ENHANCING THE EFFECTIVENESS OF MODIFIED ATMOSPHERES TO CONTROL INSECT PESTS IN MUSEUMS AND SIMILAR SENSITIVE AREAS

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Abstract—Anoxia resulting from contained atmospheres of <0.1% oxygen (1,000 ppm) was lethal to all stadia of a variety of insect pests commonly encountered in museums and other sensitive areas. Low % O_2 in a closed system was achieved and maintained by displacement with gaseous nitrogen. The effect of anoxia on representative species of cockroaches (Dictyoptera), fabric beetles (Dermestidae), stored product beetles (Anobiidae), termites (Rhinotermitidae), and wood-boring beetles (Lyctidae) was evaluated. Most insects succumbed within hours or a few days exposure. A few, such as larval carpet beetles, *Anthrenus flavipes*, eggs of cigarette beetles, *Lasioderme serrico2ne*, and powderpost beetles, *Lyctus spp*, survived >8-day exposure. Length of exposure for mortality (LT) appeared to be related to metabolic rate and to the ability of the exposed stage to regulate water loss. In this regard, LT was generally inversely correlated to % O_2 and to exposure temperature and relative humidity. Increasing O_2 to 0.32% increased time for LT for some species but not for others. Increasing O_2 to 0.62% approximately doubled LT. Adding CO_2 during anoxic exposure reduced LT, apparently by increasing futile metabolism. It was difficult and relatively costly to maintain <0.1% O_2. Higher O_2 rates may be practical for controlling some species if higher temperature or low humidity may be maintained. Methodology for maintaining between <0.1% and 0.62% O_2 is discussed.

INTRODUCTION

Rare and virtually priceless museum objects require special care to protect them from insect attack or to eliminate insects in or on them. Novel chemical spray, carbon dioxide (CO_2) fumigation, or heat or cold treatment or storage may be undesirable because of gross or microscopic effects on the artifact. There has been increasing interest in implementing the use of controlled or modified atmospheres such as anoxia to kill museum pests. Similarly, pest control practitioners are often in search of reduced-hazard methodology to control insects in items such as electronic equipment, water coolers, food carts, and household goods. Most research with *modified environmental condition* has been done in an attempt to reduce pesticide use and risk and has centered around the idea of freezing or high heat. Freezing and high heat processes have been developed commercially to control or suppress insects in commodities, especially during storage or shipment. *Modified atmosphere* generally refers to alteration of the gaseous environment in which the insect lives. Typically, modified atmospheres are produced artificially and are maintained by enveloping an object with a gas such as carbon dioxide or nitrogen (N_2). The source of gas is usually a commercial pressurized cylinder (Calderon, 1990).

There has been considerable research conducted with modified atmospheres against insects infesting stored foods and grain (Bond, 1990; Bailey and Banks, 1980; Fleurat-Lessard, 1990; Champ *et al.*, 1990). High percentage CO_2 coupled with lowering oxygen (O_2) concentration to 10–20% will kill insects and mites in stored wheat and barley without damaging the product (White and Jayas 1993). Similarly, Whiting *et al.* (1992) killed 99% of four species of grain-infesting moths by exposing them to 0.4% O_2 + 5% CO_2. The long exposure time generally needed to kill insects at low O_2 has historically been considered to be problematical, and the technology to reduce and maintain atmospheric O_2 to exceptionally low levels has only recently become available. In some instances, however, long exposure is not operationally disruptive. Such is often the case in museums where it is not uncommon for over 75% of acquired objects to be warehoused rather than being on display. Reichmuth (1990) reported that various species of stored product pests died in low O_2 atmospheres within a few days at 30° C and within about a month at 15° C. Jay *et al.* (1990) found that as the exposure temperature increased from 32° C to 43° C in 99% N_2 the time required to kill all stages of

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the cigarette beetle, *Lasioderma serricorne,* decreased from 96 to 24 hours. Although the use of modified atmospheres to control insect pests on fresh exotic fruits such as papaya and avocado may be limited because of deleterious effects that low O_2 and high CO_2 have on the fruit (Yahia *et al.*, 1992; Yahia and Carrillo-Lopez, 1993), no such effects have been observed with inanimate objects.

