Toxicity of High Oxygen Atmospheres to *Tribolium castaneum* (Coleoptera: Tenebrionidae)[1, 2]

Moshe Calderon, J. G. Leesch[3] and E. G. Jay[3]

Agricultural Research Organization, The Volcani Center
Institute for Technology and Storage of Agricultural Products
Bet Dagan, Israel

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ABSTRACT Adults, larvae, and pupae of *Tribolium castaneum* (Herbst) were exposed to atmospheres containing 70%, 80%, and 90% O_2 for 7, 14, 21, and 28 days. Ninty-nine percent morality of adults was obtained after 21 days at 90% O_2, while a maximum mortality of 80 and 38% was achieved after 28 days at 80% and 70% O_2 respectively. The effect of O_2 on larvae was found to be greater than on pupae: 100% larval mortality was obtained at 90% O_2 after 14 days while complete mortality was achieved with pupae after 28 days.

After treatment with high O_2, the appearance of abnormal insects as well as effects on their life cycle were observed. When larvae were treated for 14 days at 80% O_2, only a few individuals developed to adults. No larvae reached the adult stage after exposure to 90% O_2 for 14 days. In all treatments, except those conducted at 90% O_2, insects exposed as pupae developed into adults.

KEY WORDS Insecta, mortality, flour beetle, treatment.

In recent years, extensive research has been done on the use of modified atmospheres to control stored product insects. Results of this research and achievements in the area of controlled atmopsheres are summarized in the proceedings of two international meetings (Shejbal 1980, Ripp et al. 1984). Most research and its applications have centered on uses of carbon dioxide and/or oxygen removal from atmospheres. However, little has been published on the effects of high O_2 concentrations on these insects.

It is well known that oxygen, at high concentrations, does have definite deleterious effects on plants, micro-organisms, and animals, including insects (Stadie et al. 1944). The effect of hyperoxia on insects has been the subject of many classic investigations on insects, e. g., *Drosophila* (Williams and Beecher 1944), the American cockroach (Walter et al. 1974), a chalcid wasp (Goldsmith and Schneiderman 1960). Some investigations included stored product insects, such as *Anagasta kuehniella* (Zeller), *Tenebrio molitor* L. (Clark and Cristofalo 1961), and *Tribolium confusum* (Jacquelin DuVal (Lee and Ducoff 1983). However, these studies were mainly concerned with the physiological aspects of hyperoxia and not the resulting mortality. In the 1970's, hyperoxia in insects was investigated from

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[2] This article reports the results of research only. Mention of a proprietary product or pesticide does not constitute an endorsement or recommendation for its use by USDA, nor does it imply registration under FIFRA, as amended.

[3] Stored-Product Insects Research and Development Laboratory USDA-ARS, Savannah, GA 31403.

the space biology point of view studying problems associated with the high oxygen environment present when simulating space flights (Brown and Hines 1976; Kloek et al. 1978; Kloek and Winkle 1979).

Data on toxicity of oxygen to stored-product insects are very scarce compared to those using modified atmospheres for stored product insects control. To the best of our knowledge, the only work dealing with this problem is that of AliNiazee (1973), who exposed two *Tribolium* species to atmospheres of 100% O_2. His results suggest that high O_2 could be used for insect control. This research reports the effects of high O_2 concentrations and different exposure times on the mortality of a stored-product insect.

Materials and Methods

All tests were conducted in a room in which a temperature of 26.6 ± 1°C and a relative humidity of 60 ± 5% RH were maintained.

Gas mixture supply system. Required gas mixtures were obtained from gas cyclinders by mixing compressed oxygen and nitrogen through Fisher-Porter flowmeters which are designed to blend the gases. Delivery of the gases to be mixed was made through 2-stage pressure regulators from tanks of pure gas. After mixing, the resulting gas was regulated to flow at about 50cc/min into Fisher-Mulligan Gas washing bottles, which contained a mixture of water and glycerin in order to obtain an RH of 59.5° ± 3%. The humidified gas mixture was then split into two main streams using plastic tubes, so that flow of the same gas mixture was obtained from the main stream passing the flowmeter. All tubing used in the system was 0.6 cm. I.D. Tygon®.

The exposure chambers. Each flow of determined, modified atmosphere was then split in four, so that a separate tube conveying the test gas mixture was directed into an individual 0.24-liter glass jar exposure chamber. The chambers metal lids were fitted with inlet and outlet copper tubes of 0.6 cm I.D., and also with hooks to hang the cages containing the test insects inside each chamber. Air flow went into each chamber through the longer tube which opened near the bottom of the chamber and came out through the shorter one in the top. The intended air flow rate was 10 cc/min to each chamber.

