Use of Gaseous Nitrogen for Controlling Stored-Product Insects in Cereal Grains

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ABSTRACT

Research was conducted on several insect species to determine the feasibility of using N_2 as a means of controlling stored-grain insects. Test results show that insects can be controlled in stored grain by replacing most of the O_2 with N_2. It was shown that insect mortality is a function of the following factors: 1) temperature, 2) insect species, 3) O_2 concentration, and 4) exposure time.

At some time within the normal marketing channel, cereal grains are placed in storage facilities. Even though the length of storage will depend on ultimate utilization, most of the grain produced each year must be held in storage for periods of time which can influence quality. Since storage periods normally exceed 30 days, the major hazard in storing low-moisture grain is damage by insects.

It is possible to control insects in stored grain by frequent inspections and fumigations. However, since infestation is a cycling problem, repeated use of most fumigants has caused additional problems which in some cases are more dangerous than contaminated grain. Fumigants are dangerous, and personnel handling them may be subjected to undue health hazards. Moreover, repeated use of some fumigants produces a residue to such an extent that the grain could be condemned for food or feed. Therefore, any method of storing grain to eliminate these problems would be advantageous.

The abundance of N_2 has stimulated interest in recent years in the O_2 exhaustion method of controlling insects. Oxley and Wickenden (1) reported in 1963 that if grain insects develop in a strictly air-tight container, all are killed by exhaustion of O_2 when the concentration of this gas falls to approximately 2%. The time required to obtain 100% kill by this method depends to a great degree on the kind of insect and insect population. Low insect densities of six adults per lb. of grain required as long as 30 days before complete kills were obtained compared to 10 days at population densities of 60 adults per lb.

Commercial operations involving grain handling and storage normally will not permit the air-tight container method to be used for insect control. Not only would it be difficult to obtain a completely air-tight grain storage structure, but this method has a disadvantage in that insect populations must be allowed to build up before the oxygen supply is depleted in the grain interstices.

Research was conducted to study the feasibility of using N_2 to rapidly remove the O_2 supply from the grain interstices, thereby creating an undesirable environment for stored-grain insects. In practice, this would be done by initially

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purging the grain storage structure with N_2 until the O_2 content was reduced to a level lethal to insect populations. The high $N_2:O_2$ ratio atmosphere would then be maintained for a period sufficient for complete insect mortality. The methods used in these studies were designed to determine the influence of the following factors on insect mortality: 1) temperature, 2) insect species, 3) O_2 concentration, and 4) exposure time.

PROCEDURE

To determine the influence of certain factors on insect mortality, rice weevils (*Sitophilus oryzae* L.) and flat grain beetles (*Cryptolestes pusiluss* Scönkerr) were placed in samples of sorghum grain and exposed to gas mixtures containing the following compositions: (a) 0.5% O_2:99.5% N_2, (b) 1% O_2:99% N_2, and (c) atmospheric air (21% O_2). To conduct these tests in the range of temperatures in which major infestations normally occur, insects were exposed to the various gas compositions at 70°,80°,and 90°F. for 6, 12, 24, 36, and 48 hr. Additional tests were run at periods longer than 48 hr. in an effort to determine the minimum exposure period for 100% mortality. Data from the additional tests are included in the results but were not utilized in any statistical analysis.

A diagram of equipment and gas flow path is shown in Fig. 1. Pressure of premixed gases was reduced by a flow regulator in series with a two-stage O_2 regulator. To maintain the grain moisture content at approximately 13% (w.b.), test gases were humidified by passing a portion of the volume through a gas washer. A hand valve was located on a by-pass line around the washer bottle to regulate the relative humidity of gas entering the insect exposure containers. A sensing chamber was installed in the gas line for monitoring purposes.

The gas flow rate was maintained by flow controllers located in the supply line to each set of exposure containers. Each set represented one exposure time and was