In studies with insect pests of museums, Gilberg (1989, 1990) found that 7-day exposure at 0.4% O_2 killed adult cigarette beetle, drugstore beetle, carpet beetle, powderpost beetle, and webbing clothes moth. Valentin (1990) showed that exposure to low O_2 (1%) killed old house borer beetles, *Hylotrupes bajulus* (L.), and powderpost beetles, *Lyctus brunneus* (Stephens), within 20 days. According to Paton and Creffield (1987), those same species of beetles were not killed when maintained in 80% CO_2 , but a 5-day exposure did kill the West Indian drywood termite, *Cryptotermes brevis* (Walker). Rust *et al.* (1993, 1995) reported that approximately 72–hour exposure at <0.1% O_2 provided complete kill of 10 important museum pests, but that immature *L. serricorne* and furniture carpet beetle, *Anthrenus flavipes* LeConte, required up to 192–hour exposures for complete kill.

Museum artifacts susceptible to insect attack are often relatively large. Picture frames, furniture, and tapestries are particularly susceptible. Small sealed chambers may be used for small objects but it is unlikely that very low O_2 in the range of <0.1% can be maintained in large chambers needed to accommodate treatment of large pieces. For this reason we also studied the practicality and efficacy of what appear to be more attainable and realistic rates of atmospheric O_2. Contained O_2 concentrations >0.3% are readily attainable with present technology. The primary objectives of the study, therefore, were a.) to determine the effectiveness of <0.1% O_2 atmospheres of against several important museum pests and b.) to determine the effectiveness of 0.3, 0.6, and 0.95% O_2 against the representative anoxia-tolerant furniture carpet beetle, *A. flavipes*, and cigarette beetle, *L. serricorne.* From the onset, the principal target stadia were those which are the most anoxia-tolerant for each species. Effects of temperature, relative humidity (RH) and CO_2 as additives to low O_2 exposure also were studied.

METHODS AND MATERIALS

Each stage of test insect was exposed in sealed acrylic chambers and in oxygen-impermeable plastic bags to monitored low O_2. Post-exposure mortality was determined by direct observation. Acute and latent mortality resulting from exposures to static <0.1% O_2 and to constant-flow 0.32%, 0.62% and 0.95% oxygen at 33%, 55% and 75% relative humidity were evaluated and compared. Effects of simultaneously adding 5% gaseous CO_2 and increasing temperature during exposure also was studied. Gaseous N_2, O_2 and CO_2 were provided for the study from liquid in commercial high pressure tanks. Gas pressure from the cylinders was regulated by step-down pressure regulators and flow was controlled with adjustable flow meters.

Prescribed O_2 concentration and humidity was maintained with a metered steady flow of monitored humidified gaseous nitrogen (Figure 1). The metered N_2 essentially purged the system of oxygen to a predetermined level at which the % O_2 concentration was maintained. Oxygen concentration was monitored electronically with a Teledyne oxygen analyzer (model 316). Container air was sampled by inserting a hypodermic needle, connected with Tygon® tubing to the analyzer, through a septa in the wall of the chambers and bags.

Insect rearing and collection

Insects for the study were reared in laboratory culture in environmental chambers at 26.7° C (80°F) and 75–80% relative humidity.

Cockroaches. Cockroaches for the study were cultured on an irregular photoperiod and were fed dry dog chow and water ad libitum. *Blattella germanica* (L.) and *Periplaneta americana* (L.) were reared in 120-l rubbish bins while *Supella longipalpa* (F.) was reared in 3.9-l glass jars. Cockroaches and oothecae were selected while anesthetized with gaseous CO_2 one day prior to exposure. Depending on the species, 10 to 20 replicates of 1 to 3 adults, 5 to 10 nymphs, and 2 oothecae (n = 250 to 500) were used for exposures.

