

## **Study on the Efficacy of Freezing Against Museum Pest Insect *Demestes maculates***

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**Abstract:** Museum taxidermies have traditionally relied on toxic chemicals for the prevention and treatment of pest infestations. However, concern over the health and safety implications of the use of chemicals in the museum environment, effectiveness, and potential adverse effects on museum specimens and associated materials has promoted investigation into alternative control methods. One such method is the use of freezing. The efficacy of freezing against museum pest insect *Demestes maculates* was evaluated. Results show that all pests tested at the surface of tanned skin failed to survive beyond 6 h of exposure of the temperature -20°C. However, the insulating properties of both the tanned skin and the stuff straw affected insecticidal effect by slowing the spread rate of cold air. Moreover, results also indicated that the rate of cooling played a key role in the course of eradication with freezing. With the increasing of the time of temperature equilibrium, the time from the point of equilibrium temperature to the point of obtaining 100% mortality increased significantly. It was suggested that the adaptation of pest insect against freezing would be strengthened and the freezing time would be prolonged if the temperature decreased slowly.

**Key words:** Equilibrium temperature, freezing, insecticidal effect, museum pest, taxidermy

### **INTRODUCTION**

Recently, insect pest management for taxidermy posed a dilemma in natural history museum. On one hand, the use of chemicals to protect taxidermy against damage by pests has a long tradition, and made great achievements which is witnessed by the quality and integrity of the considerable quality of taxidermies stored in museums throughout the world (Linnie, 1999). As a result, starting in the late 18<sup>th</sup> century and until relatively recently, the use of pesticides, which included different toxic chemicals such as arsenic, mercuric chloride, strychnine, DDT, ethylene dichloride, methyl bromide, ethylene oxide, sulfuryl fluoride and numerous others, was perceived as the only effective means of preventing the loss of taxidermies to various pests (Ornstein, 2010). On the other hand, with the development of economy and the environmental protection in the awakening of the awareness of mankind, concern over the health and safety implications of the use of chemicals in the museum environment, effectiveness, and potential adverse effects on taxidermies and associated materials has led to a reappraisal of collection protection policies (Dawson and Strang, 1992; Irwin, 1987; Linnie, 1996; Peltz and Rossol, 1983). Therefore, this has focused attention on alternative methods of controlling and eradicating pests from museums.

As regards museum objects, controlled atmospheres ( $\text{CO}_2$ -rich as low- $\text{O}_2$  atmospheres) and physical methods,

predominantly freezing technique, have been used in recent years (Berkouwer, 1994; Ketcham-Trosak, 1984; Strang, 1992; Burke, 1996; Navarro, 1978). Studies indicated that freezing of the body fluids of pests involves potentially lethal effects (Linnie, 1999). Firstly, metabolic pathways are generally slowed, potentially leading to an imbalance in the overall cellular metabolism. This may lead to the osmotic swelling of intracellular and/or intra-intestinal tissues, eventually resulting in rupture. Secondly, the growth of ice crystals into the cell may cause damage to the cellular structure. Extracellular freezing may cause cells to shrink, leading to intolerable osmotic stress across cell membranes. Lastly, freezing will also increase the concentration of inorganic salts to levels that may irreversibly change the structure and function of the host enzymes.

The destructive effects of exposure to low temperature are time dependent and it is therefore not possible to quote a single lethal temperature for a pest without stating the exposure period. The most usual method of defining the lethal conditions of temperature and time for a pest is to determine the combination of temperature and exposure time that kills a given percentage of the pest (Linnie, 1999).

Although the freezing methods is routinely practiced in the UK and other advanced countries for infestation control in different museum objects, recommendations of freezing times and temperatures for museum pests vary in different literatures (Pinnige, 2003). For example, Florian

(1986) reports on freezing procedures used in museum with temperature/time relationships ranging from -12°C for 48 to -40°C for 24 h, respectively, but the research by Pinnige (2003) suggests using either -30°C for three days or -18°C for at least 14 days. Meanwhile, in the last few years, the methods of control museum pests by freezing are introduced into China, but the results obtained from some trial experiments which were conducted for the taxidermies stuffed with straws according to the above guideline in the natural history museums of some cities such as Beijing, Shanghai and Tianjin, were not satisfied. Differences maybe resulted from various conditions such as individual differences of pests, different freezers, different treatment methods and difference of taxidermy. Studies indicated that the insulating properties of materials made for museum collections could affect the freezing results (Bergh *et al.*, 2006).