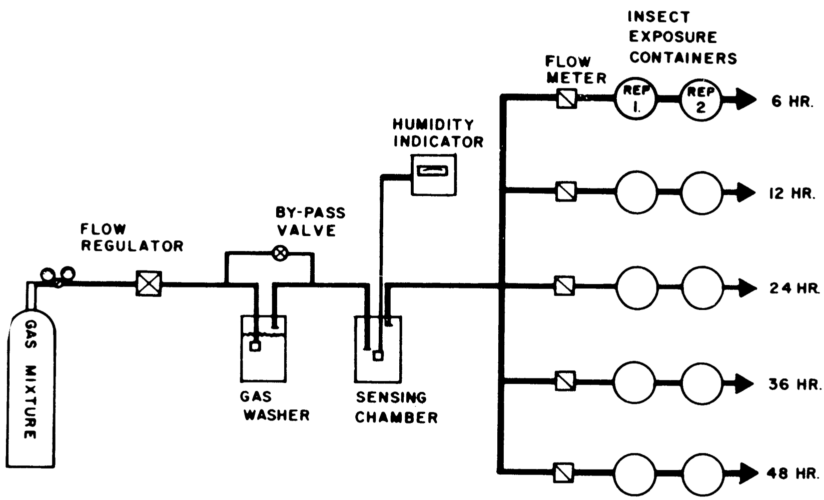

Fig. 1. Diagram of equipment arrangement and gas flow path used to study the influence of certain factors on insect mortality.

composed of replicated containers placed in series. Since the gases were premixed, a flow rate of 20 to 30 cc. per min. for each set was selected. This was sufficient to prevent any diffusion of O_2 into the exposure containers.

In each test, one insect species was exposed to one temperature at different O_2 concentrations and exposure times. Tests were run by sealing 30 adult insects and 1 lb. of sorghum grain in insect exposure containers constructed from No. 404 by 700 cans. A plenum chamber was formed in the lower section with 80-mesh wire and 3/16-in. copper tube inlet and outlet ports were soldered in the plenum and above the grain surface of each can.

The grain and insect mixtures were preconditioned in quart jars with screen tops at the test temperature for at least 12 hr. prior to each test. After the preconditioning period, the insects and grain were placed in the exposure chambers and these were sealed. The containers were then connected to the gas mixture equipment and purged at a high flow rate with the test gases. The outlet of each series was monitored with an O_2 analyzer to determine when purging was completed. The flow rate was then reduced to a level sufficient to prevent any diffusion of O_2 from the outside.

Since the 0.5 and 1% O_2 tests were run simultaneously, it was necessary to use only one set of atmospheric air (21% O_2) containers to serve as a check for each test. These insects were handled in a manner similar to that used for the other concentrations of O_2, with the exception of gas flow rate. To limit equipment requirements and at the same time make the tests as critical as possible, the 21% O_2 samples were sealed in air-tight containers without gas flow.

At the end of each exposure period, containers were removed and grain and insects were placed in glass jars for short periods of time to allow any inactive insects to revive before counting was done for mortality determinations. After the initial count, the test samples were placed in jars in controlled-temperature chambers for 2 days additional, after which time they were recounted to ensure than an inactive insect would not be accidentally counted as dead.

RESULTS

Results of these tests were tabulated as percent live and percent dead insects in each treatment, and a statistical analysis was performed on the percent dead. Some of the results are also presented as percent mortality. These data were obtained through the use of Abbott's formula (2), which states:

$$\%\ \text{Mortality} = \frac{\%\ \text{live in check} - \%\ \text{live in treatment}}{\%\ \text{live in check}} \times 100$$

This formula adjusted the treatment data for any naturally occurring deaths in untreated groups.

The major mortality findings are given in Figs. 2 and 3. These results indicate that all stored-grain insects do not react the same under identical conditions, nor will like species respond the same under different storage conditions. Statistical analyses of the results show that there were highly significant differences at the 1% level in the percent of insects dead due to temperature, insect species, O_2 concentration, and exposure time. There was no significant difference associated with the test replications.