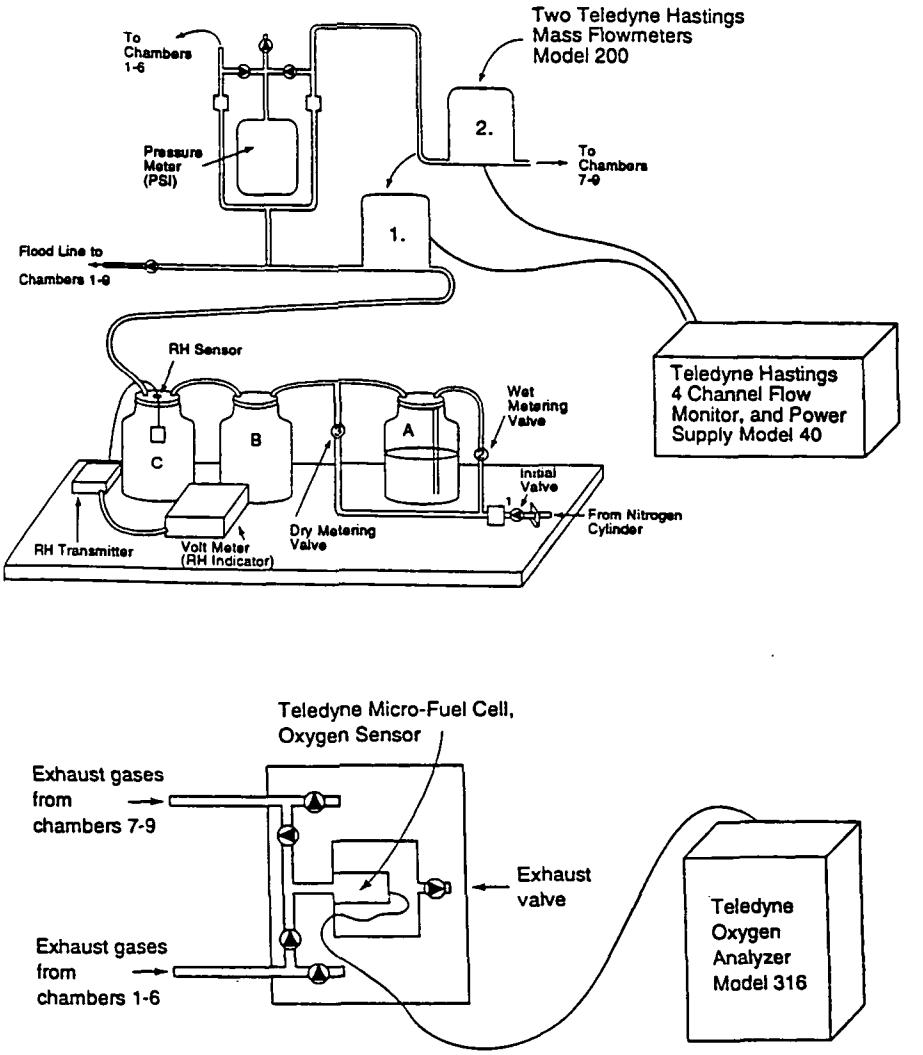

Figure 1. Schematic diagrams. *Upper* – Gas flow system, including apparatus for humidifying and monitoring relative humidity and N_2 flow. *Lower* – Instrumentation for monitoring O_2 in a continuous flow of N_2 in chambers.

Webbing clothes moth. Tineola bisselliella (Hummel) was cultured on mixed ground dog chow and modified brewer's yeast. Adults oviposited on folded paper in jars containing a small amount of media. Eggs and larvae for study were collected from flannel fabric swatches placed in the oviposition jars. Cocoons and larvae were picked from culture about 24 hours before exposure. Adults were selected while anesthetized with CO_2. Eggs were collected by allowing adults in an inverted jar to oviposit through a 20-mesh screen onto 60-mesh diet in a petri dish. One- to 3-day-old eggs were picked with a dampened #000 camel's hair brush and fastened to a piece of double stick transparent tape attached to black construction paper. The dark background provided contrast so that the eggs could easily be inspected. Several hundred of each stadia were used for the study.

Firebrat. Thermobia domestica (Packard) were cultured in a modified 80-l ice chest in which 90° C was maintained with a thermostatically controlled 15-watt lamp. The insects were given dry dog chow and water ad libitum. Nymphal firebrats were selected according to small (<5 mm), medium (6–8 mm), and (8–10 mm) by collecting them on sieve screens. Replicates of 10 firebrats were used for each exposure.

Cigarette beetle. Procedures for rearing *L. serricorne* (F.) were modified from Rust *et al.* (1993).

