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Research Article

The Feasibility of Using Low-oxygen Atmospheres to Control Insect Pests for Taxidermies in Natural History Museums

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Abstract: To find the environmental friendly alternative methods for control taxidermy pests in natural history museum, six species insect pests at various stages of their development were exposed to a low-oxygen atmosphere of 1.5% for a period of one week. Apart from a 50% survival rate for the larval stage of *Anthrenus verbasci*, the modified atmosphere was observed to have a lethal effect on all insect stages tested. When the exposure period was extended to periods of 2, 3, 4, 5 and 6 weeks, respectively 100% mortality was recorded for all insects tested. Evidence from this investigation supports the view that atmospheres reduced in oxygen may represent a viable alternative to chemical control methods. The feasibility of using this technique for the routine control and eradication of insect pests in natural history museums is discussed.

Keywords: Eradication, insect pest, low-oxygen atmosphere, natural history museum, taxidermy

INTRODUCTION

Methods used for the eradication of insect pests in natural history museum taxidermies have traditionally involved the use of chemicals including arsenic, mercuric chloride, strychnine, DDT, ethylene dichloride, methyl bromide, ethylene oxide, sulfuryl fluoride and numerous others (Ornstein, 2010). Those chemicals may react adversely with museum materials and taxidermies, can be toxic to the public, may be harmful to the environment and their use is, in many cases, highly regulated (Dawson and Strang, 1992; Jedrzejewska, 1967; Linnie, 1987, 1994). This has focused attention on alternative methods of control. The use of carbon dioxide, low oxygen atmospheres and inert gases (nitrogen, helium and argon) have been considered alternatives to chemical control (Daniel *et al*., 1993; Valentin, 1993). The advantage of the use of some of these atmospheric gases under controlled conditions is that the toxicity risk to users is reduced and the reactive effects on taxidermies and materials are considered less damaging than conventional chemical or fumigant treatments.

A modified atmosphere may be used to destroy or prevent infestations, as a control method in situations where infestations are suspected and as a general "quarantine" treatment for incoming material. Modified atmospheres are created by replacing the existing ambient atmosphere with one lethal to insects. An environment low in oxygen may be achieved by adding carbon dioxide, nitrogen, or air depleted in oxygen under sealed conditions. This technique is a modification of the practice of hermetic storage where food commodities such as grain and beans were sealed in underground pits. The respiration of the harvested product combined with the metabolism of the invading insect pests reduced the available oxygen to a lethal level. The resulting atmosphere also inhibited fungal growth and maintained the quality of the food product over an extended period.

Although research into modified atmospheres began in the mid-19th century, interest in applying the technique in a routine manner did not occur until approximately 50 years ago and serious interest about 30 years ago, probably resulting from the success of and growing concern of health risks associated with conventional fumigants and grain protectants. Most recent research relates to the use of gases in silos, granaries and other forms of bulk grain storage as a direct alternative to traditional chemical fumigation methods.

Studies have shown that the survival of stored-product insect pests is restricted in atmospheres containing low-oxygen concentrations (Conyers and Bell, 2007; Navarro, 1978; Rust *et al*., 1996; Soderstrom and Brandl, 1982; Storey, 1978). It has also been demonstrated that to produce a lethal environment for even the most susceptible stored-product insect, the oxygen concentration should be maintained below 4% (Bailey, 1965; Bailey and Banks, 1974). Burke (1996) recommends concentrations of 0.5% or lower for museum insect pests and states that concentrations greater than 1% do not appear to be effective, even for longer exposures. Rust *et al*. (1996) found that the exposure time in a low-oxygen atmosphere is likely to be dramatically decreased by increasing the temperature above 25.5°C and lowering the relative humidity below 55%.