The test insects. The red flour beetle, *Tribolium castaneum* (Herbst), was used for all tests. The insects were reared at 27 ± 1°C and 60 ± 5% RH, on a 1:1 mixture of wheat flour and maize meal, supplemented by 5% Brewers' yeast (by volume). The ages of the life stages used in these tests (means ± S.D.) were as follows: larvae - 22.3 ± 3.6 days, pupae - 30.0 ± 3.7 days, and adults - 41.0 ± 2.7 days.

Age was determined from the time parent adults were added to the media until the insects of different life stages were selected for exposure. Pupae and adults were placed in 40 × 30 mesh monel wire cages, 6.4 cm high by 1.9 cm in diameter. Larvae were placed in 76 × 76 mesh cages as above, measuring 5.1 cm high by 1.3 cm in diameter.

Exposure and postexposure procedures. Cages containing 50 adults, 25 larvae or 25 pupae each, were suspended from the lid of each chamber. After sealing the chamber, insects were exposed to atmospheres containing 70%, 80% and 90% O_2 (balance made up with N_2) for periods of 7, 14, 21 and 28 days. Each combination of O_2 concentration and time of exposure was replicated 3 times for

each of the 3 stages of the insect tested. Control insects were exposed in a similar manner in containers in which the air flow came from a cylinder with compressed air. The air flow rate maintained in all jars was 10.9 ± 3.8 cc/min. The constant air flow eliminated the possible effect of accumulation of CO_2 due to insect metablolism. The gas composition was determined twice a day during the experiment using a Fisher-Hamilton Model 29 Gas Partitioner®.

At the end of each exposure period, the insects were removed from exposure chambers and placed in plastic Petri dishes containing a few grams of rearing medium and placed in a postexposure room maintained at 26.6 ± 1°C, and 60 ± 5% RH. Mortality counts were made 14 days after exposure. Counts to determine the percentge of each stage in the population were made 42 days after exposure.

Results and Discussion

Mean compositions (% ±SD) of atmospheres flowing through the exposure chambers were as follows:

Conc.

Intended O_2	% O_2 Found	% N_2 Found
70%	70.0 ± 1.8	29.8 ± 1.7
80%	78.6 ± 3.1	21.3 ± 1.6
90%	90.4 ± 2.0	9.6 ± 1.5
Control (air)	21.6 ± 0.4	78.8 ± 6.0

Effect on adult mortality. The effect of the three high O_2 concentrations on adults exposed for different times can be seen in Fig. 1. The corrected adult mortality is plotted as obtained from probit analyses (Daum 1970). Only exposure to 90% O_2 resulted in 99% mortality after 21 days (Table 1). The maximum mortality obtained with 80% O_2 was 80%, which occurred after 28 days of exposure. Probit-mortality curves indicate the decreasing slope of the response of this species to decreasing concentrations of O_2. At 70% O_2, LT_{95} was predicted to be about 200 days, while the LT_{99} for 80% O_2 was about 60 days. These results indicate that 90% O_2 may be promising for the control of *T. castaneum* adults while the two lower O_2 concentrations do not seem to be of practical importance.

Effect on pupal and larval mortality. Table 1 shows the relative susceptibility of the 3 developmental stages to the given O_2 concentrations as a function of exposure time. The larval stage is most susceptible to these treatments when the exposure is for 14 days or more. While 90% O_2 caused 100% mortality after only 14 days, at 80% O_2, 28 days were required to give 100% mortality. The effect on pupae was similar to that on adults, except for the shortest exposure (7 days) to 90% O_2, when pupal mortality was much higher than for adults.

Counts made 42 days after exposure recorded all different developmental stages of live and dead insects 6 weeks after treatments. Treated larvae or pupae which developed into adult beetles were considered alive even if the adult was found dead. So, long exposures of larvae may have affected pupal development from surviving larvae, or adults in the case of treated pupae. Therefore, the mortality figures do not show at what stage death occurred, or to what stage the immatures were able to develop.

Table 1. Percent mortality (corrected means ± SE) of *Tribolium castaneum* exposed to high O_2 concentration for various exposure times. Mortality was determined 14 days post-exposure.