Two rearing jars were prepared every three days making it possible to accurately age the life stages. Eggs were collected every 3 days from 30 g diet in 0.9-l jars in which approximately 200 mixed-sex 1 to 7-day-old adult beetles were allowed to mate and oviposit. Adults were collected from 6 different rearing jars and mixed together to minimize inbreeding. At 2 days the adults were removed with a 20-mesh screen and the eggs were collected with a 60-mesh screen. Eggs were used in the study or were placed on diet so that hatching larvae could develop.

Furniture Carpet Beetle. Two rearing jars for *Anthrenus flavipes* LeConte were set up monthly. Approximately 30 ml of adult beetles from culture jars were allowed to oviposit in 3.9-liter glass jars provisioned with sterilized duck feathers. Eggs were collected by allowing adults to oviposit through a screen. About 50 young adults and pupae ready to emerge were placed in plastic vials (7 cm by 3 cm diam) provisioned with feathers approximately 3 to 7 days before eggs were needed. Each vial was covered with a snap-cap lids with 20-mesh screen openings. Eggs were collected from the bottom of the vials. Larvae were selected according to size rather than by known age.

Other stored product beetles. Culturing and handling of the cabinet beetle, *Trogoderma inclusum* LeConte, the larder beetle, *Dermestes lardarius* L., the confused flour beetle, *Tribolium confusum* J. du Val, was also as detailed in Rust *et al* (1993). Adults, eggs and larvae were tested in instances were those stages were available. In a few instances where the life cycle may be many months or years, only adults were included.

Drywood termite. Western drywood termites, *Incisitermes minor* (Hagen) for the study were extracted from infested wood. One or two weeks before exposure pieces of stored infested wood were split with wedges and the termites gently extracted. The termites were housed with thin pieces of douglas fir and paper toweling in sealed food containers until they were selected for exposure.

Powderpost beetles. The procedure used to culture and expose powderpost beetle was modified from Rust *et al.* (1993). The primary species tested was *Lyctus brunneus* (Stephens), but *L. linearis (Goeze)* and *Trogoxylon prostomoides* (Gorham) also were included in some exposures. The beetles were allowed to develop in 0.9-l jars containing powdered media (wheat flour, corn meal, yeast extract, methyl-p-hydroxybenzoate, and ascorbic acid) and baked diet (fibrous cellulose, yeast extract, and wheat flour). Adults oviposit in loose or baked diet. Larvae migrate to the soft diet, making them easy to collect by sieve screen. To collect eggs, 30 to 50 adults were placed in petri dishes with black construction paper about 4 days before the eggs were needed. A pinch of sifted diet in the bottom of each dish encourage oviposition on the black paper. Larvae were selected ten days prior to exposure. Larvae were grouped and exposed according to size, rather than days of age, with sieve screens. Eggs were selected one day prior to exposure. Eggs were collected with the aid of a microscope. Eggs were individually transferred with a camel-hair brush to double-stick tape fastened to black construction paper. Typically, 50 eggs were placed on each strip and the strips of eggs were transferred to sealed food containers until they were used in the exposures.

Exposures

Controlled atmospheres were achieved by continuously purging ambient oxygen from the chambers with specially prepared combinations of nitrogen-oxygen gases. The system consisted of two banks of six 4.2-l methacrylate chambers that could be simultaneously flushed with N_2–O_2 mixtures. An apparatus for adjusting RH and gas flow was inserted between the gas supply cylinders and the input manifold to the chambers (Figure 1). Exhaust O_2 concentrations were continuously monitored. Chamber atmospheres were humidified by passing the gas through a water bottle. The flow of gas at the desired RH was split into two lines. One line could be used to purge all chambers. The other line led to chambers used for static or continuous-flow experiments. In static chambers the desired O_2 concentration was reached and reestablished daily to account for inevitable leakage of O_2 into the chambers. Flow-through condition was maintained by a calibrated flow of N_2. The flow-through condition ensured that O_2 concentrations did not fluctuate.

The RH in the static chambers was maintained at 55% RH with a saturated solution of magnesium nitrate. One 14-g packet of AGELESS® (Mitsubishi Gas Chemical Co., Inc.) was placed in each chamber to scavenge excess O_2.

