9.0-9.5% CO_2, the balance principally N_2.

Temperature	S. oryzae	S. granarius	R. dominica	T. castaneum	O. surinamensis
32°C	19	20	17	8	4
27°C	48	55	31	17	11
21°C	200	145	79	32	18
15°C	297	228	175	67	47

this temperature range the treatment times were reduced more for Sitophilus spp. as temperature was increased from 21 to 27°C. Other species responded most to an increase from 15 to 21°C. Treatment times for developing stages of S. oryzae (L.) ranged from 48 hours for adults to 246 hours for 4th-instar larvae when the temperature was 27°C, and was 108 hours for 1st-instar larvae and 653 hours for 1-day-old pupae when the temperature was 21°C (Table 3).

Although death was the principal criterion used in evaluating the effectiveness of the generated atmosphere, other responses were observed. Insects were immobilized soon (generally in less than 60 seconds) after exposure to the atmosphere and did not become active throughout the period of exposure. Some immobilized insects exposed for sublethal subsequently had only a delay in development time approximately equal to the period of exposure. Others similarly exposed developed partial paralysis, particularly in the posterior segments, and appeared incapable of

TABLE 3.

Time required (hours) to obtain 95% mortality of developing stages of rice weevil (Sitophilus oryzae) exposed at two temperatures to a modified atmosphere produced by an exothermic, inert atmosphere generator: composition 1.0% O_2 and 9.0-9.5% CO_2, the balance principally N_2.

Temperature	Eggs	Larvae	Pupae	Adult
27°C	70	79-246	245-107	48
21°C	162	108-562	653-537	200

defecation. Larvae, so stricken, were often observed with dried fecal material protruding from the abdomen; none survived to the pupal stage. Some adults emerging from exposed pupal stages of the flour beetle, *T. castaneum*, and the cowpea weevil, (*Callosobruchus maculatus* (F.) were less than normal size and had rudimentary wings; others never developed adult characteristics in the posterior segments: In addition, sublethal exposures of the adult navel orangeworm reduced the number of progeny produced by surviving adults (Table 4): exposures of 1/2 to 3 hours did not kill

TABLE 4.

Percent mortality and number of progeny of adult navel orangeworms exposed at 27 ± 1°C to low O_2 atmosphere produced by an exothermic inert atmosphere generator.

Exposure hours	% Mortality[a] in 48 hours	Progeny/25 adults[a]
Control (untreated)	0	122.0
1/2	0	123.0
1	0	88.0
2	0	1.3
3	0	1.0
4	6	1.0
8	26	0.0
12	64	0.0
24	100	0.0

[a]Avg of 3 replicates.

any exposed adults, but the number of 2nd-generation adult progeny declined from 123 among adults exposed for 1/2 hour to only 1 progeny from adults exposed for 3 hours. Adults surviving 4-hour exposure deposited viable eggs, but the newly hatched larvae had limited mobility and most died during the first week of development. Some eggs were deposited by adults surviving 8-hour exposures, but the red coloration typical of developing eggs of the navel orangeworm was not observed and none of the eggs hatched. None of the adults surviving 12-hour exposures deposited eggs.

CONCLUSIONS

Four general conclusions can be drawn from results obtained in studies of the insecticidal activity of generated low oxygen atmospheres: (1) the atmosphere is lethal to all life stages (egg, larva, pupa and adult) of the common beetles and moths that infest stored-products, (2) susceptibility to the atmosphere varies substantially between species and between the various stages of development within each species, (3) effectiveness of the generated atmosphere is primarily a product of exposure time and treatment temperature; time periods required for control decrease as treatment temperature increases, and (4) sublethal exposures to the generated atmosphere often result in morphological abnormalities and physiological damage that disrupts or prevents normal development.

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