Stage	7 days	14 days	21 days	28 days
		Mortality at: 70% O_2		
Pupae	1.6 ± 1.4	11.4 ± 3.7	8.1 ± 2.0	25.6 ± 6.9
Larvae	0.0	75.2 ± 0.3	82.0 ± 4.1	85.2 ± 6.1
Adults	0.7 ± 0.7	15.2 ± 1.1	27.0 ± 6.3	37.6 ± 6.1
		80% O_2		
Pupae	24.7	28.1 ± 22.0	43.4 ± 25.5	80.0 ± 10.9
Larvae	0.0	93.5 ± 1.2	91.3 ± 8.7	100 ± 00
Adults	4.34 ± 0.9	41.5 ± 1.9	54.9 ± 3.8	80.5 ± 3.7
		90% O_2		
Pupae	86.5 ± 1.5	91.7 ± 14.3	98.6 ± 2.4	100.0 ± 00
Larvae	83.7 ± 1.6	100 ± 00	100 ± 00	100 ± 00
Adults	4.03 ± 1.0	84.1 ± 0.6	99.7 ± 0.3	99.3 ± 0.4

The effect of high O_2 atmospheres on the ability of larvae and pupae to complete their life cycle is shown in Figures 2 and 3. At the 7 and 14-day exposures to 70% and 80% oxygen, very few of the treated larvae were affected and most completed their life cycle to adults (Fig. 2). However, few insects exposed as larvae developed to adults after exposure to any of the O_2 concentrations for 21 or more days.

Figure 3 shows that in the 42-day post-exposure counts the pupal stage is more tolerant to high O_2 exposures than the larval stage as was true in the 14 day post-exposure counts. Regardless of the length of exposure to 70% O_2, all pupae reached the adult stage and at 80% O_2, only low pupal mortality resulted. The results show the higher susceptibility of larvae to the treatments as compared to that of pupae and adults. The results also demonstrate the high mortality and lack of development of all stages after exposure to 90% O_2 for 14 days or more.

These results differ from these of Clark and Cristofalo (1961) who found that "larvae are more oxygen resistant . . . than are pupae." This disagreement is difficult to reconcile because of the differences in the method of treatment, concentrations of O_2 used and difference in species of insects tested. We found that the shortest exposures to 70% and 80% O_2, larvae were less affected than pupae and adults, but that larvae had a much greater susceptibility than pupae or adults at the longer exposures (Table 1). The inability of treated larvae, in most treatments, to complete their life cycle was also obvious (Fig. 2). AliNiazee (1973), treated two *Tribolium* species with 100% O_2 and found that the immature stages of these insects were more susceptible to oxygen poisoning than adults. However, the author did not specify any differences in susceptibility between larvae and pupae.

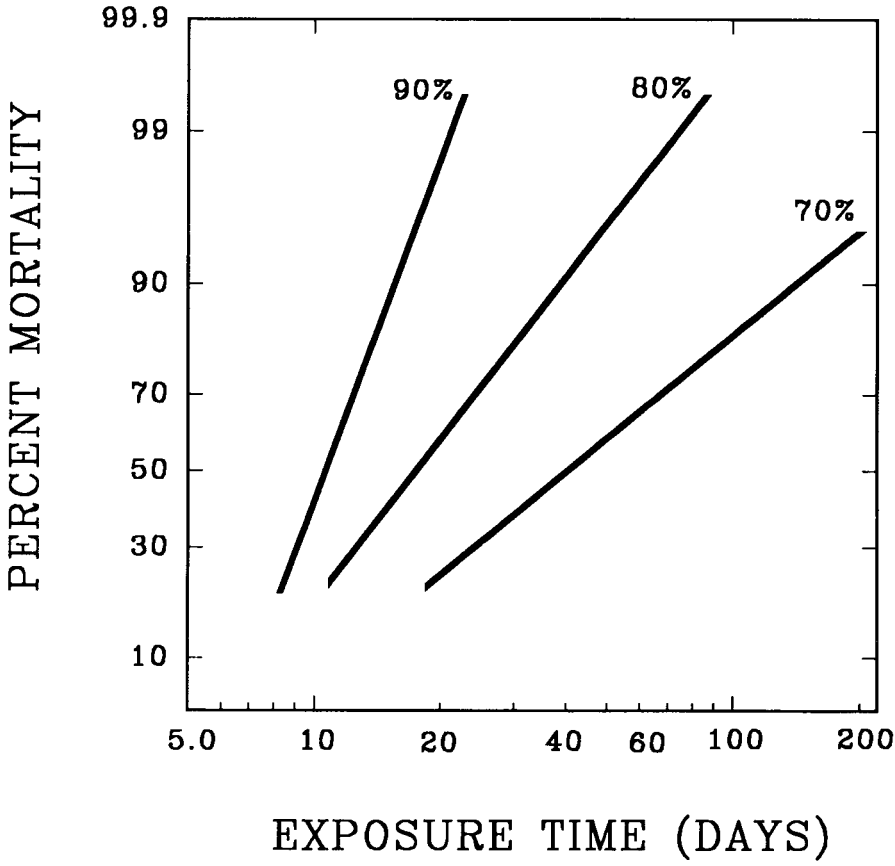

Fig. 1. The predicted mortality of *T. castaneum* adults versus time of exposure to 3 concentrations of oxygen. Mortality determined at 14 days post-exposure.