Before the modern form was evolved, taxidermy was stuffed with rags, cotton and straw as crude form. So there existed in natural history museums and private collections a large number of taxidermies stuffed with straws including many precious and scarce type specimens (Ornstein, 2010). All the time those taxidermies faced large challenge in pest control because of unsatisfied taxidermy technology limited by past low social development level. On one hand, the tanned skin made by old methods could be attacked easily by all kinds of pests; on the other hand, the stuffs such as straw, cotton and rags easily affected by different weather condition always provided favorable living environment for pests. However, no research about the control effects of freezing on pest infecting inside taxidermy stuffed with straws was reported.

Therefore, the present study was to investigate the control effects of freezing on the museum pest *Demestes maculatus* adult which simulated an infestation inside taxidermy made of tanned skin of *Bubalus bubalis* and straw blankets.

## MATERIALS AND METHODS

The experiment was carried out from October 08<sup>th</sup>, to December 30<sup>th</sup>, 2010 at the Animal & Ecology Test Lab, School of Life Sciences, Sun Yat-sen University, China.

The test insect used was *D. maculatus*, which was recognized as serious museum pest and selected to tested pest for all kinds of pest control methods because adequate number of its life stages can be easily reared from culture (Linnie, 1987). Stock cultures of strains of *D. maculatus* were obtained from School of Life Sciences, Sun Yat-sen University, China. Adult beetles of *D. maculatus* were selected from stock cultures and were provided with 200 g of ground culture media. Water was provided daily for five days by pipetting a small amount onto folded filter paper placed on the surface of media.

Adults of known age group (14-20 days) were selected from serial cultures using a spatula. Mature adults were selected to avoid immature cuticle susceptibility inherent in young beetles (Parkin, 1966).

Prior to the experiment, selected adults were transferred to 9.0-cm-diameter plastic petri dishes with lids and underwent a 3-day acclimation period at 20°C, 70-80% Relative Humidity (RH). Each dish contained 40 tested individuals.

The dishes were wrapped in straw blankets (density 0.06 g/cm<sup>3</sup>) at three distances from the upper surface to the middle approximately 0, 5, 10 cm from the surface (noted L2, L3 and L4), respectively. Then the blanket package as stuffing material was wrapped in the tanned skin of *Bubalus bubalis* used for taxidermy. Meanwhile, a dish noted L1 was attached on the skin surface by plastic film. Temperature sensors connected to a Squirrel data logger were placed close to each dish. Finally, the whole package was wrapped in plastic film and placed in a Cylinda AFB 500 chest freezer at -20°C. Control batches were held at 20°C in parallel with the test samples.

The freezing test lasted for 6, 12, 18, 24, 30, 36, 42, 48 and 54 h, respectively. The temperatures were recorded at the end of the test. After the freezing period had been completed, insects were removed and held at 20°C for monitoring and subsequent determination of mortality levels. For adult beetles, the lack of movement and failure to respond to gentle prodding, have been considered a moribund condition (Boles *et al.*, 1974). However, such a condition could also indicate paralysis (O'Brien, 1960). In this study the loss of the righting posture and absence of spontaneous movement or an irreversible, uncontrolled, lethargic condition was used to determine death. Each test was replicated three times and mean values were calculated.

Data were corrected for control mortality (Abbott, 1925), Corrected mortality = (Total mortality - Control mortality) / Total number of insect used × 100. Mean values of mortality and temperature were analyzed using SPSS 16.0 statistical software.

## RESULTS

The obtained temperatures of different depths of simulated specimen for different exposure time under -20°C were shown in Fig. 1. The temperatures of all depths decreased gradually as the exposure time increased and finally reached the lowest temperature of -20°C. Meanwhile, the times to -20°C increased as the depth increased. The depth of L1 reached -20°C less than 6 h, while it took nearly 18, 30 and 36 h to reach same temperature at the depths of L2, L3 and L4 respectively.

During the course of experiment, no death of pests were found in all control groups, but different mortalities

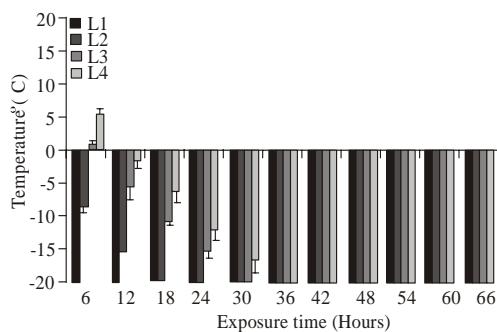


Fig. 1: Mean value ( $\pm$ SD for replicate) of obtained temperatures  $^{\circ}\text{C}$  of different depths of simulated specimen (L1, L2, L3 and L4, respectively) for different exposure time (6, 12, 18, 24, 30, 36, 42, 48, 54, 60 and 66 h, respectively) under  $-20^{\circ}\text{C}$