The application of modified atmospheres for the control of museum insect pests was first investigated approximately 20 years ago. Since then, considerable work has been done on the efficacy of using the technique for the control of pest insects in museum collections (Elert and Maekawa, 1997; Rust *et al*., 1996; Valentin, 1993). Theoretically, the concept of exposing museum specimens to modified atmospheres should not present undue difficulties considering the technology available and standards achieved in ensuring safe chemical treatment by traditional fumigation methods. The advantage of low health risks combined with the residue-free benefits of the technique appear to offer a suitable alternative to chemical fumigation treatments (Daniel, 1995).

In this study several species of recognized pests of museum collections were exposed to a low-oxygen atmosphere over varying time periods. Egg, larval, pupil and adult stages were used to determine potential susceptibility differences. An evaluation of the effectiveness of the technique is made as a pest-control tool in the museum environment.

MATERIALS AND METHODS

The experiment was carried out from June 10th, to December 30th, 2012 at the Animal and Ecology Test Lab, School of Life Sciences, Sun Yat-sen University, China.

The selection of particular insect pest species was made on the basis of their recognized status as pests of museums, their fecundity rates and their availability. All of the species chosen are cosmopolitan and are considered among the most serious of the taxidermy pests. Those species tested are included in a reference listing of museum pests and are known to feed directly on produce containing high protein content (Beauchamp *et al*., 1981). All tested insects were obtained from School of Life Sciences, Sun Yat-sen University, China. Egg, larval, pupil and adult stages were used. A list of the species, experimental temperature conditions, numbers of individuals and lifecycle stages used is given in Table 1.

The six insect species selected for study were: *Anthrenus verbasci* (L.), Varied carpet beetle (Coleoptera; Dermestidae); *Dermestes maculatus* De Geer, Hide beetle (Coleoptera; Dermestidae); *Oryzaephilus surinamensis* (L.), Sawtoothed grain beetle (Coleoptera; Silvanidae); *Plodia interpunctella* (Hubner), Indian meal moth (Lepidoptera; Pyralidae); *Stegobium paniceum* (L.), Biscuit beetle or Drug-store beetle (Coleoptera; Anobiidae) and *Tribolium confusum* Jacquelin du Val, Confused flour beetle (Coleoptera; Tenebrionidae).

All the egg, larval, pupil and adult stages were collected, sorted and counted immediately prior to their exposure to the low-oxygen atmosphere. Eggs were examined under light microscopy to confirm that they were in good condition and probably viable. Larvae were chosen from laboratory cultures and, in all cases, final instar stages were preferred. Pupae were gently prodded with a blunt probe to confirm evidence of viability by their exhibition of a reflexive response to the stimuli of light and touch. Adult stages that were observed to be mobile and responsive were also selected from stock cultures. The precise number of individuals and the stages used for each species were dependent on their availability at the time of selection. Insects were retained in plastic petri dishes or in screwcap jars, where appropriate, with small amounts of culture media until the insects' exposure to test conditions.

Table 1: Percentage of mortality of various insect species following their exposure to a low-oxygen atmosphere for one week at 25°C

Species	Stage	Number used	Mortality (%)
D. maculatus	Adult	40	100
D. maculatus	Pupa	40	67
D. maculatus	Larva	40	100
D. maculatus	Egg	40	100
A. verbasci	Adult	40	100
A. verbasci	Pupa	40	100
A. verbasci	Larva	40	50
A. verbasci	Egg	40	100
O. surinamensis	Adult	40	100
O. surinamensis	Pupa	30	100
O. surinamensis	Larva	40	100
O. surinamensis	Egg	40	100
T. confusum	Adult	40	100
T. confusum	Pupa	20	100
T. confusum	Larva	20	100
T. confusum	Egg	20	100
P. interpunctella	Adult	40	100
P. interpunctella	Pupa	40	100
P. interpunctella	Larva	40	100
P. interpunctella	Egg	40	100
S. paniceum	Adult	40	100
S. paniceum	Pupa	30	100
S. paniceum	Larva	30	100
S. paniceum	Egg	30	100

For their exposure to the low-oxygen atmosphere, each batch of test insects was placed in a separate metal container with a small amount of culture medium. The containers were tin cans, approximately 1 L in capacity and measured 17 cm in height by 13 cm in diameter. The evacuation of oxygen from the cans involved an initial crimping and sealing of a metal lid onto each container. Air inside the cans was then removed using an industrial degassing unit that provides a negative pressure of 0.95 bar. Immediately after this process, nitrogen was added at a pressure of 1 bar, resulting in an atmosphere that was reduced in oxygen. The concentrations of oxygen were initially recorded as 1.1, 1.6 and 1.3%. A second set of concentrations (taken 30 min later) were recorded as 1.5, 2.0 and 1.6%, respectively. The mean oxygen concentration was 1.5%.