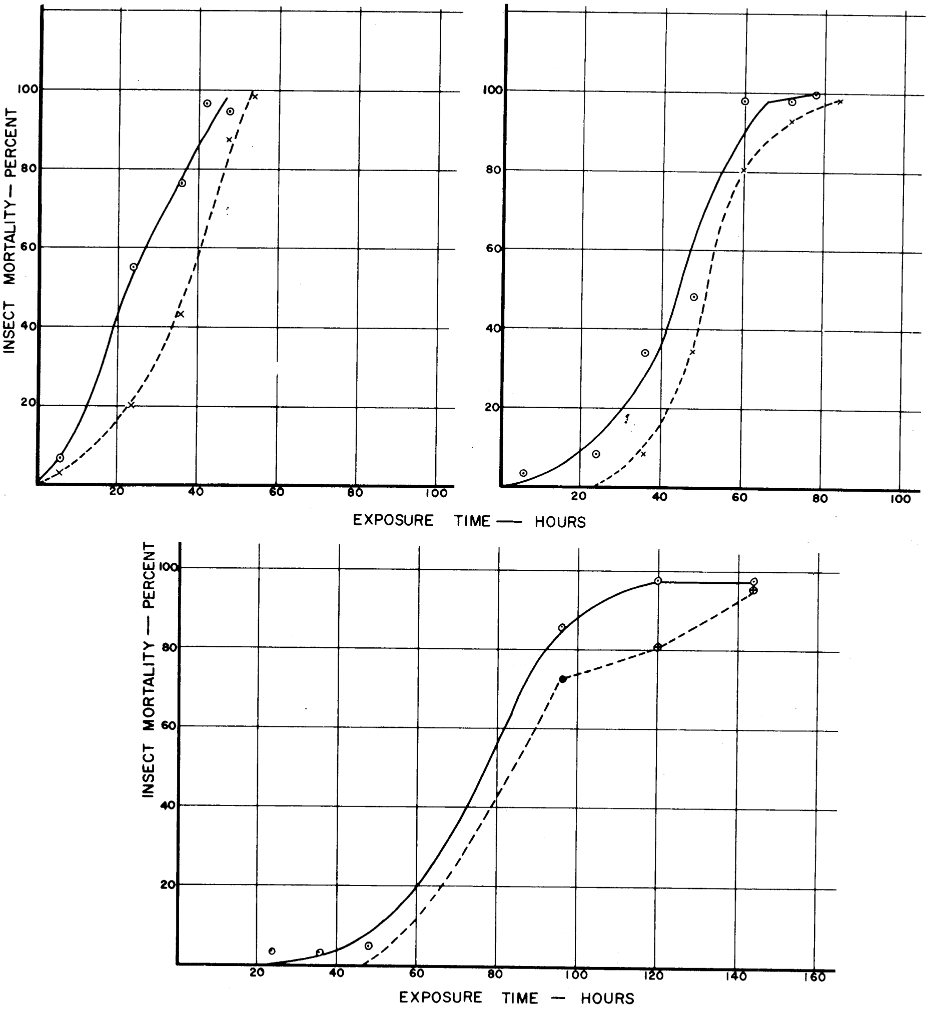

Fig. 2. Effect of exposure time on mortality of rice weevils exposed to: upper left and upper right, 90° and 80°F., respectively, and lower, 70°F.; and oxygen concentrations of: continuous line, 0.5%; dashed line, 1.0%.

Temperature

Results of these tests showed that temperatures normally found in stored grain affect the life span of stored-grain insects. Generally, the higher the temperature, the shorter the life cycle of the insects because of higher rates of respiratory metabolism. This temperature relation is very apparent in storage atmospheres containing low concentrations of O_2. Raising the storage temperature in the absence of normal concentrations of O_2 caused a rise in the normal O_2 requirements of the insects.