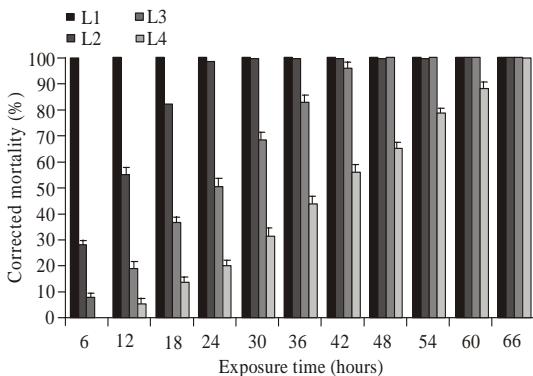


Fig. 2: Mean value ( $\pm$ SD for replicate) of corrected mortality (%) of adults of *Demestes maculatus* placed at different depths of simulated specimen (L1, L2, L3 and L4, respectively) and for different exposure time (6, 12, 18, 24, 30, 36, 42, 48, 54, 60 and 66 h, respectively) under  $-20^{\circ}\text{C}$

of adults of *D. maculatus* placed at different depths of simulated specimen (L1, L2, L3 and L4, respectively) were obtained from different exposure time (6, 12, 18, 24, 30, 36, 42, 48, 54, 60 and 66 h, respectively) under  $-20^{\circ}\text{C}$  (Fig. 2). The freezing time leading to 100% mortality of pests tested prolonged as the depths increased. All pests tested at L1 failed to survive beyond 6 h of exposure, while no mortality recorded at L4 after 6 h of exposure. Observation of 100% mortality of pests at L2, L3 and L4 appeared at least 30, 48 and 66 h of exposure respectively.

## DISCUSSION

The present study indicated that both the tanned skin and the stuff straw had the insulating properties and could affect the spread rate of cold air inside the taxidermy during the freezing course. It took nearly 18 h for the opposite of the tanned skin and 36 h for the core of the

straw blocks wrapped in tanned skin to reach equilibrium with the freezer temperature ( $-20^{\circ}\text{C}$ ). The similar findings also were found in the collection of other materials. The research of Bergh *et al.* (2006) illustrated that the middle layer of collections made of wood and wool reached  $-10^{\circ}\text{C}$  after 12 h and the temperatures did not decrease further even after expose of five weeks under the freezing temperature  $-20^{\circ}\text{C}$ , and only after air circulation in the freezer had been improved, did the middle layer reach temperatures of approximately  $-20^{\circ}\text{C}$  after 20 h.

In the study, all pests tested at the surface of tanned skin failed to survive beyond 6 h of exposure of the temperature  $-20^{\circ}\text{C}$ . It was suggested that freezing at  $-20^{\circ}\text{C}$  was an effective method for eradication of *D. maculatus* adults. The opinion was in agreement with the finding of previous report which demonstrated that although there was some variation in susceptibility between two museum insect pests species (*D. maculatus* and *Anthrenus verbasci*) evaluated and also between the different life-cycle stages (egg, larval, pupal and adult), exposure to a temperature regime of  $-20^{\circ}\text{C}$  for 6 h was sufficient to achieve 100% mortality throughout all stages of both species (Linnie, 1999). Strang (1992) in a review of literature relating to insect thermal mortality found no evidence of museum insect pests exhibiting a high degree of either freeze-tolerant or freeze-avoidance behaviour that would prevent their death below  $-20^{\circ}\text{C}$ .

Results also indicated that the rate of cooling played a key role in the course of eradication of *D. maculatus* adults with freezing method. With the increasing of the time of temperature equilibrium of the layer to  $-20^{\circ}\text{C}$ , the time from the point of equilibrium temperature to the point of obtaining 100% mortality increased significantly. Such variance could be related to the viability of the pest insects. Typically, viability was dependent on the rate of cooling, with greater mortality observed following rapid rather than slow cooling (Linnie, 1999). So, it was suggested that the adaptation of pest insect against freezing would be strengthened and the freezing time would be prolonged if the temperature decreased slowly. This is an important consideration in a museum context because the insulating properties of bulk materials containing suspected insect infestations are likely to slow down the rate of freezing.

## CONCLUSION

The present results demonstrated that freezing at  $-20^{\circ}\text{C}$  was an effective method for eradication of *D. maculatus* adults. However, this is an important consideration in a museum context because the time of temperature equilibrium which would be increased by the insulating properties of both the tanned skin and the stuff straw, could slow the insecticidal speed.

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