Experiments also were done to determine the feasibility of using O_2-impermeable plastic instead of chambers to provide an enclosure for lethal low O_2. Custom-made bags of special plastic were

used as enclosures for insects exposed to N_2-purged atmospheres. Atmospheric O_2 in the bags was measured electronically as was done with the chambers and the mortality of exposed insects was determined in the same manner.

Test insects were placed in the chambers, the door was sealed and the desired O_2 and environmental condition was established. Tests were conducted at room temperature or elevated with a space heater.

Determining mortality

In instances of short life expectancy (eg. adult moths), the number of dead adults was counted 24 hours post-exposure. Mortality was determined by examining the insects microscopically for any perceptible movement. Larvae and eggs were held for subsequent development. Immatures that did not develop were considered dead. Pupae were dissected from cocoons 3 weeks post-exposure and the number live was counted.

Unexposed controls. On the day exposures began, same species and stadia were set up as unexposed controls. Insects used as comparative controls were placed in sealed acrylic chambers at ambient O_2 and identical RH and temperature as the insects being exposed. The control insects were held for the same time period of time as those exposed to low O_2. After exposure, the treated and control insects were transferred to plastic holding boxes in an environmental chamber at 25.6° C (78° F) and 75 to 80% RH. Abbott's Formula (1925) was used to correct for mortality among the unexposed controls.

RESULTS

The resultant kill of the 12 species of insects exposed to <0.1% O_2 is summarized in Figure 2. There was no obvious relationship between species and anoxic sensitivity. The longest exposure required for 100% mortality (LT) was for the egg stage of the cigarette beetle, which took 192 hours. The cigarette beetle, *L. serricorne,* was the most anoxia-tolerant insect tested. All stadia of all the other species tested died with 6 days or less exposure. Several days exposure <0.1% O_2 was lethal to even the most anoxia-tolerant species. The egg stage was generally more anoxia tolerant than the other stages, but that was not the case for every species. Fastest kill occurred with firebrats and cockroaches, apparently independent of size. Firebrats and cockroaches succumbed within 3 to 6 hours. As a group, wood-boring beetles and termites were among the most anoxia-tolerant insects, some of them surviving about 4 to 5 days exposure at <0.1% O_2.

Most insects survived longer as O_2 concentration increased, but even 0.95% O_2 was lethal to the anoxia-tolerant cigarette beetle within days. This typical relationship was evident against the egg stage of *L serricorne* (Figure 3) and was also evident against the stages of *A. flavipes.*

As shown in Figure 4, the LT at low % O_2 increased with increasing RH. With larval *L. serricorne* for example, 48-hour exposure in 0.32% O_2 provided 94% mortality at 33% RH but only 25% mortality at 75% RH. At 96 hours all the larvae exposed at 33% were dead but about 5% of those exposed at 75% RH were still alive. These results suggest that desiccation is involved in the lethal action of anoxia.

The effect of adding CO_2 was studied in only a few instances, with cigarette beetles and carpet beetles. Generally, 5% CO_2 increased mortality about 20% for any given period of anoxic exposure.

Increasing exposure temperature from 25.6°C to 30°C (from 78°F to 86°F) reduced the time for 100% kill of cigarette beetle eggs (Figure 5). Except for stadia that died quickly in virtually any anoxic condition we tested, increasing the temperature a few degrees consistently shortened LT.

DISCUSSION

The efficacy of nitrogen-purged atmospheres for long-term storage of sensitive organic antiquities has been investigated for nearly a decade. Initially designed to protect the Royal Egyptian Mummies in the Egyptian Museum in Cairo from selected insect attack, hermetically sealed storage