Abnormal insects. Following exposures to high O_2 concentrations, and particularly during longer exposures to 80% and 90% O_2, partial or full paralysis of some insects was observed. The most common phenomenon was the inability of larvae to pupate or of pupae to eclose as adults. There were abnormal wingless pupae-adult forms, which remained alive for several days. The deformation and the partial paralysis were also seen in adults after the longer exposures to 80% and 90% O_2. Abnormalities caused by oxygen toxicity have been reported previously (Clark and Cristofalo 1961, AliNiazee 1973).

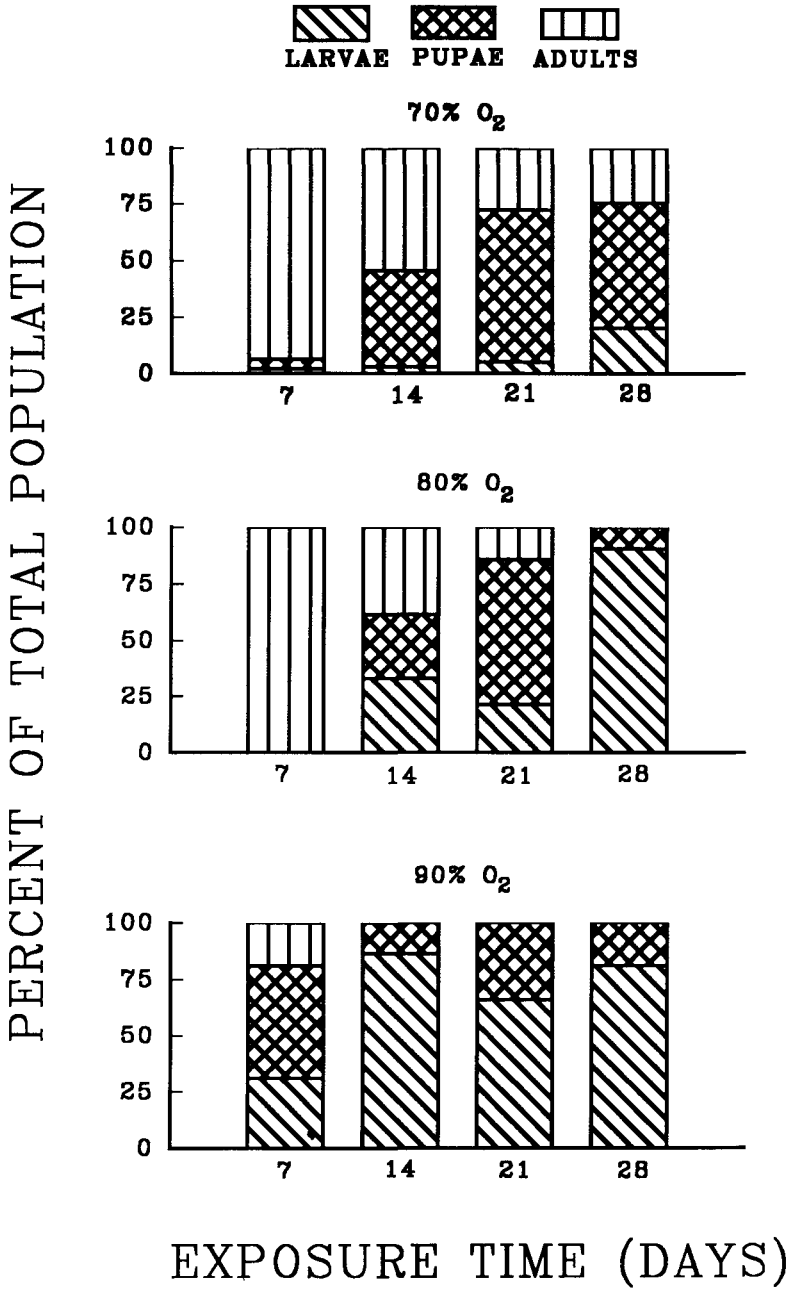

Fig. 2. Composition of populations of *T. castaneum* both dead and alive 42 days after exposure of the larvae to 3 concentrations of oxygen for various times.