Groups of control insects were maintained under identical conditions but were held in cans containing an ambient atmosphere. All cans were transported to the laboratory and maintained under constant temperature conditions at 25. After one week the cans were opened

for inspection. Death was determined by the absence of spontaneous movement or evidence of an irreversible, uncontrolled, lethargic condition in larval or adult stages. However, these criteria cannot be used for egg and pupil stages, therefore the cessation of normal development followed by obvious physical deterioration was considered an indication of death for egg and pupil stages. All insect stages, whether considered viable or moribund, were held at 25°C for monitoring. This was performed at one week intervals over a period of 6 weeks.

RESULTS

Upon opening the containers with the low-oxygen atmosphere, it was evident that most insect stages had failed to survive. In contrast, control insects that had been held under ambient atmospheric conditions exhibited normal behavioral activity. Table 1 shows the percentage of mortality of the various insect stages and species following their exposure to the low-oxygen tension for a period of 1 week.

The only stages that exhibited any indications of viability were pupil stages of *D. maculatus* and larval stages of *A. verbasci*. Upon initial inspection, the pupae of *D. maculatus* appeared dead, although there was a reflexive response to gentle touching with a blunt probe. Subsequent monitoring indicated signs of further development, including the progression of elytral formation and head capsule growth. However, full development was impaired and none of the pupae progressed to the adult stage. They were finally diagnosed as dead after the 6-week monitoring period had elapsed and when emergence had been completed in the control samples.

Larval stages of *A. verbasci* exposed to the low oxygen atmosphere, although not mobile on initial inspection, responded to gentle touching with a blunt probe. Fifty percent of larvae continued to improve and eventually became capable of complete and unimpaired movement. Development continued into the pupil stage and subsequent emerging adults appeared normal. All other insect stages failed to show any sign of viability during the 6-week monitoring period. At the end of this period a final assessment of their condition was made using the methods described above and by comparison with the control samples.

Of all the species and life-cycle stages tested, larvae of *A. verbasci* were the only insects to survive exposure to the test conditions. This ability was further investigated by repeating the experimental procedure; although in this case, the period during which the test insects remained in the low-oxygen atmosphere was extended to periods 2, 3, 4, 5, or 6 weeks at 25°C, respectively. Test conditions and oxygen tensions were maintained as described above. Forty mature larvae were used for each time period. Each test was duplicated and control tests using larvae held under the same conditions but with cans filled with ambient atmospheric air were also performed.

Table 2: Percentage of mortality of mature larvae of *Anthrenus verbasci* following their exposure to a low-oxygen atmosphere over a variety of time periods at 25°C

Exposure period (weeks)	Mortality (%)
1	50
2	100
3	100
4	100
5	100
6	100

After each tested time period had elapsed, the test cans and the control cans were opened and inspected. Mortality in the control samples, even after 6 weeks in the sealed cans was negligible with larvae exhibiting normal behavior such as movement and response to light and touch. However, all larvae exposed to the low-oxygen atmosphere over these time periods appeared moribund on removal from the deoxygenated containers. Subsequent monitoring of the test larvae over a 6-week period following removal from the test conditions failed to record any survivors. Larvae were finally diagnosed as dead after the completion of the monitoring period or when emergence was completed in the control samples (Table 2).