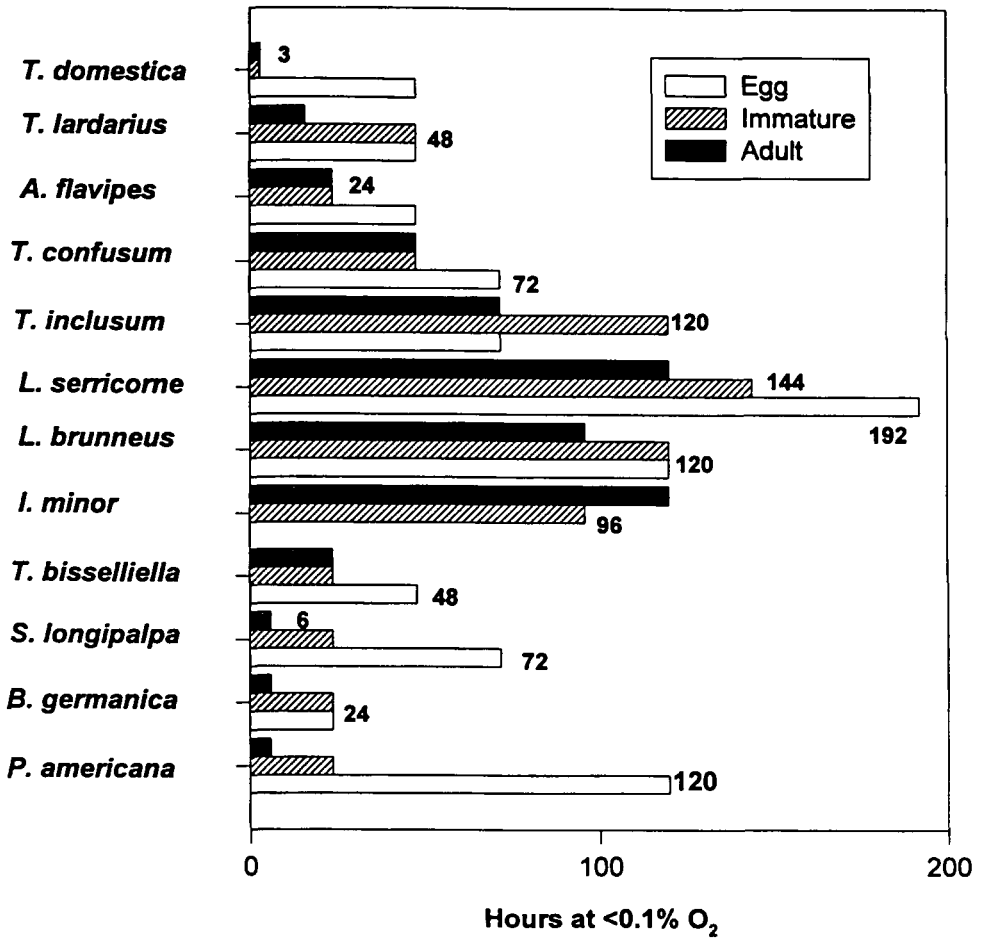

Figure 2. Hours for 100% mortality of 12 species and stages of insects exposed to <0.1% O_2 at 26.5°C and 55% relative humidity. These or closely related species commonly infest organic artifacts in museums.

cases in which atmospheric O_2 was reduced to fairly low levels for weeks or months proved to be very effective (Maekawa, 1995).

In this study we found that mobile 'chambers' and shorter anoxic exposure times could be used to control important museum insect pests. Exposure time for eradication of 12 species of insects representing a range of ecological situations was reduced to just a week or so. Nitrogen-purged atmospheres of <0.1% O_2 in solid chambers resulted in 100% mortality of all stages of some insects within 48 hours or less. Among the most anoxia-tolerant species were the cigarette beetle, *L. serricorne*, the cabinet beetle, *T. inclusum*, powderpost beetles, *Lyctus spp.*, and the drywood termite, *I. minor*. Except for *Lyctus spp.*, even those species succumbed within 5 to 8 days. Some powderpost beetles, however, survived >14-day exposure

Although highly effective, it was expensive to maintain <0.1% O_2 for long periods of time. Medically pure N_2 was needed to ensure O_2 at <0.1%. On the other hand, 0.32 to 0.95% O_2 was readily attained by purging with less pure and less costly commercial pressurized N_2. Generally, LT increased with increasing O_2 concentration. Reduced efficacy was not pronounced at 0.32%, but was at 0.95%. The 0.62% rate was intermediate. For example, larvae of *L. serricorne* are anoxia-tolerant but they died with 4 days exposure at either <0.1% or 0.32% O_2. Some larvae (4%) survived 11 days at 0.62% and 36% survived at 0.95% O_2. This pattern of activity was seen with most insects and stadia tested.