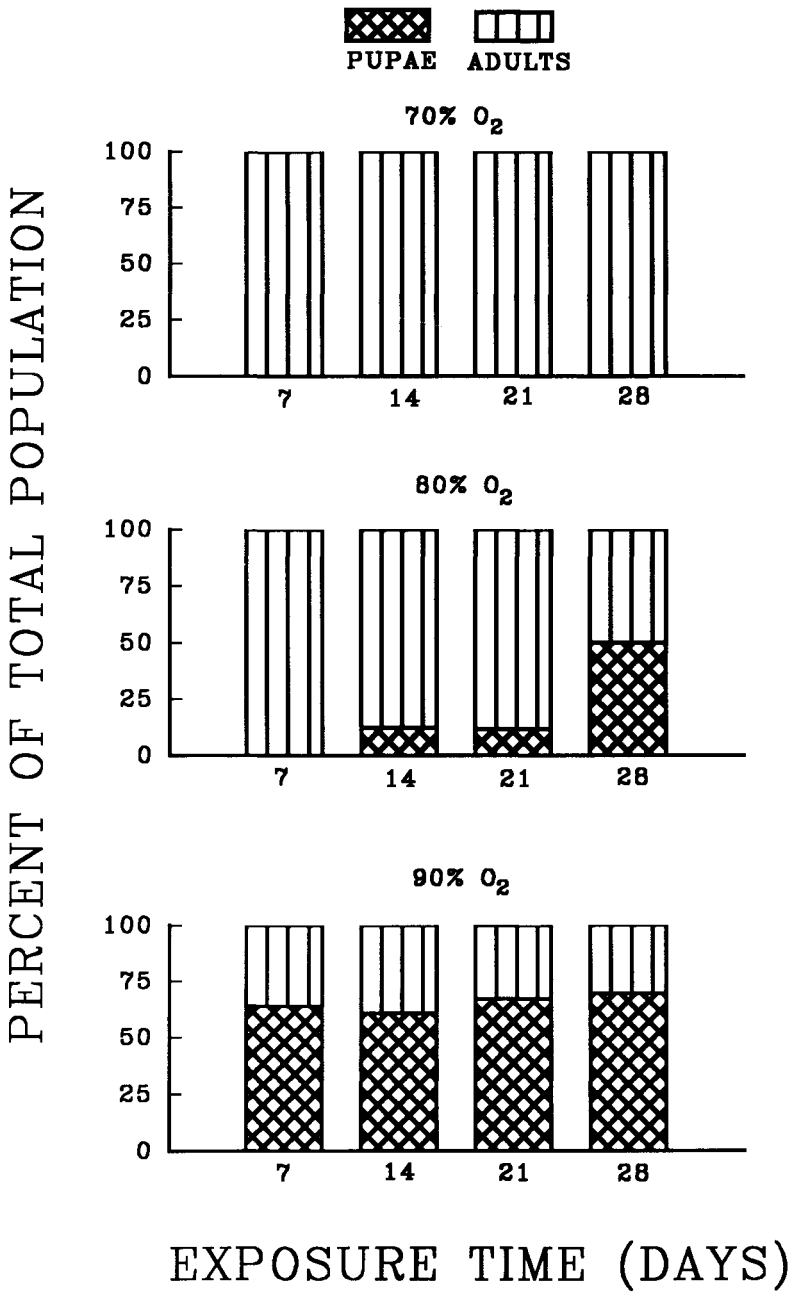

Fig. 3. Composition of populations of *T. castaneum* both dead and alive 42 days after exposure of the pupae to 3 concentrations of oxygen for various times.

Technology use. Although the economic feasibility of using high O_2 concentrations for insect control remains questionable, the vulnerability of insects to these treatments makes them attractive for possible use. There is a concern of the safe use of 90% O_2 in grains or areas where expolosions or fires may occur; however, it is of scientific interest to know the effects of high O_2 levels on insects for future research on combinations of gases to control insects. It should also be pointed out that small-scale use of high-level O_2 treatments may prove feasible if safety techniques can be applied. The development of methods in which stored product insects could be exposed to 90% O_2 in safely equipped fumitoria may become in some cases, a practical proposition. The use of the technique for small-scale disinfestation of commodities or museum specimens may be feasible.

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