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Heat Eradication of Insect Infestations: The Development of a Low Cost, Solar Heated Treatment Unit

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Introduction

Above a certain funding threshold, a range of thermal treatment options become available to institutions wishing to rid their collections of insect pests. These include chest freezers, thermostat controlled laboratory ovens and the like at the low end of the price range; and purpose built, temperature and humidity controlled treatment rooms at the high end of the range.

The intent of this research is to provide a cheap, safe and controllable thermal treatment option, available for use by institutions whose circumstances or funding dictate that even the cheapest of the above treatment methods are beyond reach.

Previous research conducted by (amongst others) Thomas Strang of the Canadian Conservation Institute has shown that all life cycle stages of the insect pests usually responsible for insect infestation can be effectively eradicated by elevated temperature treatment at 60 degrees Celsius for a period of 4 hours (Strang 1992, 48).

What is required, is a treatment unit capable of raising the temperature throughout the object to be treated to the target temperature and then maintaining this temperature for the duration of the treatment time.

Preliminary low cost solutions based around the “black bag in the sun” approach, while often successful and unquestionably cheap to implement, can and often do exhibit significant drawbacks:

1. Uneven heating due to sunny side / shaded side effects.
2. Limited thermal gain resulting in incomplete/unsuccessful or overly extended treatments.
3. No control of maximum temperature resulting in excessive temperature exposure.

Understanding the risks

Understandably, the risks posed to collection items by elevated temperature treatment have raised concerns. These concerns are valid and some materials such as items containing wax or resin elements, deteriorated or improperly tanned collagenous or leather objects and items repaired with thermoplastic resins of low Tg such as Paraloid B72 are inappropriate for treatment within an elevated temperature facility.

The other primary areas of concern relating to elevated temperature treatment are:

1. Reduction of lifespan due to an acceleration of the deterioration rate.
2. Damage and distortion (primarily with regard to wooden artifacts) due to relative humidity changes.

- **Accelerated Deterioration**

With regard to accelerated deterioration; it is thought that the rate of reactions such as oxidation and cellulose chain-cision will approximately double for every 5 degree Celsius rise in temperature (Strang 1995, 202).

Deterioration can be predicted by a re-arrangement of the Arhenius equation:

Rate $\propto$ exp.(-500E/T)
Where E is the activation energy of the reaction in kcal/mol (in the case of cellulose chain-cision this is around 25kcal/mol) and T is the temperature in degrees Kelvin (Thomson 1978, 185).

A sample equation calculated at 20^{o}C (room temp.) shows that deterioration rate is proportional to: exp.(-500 x 25/293)
resulting in a value of 2.96537 x 10^{-19}

Working an equation at 5 degrees greater (25^{o}C) produces the equation: exp.(-500 x 25/298)
This results in a value of 6.06659 x 10^{-19} or a deterioration rate around 2.05 times faster.

Given the above we can deduce that a sample of low grade newsprint with an expected lifetime of 50 years if maintained at 20 degrees Celcius can be calculated to lose 64 days of life as a result of being exposed to a 6 hour treatment at 60 degrees Celcius (Strang 1995, 203).
If this is taken in context that the item to be treated is currently infested with termites, silverfish, book lice, clothes moth or similar, then while the loss of some two months of lifespan is regrettable, the likely losses resulting from the object remaining untreated are almost certainly more damaging.

- Temperature, humidity and equilibrium moisture content

In the case of damage due to applied changes in relative humidity, it is important to have an appreciation of the concepts of absolute humidity, relative humidity and equilibrium moisture content in order to understand the risks posed by thermal treatment.

As temperature changes, the ability of hygroscopic materials to hold water changes. Absolute humidity is defined as the mass of water (in grams) that is held as a vapor within a cubic metre of air. The maximum absolute humidity of air is not a constant value; rather it rises with an increase in temperature. At 0^{o}C saturated air can hold 4.8 g/m^{3} of water while at 20^{o}C it is capable of holding 17g/m^{3} (Derived from Richard 1991, 284).