DISCUSSION

The use of modified atmospheres to control pests has largely developed in response to demands by the food industry to produce methods of control that are nontoxic and residue free. Similarly, the disadvantages associated with the use of chemicals in the museum environment have promoted interest in alternative nonchemical control methods. Although the use of sealed environments low in oxygen to control museum insect pests has attracted considerable interest, further research is required to investigate fully, those factors that may assist or promote the survival of insects in anoxic conditions.

These results show that in general, exposure to an atmosphere of approximately 1.5% oxygen and 98.5% nitrogen for a period of one week was successful in limiting survival of the test insect species among the various stages used. Adults of *T. confusum*, *A. verbasci*, *D. maculatus*, *O. surinamensis*, *P. interpunctella* and *S. paniceum* all failed to survive the reduced oxygen atmosphere. Of the pre-adult stages tested, larvae of *A. verbasci* were the only stage to survive the treatment with 50% eventually making a full recovery. Further tests on larvae of this species over extended time periods showed that an exposure period of 2 or more weeks achieved 100% mortality.

All insects require oxygen to survive and continue normal development and activity. The uptake and transport of oxygen from the surrounding atmosphere into the insect respiratory system is related to the ability of the insect to tolerate certain conditions. Given that

certain stored-product insects can survive at very low humidities, the means of conserving water is an important and fundamental feature of their physiology and structure. In effect, this means that insects that have adapted to survive under dry conditions, such as those found in the bulk storage of grain and other food products, require efficient respiratory systems that allow rapid exchanges of oxygen and carbon dioxide while at the same time restricting water loss. This is an important consideration because anoxia in insects works by dehydration rather than by suffocation. This has also been suggested by other workers, where water loss was found to be a major contributory factor in the mortality of *T. castaneum* held under high nitrogen concentrations (Jay, 1971; Jay and Cuff, 1981). At very low oxygen concentrations the spiracles of the insect open to such an extent that desiccation occurs. Significant weight loss caused by desiccation in certain insect larvae exposed to a range of modified atmospheres has been reported (Valentin, 1993). This condition is further accelerated by dry, warm conditions. In contrast, if ambient humidity is high and combined with low temperature, the insect may receive sufficient moisture to avoid death and render anoxia unsuccessful as a method of control.

In the culture conditions described here, *A. verbasci* did not require direct contact with water, deriving sufficient moisture requirements from the surrounding media. This ability to survive very dry conditions may have contributed to the survival rate. Another factor which may have contributed to the survival rates in *A. verbasci* may be the ability to enter a diapause state when conditions become unfavorable for further development. This facility has been defined by Hanski (1988) as an arrested development phase that minimizes the utilization of body reserves, thereby reducing the risk of an individual dying as a result of unfavorable conditions. This ability provides a natural defense mechanism during conditions of particular hardship and can persist for considerable time periods. In natural populations diapause typically occurs in response to unsuitable ambient temperature or food scarcity.

The results of this study indicate that the use of an atmosphere reduced in oxygen has a lethal effect on a range of insect species. The main difficulty facing conservators is the application of the correct combination of oxygen concentration, exposure, temperature and humidity required to achieve a lethal effect on the particular pest species under treatment. Beauchamp *et al*. (1981) in a reference listing of museum pests, loosely describe 123 pests of museums, of which 98 are insects. The variability in response of different insect species to different concentrations of oxygen indicates that no general conclusions can be drawn; for example, the response of one species cannot be used as an indicator of the response of other species. Also, other potential museum pests, such as mites, typically occupy relatively dry habitats, possess a highly specialized cuticle to prevent water loss and the spiracles are able to regulate gas exchange through a complicated mechanism resulting in low water loss rates and good osmoregulatory ability (Fleurat-Lessard, 1990).

Until recently, modified atmospheric conditions have been applied mostly to controlling insects in large bulk materials containing dry grain or cereals. These methods have also proved useful in destroying infestations where chemical insecticide control was considered unsuitable or impractical. The apparent success of the use of modified atmospheres for the control of stored-product insects has led to interest among museum workers as to whether the technique could be successfully applied to pests of museum collections. Recent advances in the development of oxygen scavengers, oxygen barrier films and fumigation "bubbles" have facilitated this trend and several museums are now using the technique routinely (Elert and Maekawa, 1997; Gilberg and Roach, 1992; Maekawa and Elert, 1996; Valentin, 1993).