Exposure time to death was decreased by raising exposure temperature a few degrees, by adding 5% CO_2, or by decreasing humidity. This suggests that anoxia symptomology may be related to

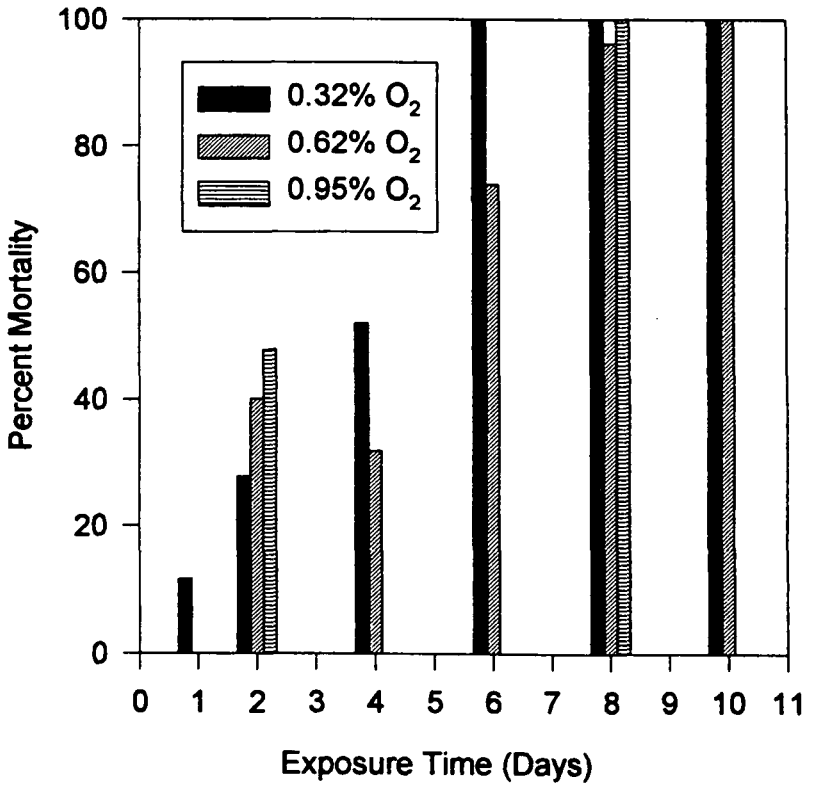

Figure 3. Effect of low oxygen concentrations on the mortality of the egg stage of the cigarette beetle, *Lasioderme serricorne*, exposed at 25.6°C and 55% relative humidity.

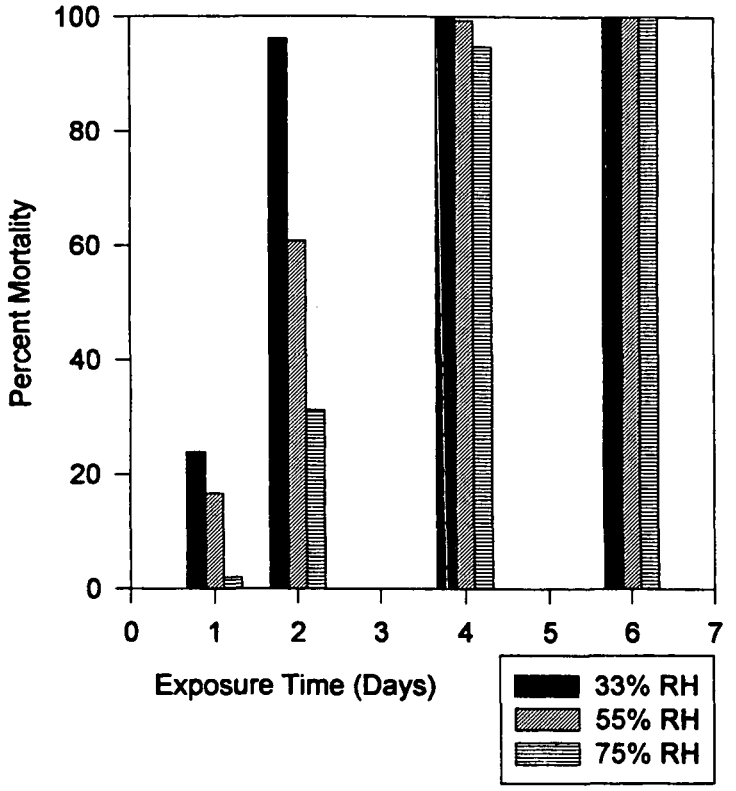

Figure 4. Effect of relative humidity (RH) on the mortality of cigarette bettle larvae exposed to 0.32% O_2 at 26.5°C. Cigarette beetles are among the most anoxia-tolerant species tested.