Regardless of insect species, raising the storage temperature always shortened

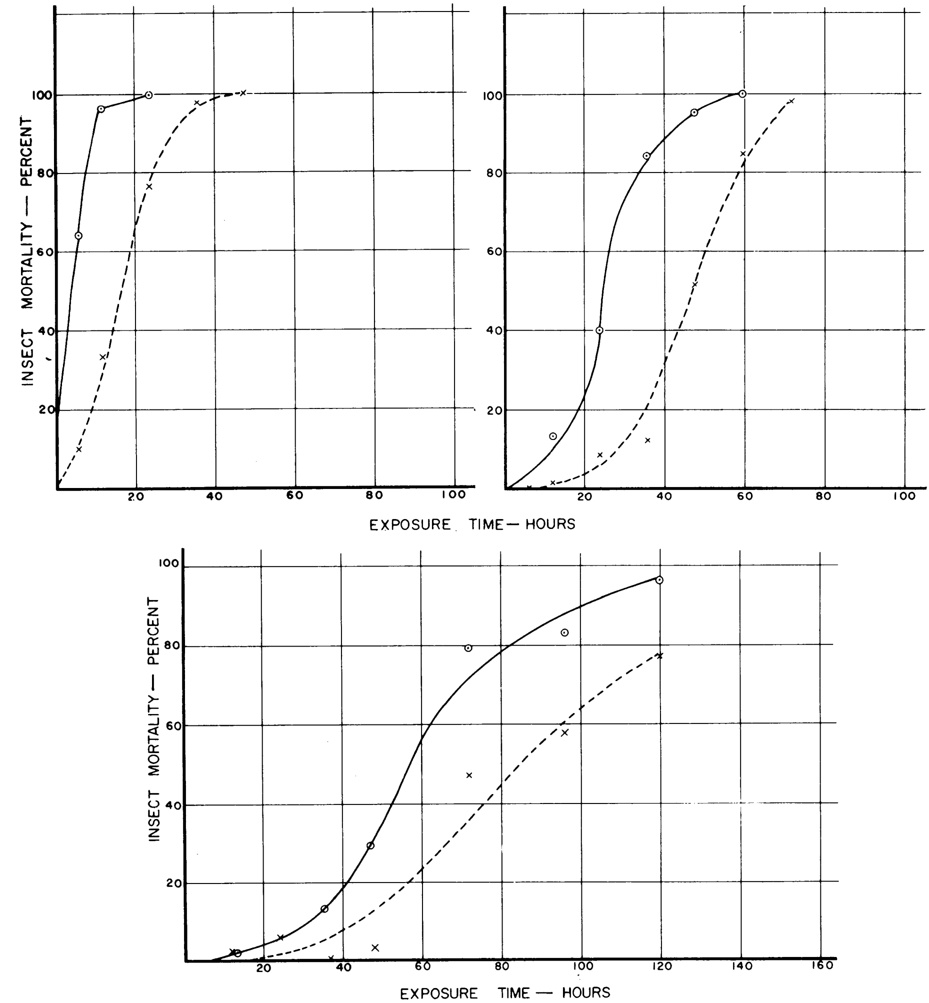

Fig. 3. Effect of exposure time on mortality of flat grain beetles exposed to different temperatures and oxygen concentrations (same as in Fig. 2).

the exposure time required to obtain any level of mortality. For example, the exposure time required to obtain 90% mortality of rice weevils stored in 0.5% O_2 atmospheres was 102, 60, and 42 hr. when the temperatures were 70°, 80°, and 90°F., respectively. The time required to obtain 90% mortality of flat grain beetles was 100, 41, and 10 hr., respectively, over the same range of temperatures.

The results in Figs. 2 and 3 indicate that less exposure time is actually required at any level of mortality when the temperature is raised as compared to a decrease in the O_2 concentration. A decrease of 9 hr. in exposure time required for 90% rice weevil mortality at 80°F. resulted when the O_2 concentration was reduced from 1 to 0.5%. When the O_2 concentrations were held constant, a reduction in exposure time of approximately 18 hr. resulted when temperatures were raised from 80° to 90°F.

Insect Species

Analyses of research data yielded a very high significance in insect mortality due to species. The flat grain beetle was less resistant to O_2 depletion than the rice weevil. This held true for all temperatures, O_2 concentrations, and exposure times. There was a significant interaction at the 1% level between all variables tested and insect species.

The significant difference in the interactions indicate that both insect species did not react the same under all conditions. However, of the two insect species, the flat grain beetle was always the easiest to kill.

Oxygen Concentration

Ross (3) has stated that insects are remarkably resistant to O_2 deficiency and that the rate at which they are able to extract O_2 from the atmosphere remains the same down to a very low level of O_2 pressure. All insects confined in inert atmospheres react in very much the same way. Cotton (4) has observed that after a brief period of intense activity, occasioned by their efforts to obtain air and to escape from an undesirable environment, they sink into a state of coma in which all muscular activity ceases and metabolism proceeds at a much retarded pace. If the insects are not restored to a normal atmosphere after a certain period of time, death results.

Tests were run on rice weevils at 90°F. to determine if small quantities of O_2 in the atmosphere would result in faster kill rates than zero O_2 concentrations. It appeared at the beginning of this research that keeping the insects as active as possible to increase their O_2 requirements would actually be better than having zero O_2 tolerance. Results presented in Fig. 4 did not substantiate this. There was essentially no difference in the mortality of insects exposed to 0 and 0.5% O_2 atmospheres. There was a considerable difference, however, when compared to 1 and 3% concentrations. Practically no mortality was obtained when rice weevils were exposed to O_2 concentrations of 3% at 90°F. for exposure periods up to 36 hr. After exposure for 48 hr., mortality increased to approximately 38% compared to 95 and 100% for insects exposed to O_2 concentrations of 1 and 0.5%, respectively.