Oxygen-absorbent chemicals, when used in sealed conditions, have been shown to be effective against certain pests of stored products (Burke, 1996; Ohguchi *et al*., 1983). One such commercially available product is the oxygen scavenger "Ageless" manufactured by the Mitsubishi Gas Chemical Company of Japan. Ageless is prepared from powdered iron oxide and is manufactured in packet form, designated according to type and size. Gilberg and Roach (1992) describe the successful use of Ageless at the Australian Museum. Objects are packaged within a flexible, low-oxygen permeability barrier film along with the requisite amount of Ageless, heat sealed and placed within a temperature controlled cabinet for 3 week at 30°C. Subsequent examination of infested objects failed to indicate any evidence of continued insect activity.

The use of oxygen scavengers is limited to objects of small to moderate size. For the treatment of large items, alternative methods of producing a modified atmosphere lethal to insects is required. These methods rely on creating an airtight enclosure that is flushed with a humidity-controlled nitrogen source to achieve the desired low-oxygen level. The technique may be used as an alternative to subzero temperature control, where the size of material requiring treatment limits freezing by conventional methods or where concerns of potential deleterious effects to specimens and materials may limit its use. Specific applications that may be appropriate include treatment of infested bulk items and as a quarantine treatment for incoming material. The technique may be usefully employed in conditions that are gas proof, such as fumigation chambers or in areas that can be sealed against gas loss. Gas-proof fumigation sheets may also be suitable and the recent development of a fumigation "bubble" by Rentokil Ltd.

that is portable and has a built-in inlet and venting system is a promising development. The "bubble" was originally manufactured in 1988 for use with toxic fumigants such as methyl bromide but was later redesigned for use with nitrogen and consists of a heat-sealable, aluminized barrier film. Maekawa and Elert (1996) describe the use of an anoxic treatment system for the disinfestation of museum objects. The system consists of a high-volume nitrogen source, a gas humidification module, the anoxic enclosure, environmental sensors and a vacuum pump. The system had a leak rate of <0.005% (50 ppm) and this was found sufficient to maintain the required anoxic environment with no nitrogen flow for several weeks once an initial oxygen level of 0.1% had been achieved.

The use of carbon dioxide should also be investigated, because it has been shown to be effective at levels as low as 30% (Story, 1985). This makes it less difficult to maintain at a level toxic to insects than other gases such as oxygen, that require a level <2%. However, the use of carbon dioxide requires special handling, because as little as a 2% rise in air concentration can cause increased respiration in humans. A self-contained breathing apparatus is therefore essential before entering areas undergoing carbon dioxide fumigation. For this reason, reduced oxygen atmospheres may be more appropriate for museums when treatment of bulk items is necessary.

Although further research is required, low-oxygen atmospheres offer the potential of effective, residue-free pest control, combined with a reduced environmental hazard, providing a viable alternative to chemical fumigation. The advent of plastic barrier films of low-oxygen permeability combined with commercially available oxygen absorbent agents, facilitate the ease of use for most museums. Similarly, the use of air-tight enclosures under anoxic conditions are now well documented and represent a welcome development for the in-house treatment of bulk items.

CONCLUSION

The use of low oxygen atmospheres to control insect pests in museum looks extremely promising. Six species insect pests at various stages of their development were exposed to a low-oxygen atmosphere of 1.5% for a period of one week. The results showed that the modified atmosphere was observed to have a lethal effect on all insect stages tested apart from a 50% survival rate for the larval stage of *Anthrenus verbasci*. When the exposure period was extended to periods of 2, 3, 4, 5 and 6 weeks, respectively 100% mortality was recorded for all insects tested. Evidence from this investigation supports the view that atmospheres reduced in oxygen may represent a viable alternative to chemical control methods.

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