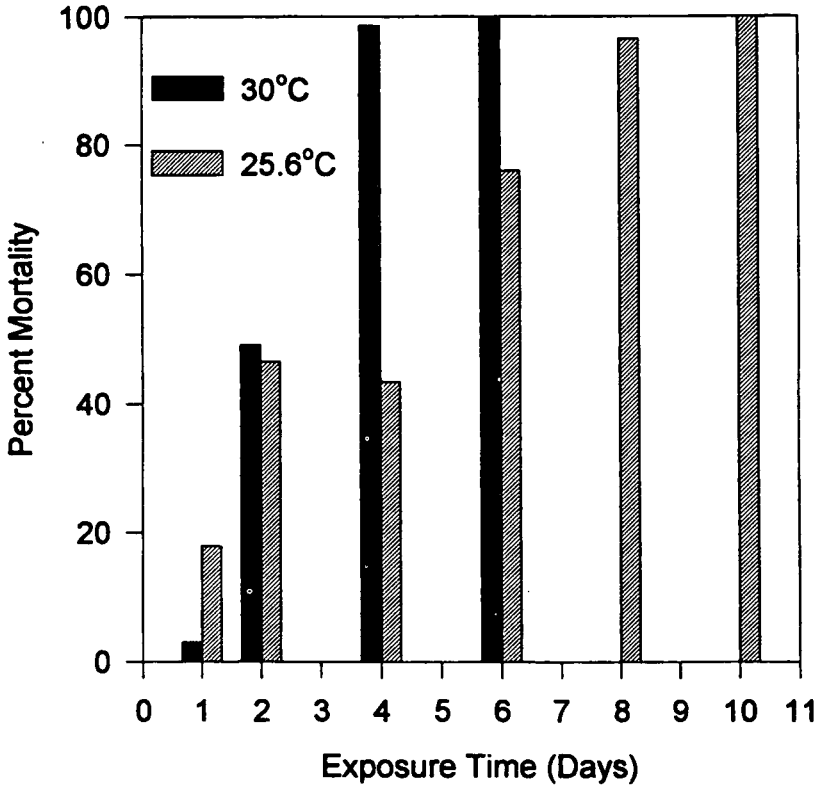

Figure 5. Effect of increased temperature on anoxia. Shown here is mortality of the egg stage of the cigarette beetle exposed to 0.62% O_2 at 2.5°C and 55% relative humidity. Similar results were found for other species.

metabolic rate. Decreasing exposure RH decreased exposure time to mortality, suggesting that anoxia may also be related to body water regulation among exposed insects. Because saturation deficit is a better measure of the drying capacity of air, there is probably a better relationship between LT and saturation deficit than with relative humidity. It is not always possible or desirable, however, to expose artifacts or antiquities to even minimal increases in heat or to CO_2. Most museums maintain conditions at about 25.6° C and 55% RH and are understandably reluctant to alter those conditions. Expansion, contraction, desiccation and acidification may adversely affect stored or displayed objects, but there are some instances where it may not be objectionable to expose less valuable items to slightly higher temperature for relatively brief periods of time.

High levels of efficacy also were attained by purging special heat-sealed plastic bags with N_2. Insects in containers placed in the bags died from about the same period of exposure as in the solid plastic chambers. Packets of the Ageless® oxygen scrubber compensated for minimal O_2 leakage and helped maintain lethal anoxic condition in the bags. Purging objects of oxygen and storing them in an O_2-impervious wrapping may be an important way to prevent objects from insect attack and to protect them in storage. This may be especially true of susceptible objects stored for long periods of time. In any event, reduced-oxygen atmosphere is highly effective against a wide variety of important insect pests, and was shown to be practical under a range of conditions. Anoxia is a particularly appropriate strategy for deinfesting and protecting valuable and delicate artifacts and may also have practicality in many commercial situations.

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