Exposure Time

The time which insect species must be held in low-O_2 atmospheres is of considerable economic importance. Very few grain storage structures can be classified as air-tight, and therefore a constant purge of inert gases must be maintained to exclude outside O_2. The quantity of gas required for insect control will depend not only on the desired O_2 level, but also on the total exposure time necessary for 100% mortality. Statistical analysis of the results indicates that there was a significant difference in the percent kill due to exposure time. The longer the exposure period for any O_2 concentration, the higher the percent mortality (Figs. 2 and 3).

A significant interaction existed between exposure time and temperature. In

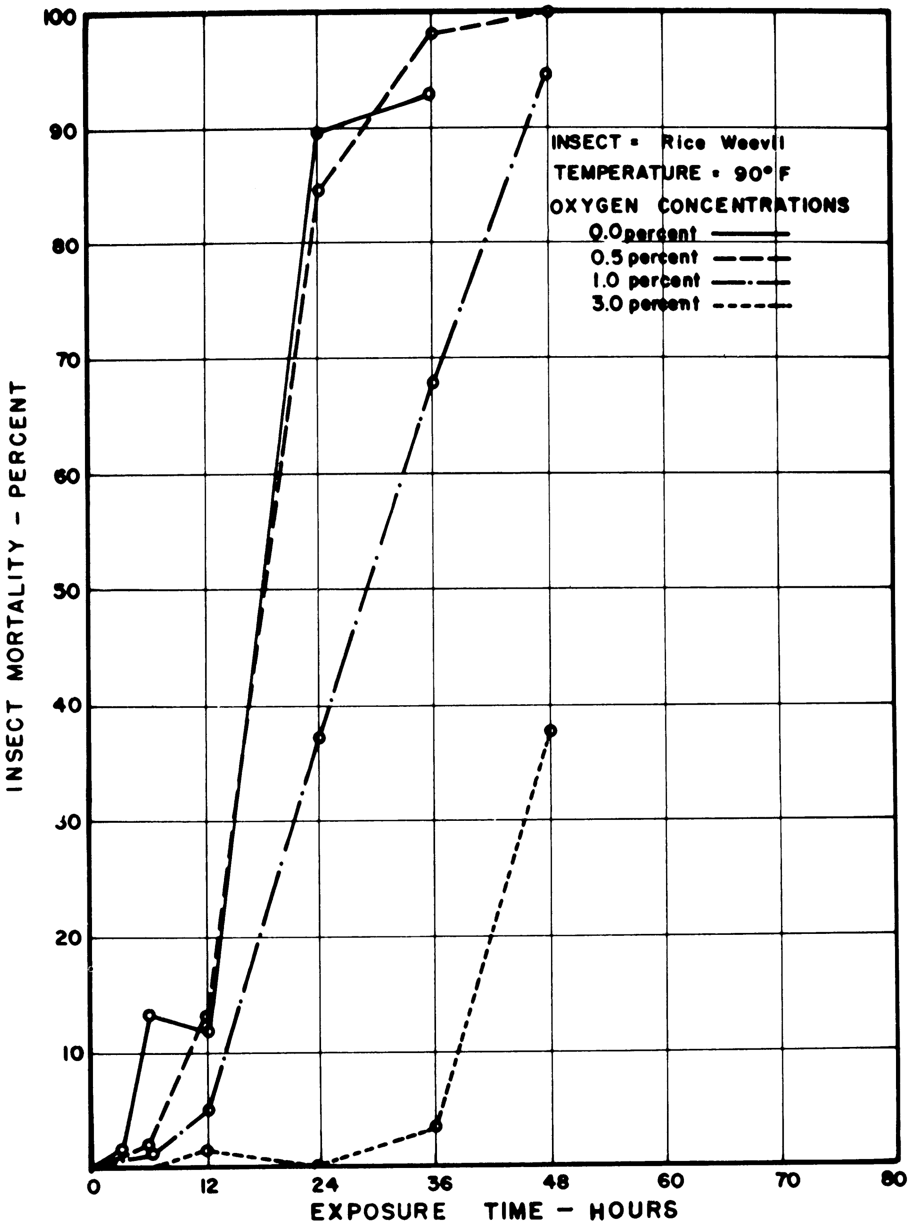

Fig. 4. Effect of exposure time on mortality of rice weevils exposed to several levels of oxygen.

other words, the exposure periods and O_2 concentrations did not cause the same results for all test temperatures. There was a relatively small difference in percent dead at 70°F. due to exposure time, but this difference increased at 80° and 90°F.

Regardless of temperature level, however, the longer exposure times always resulted in greater kills.

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