Relative humidity (R.H.) of air is a percentage following the formula:

$$RH = \frac{\text{Absolute humidity of air sample}}{\text{Absolute humidity of saturated air at the same temperature}}$$

In wood, the mass of moisture held per unit volume is termed equilibrium moisture content (E.M.C.). A change of 10% R.H. will induce a change of approximately 1.8 E.M.C. Thus, a change of 1% E.M.C. can be said to be approximately equivalent to a change of 5.555% in R.H. (Richard 1991, 282).
Changes in E.M.C. cause wood to expand and contract. A change in R.H. will cause a movement of moisture to or from the wood, varying its E.M.C. resulting in expansion or contraction. The dimensional changes resulting from a change in E.M.C. are not uniform but are affected by species, sample and grain orientation. Wood expands least along the grain (longitudinally) where changes of 1% in R.H. will induce variations in the order of 0.00114%. This change is so small as to be considered insignificant (Thomson 1986, 223).

In contrast, tangentially the same 1% R.H. change will result in approximately 0.065% fluctuation in dimensions while in the radial direction around 0.040% change in size can be expected (Richard 1991, 282).

With reference to the two examples shown below, the author wishes to state his view that in all cases where objects are to be subjected to elevated temperature treatment, the objects in question should be sealed within airtight plastic bags.

- **The Unbagged situation**

If it is intended to expose wooden items to substantial variations in temperature, the implications of these expansions and contractions become significant. If a change in temperature from 20°C to 60°C is applied to a wooden object acclimatised to 50% R.H., as the wood is heated, moisture can be taken up by the surrounding air so the E.M.C. of the wood will fall. A 40°C change in temperature is expected to induce a change in E.M.C of around 2%.
A change of 1% E.M.C. is capable of causing 0.45 % tangential distortion. (Richard 1991, 282).
The 2% E.M.C. change resulting from the 40°C change in temperature can therefore be expected to result in a shrinkage of 0.9% which is capable of causing significant damage to the object in the form of warping or cracking. It is also worryingly close to the bottom end of the 1-3% elastic threshold of the constrained glassy polymers found on polychrome items and therefore has the potential to result in the shearing or fracture of the paint film (Strang 1995, 205).

- **The Bagged situation**

If a wooden object (for example a chair) is sealed within an airtight plastic bag and subjected to the same 40°C change in temperature, the mechanisms of expansion and contraction are significantly altered and the previous calculations for R.H. and E.M.C. no longer apply.

In the airtight sealed circumstance, a 5Kg chair constructed from pine will contain in the order of 420g of water. Assuming loose bagging, the 0.3m^3 surrounding the chair is only capable of absorbing 2.55g of water (Richard 1991, 284).

Taking the impossible circumstance that the air surrounding the chair were to begin with an absolute humidity of 0% and when heated absorbed all of the moisture it could until saturated, it could therefore only inflict a change of 0.6% on the E.M.C. of the chair. This would result in a maximum tangential distortion of 0.27%, well below the level expected to cause damage. Under normal tropical circumstances where R.H. is usually above 80% and starting temperatures above 30°C the distortion could be expected to be an order of magnitude smaller than the 0.27% calculated.
In the bagged case, thermal expansion is expected to play a greater role in the distortion of wooden objects. A change of 40°C is capable of inducing a maximum swelling (dependant upon the species of wood) of 0.3%. To put a dimensional change of 0.3 % in context, this would be expected were a display item to undergo a 0.7% change in E.M.C. equivalent to a 4.3% change in R.H. The stresses imposed on the bagged object being heated from 20°C to 40°C are therefore less than those encountered in a museum maintaining a +/- 2.5% R.H. air conditioned environment.

Obtaining more heat

In order to achieve a greater level of thermal gain than that offered by the "black bag in the sun" solution discussed earlier, the concept of the solar oven is used.

During the "green" boom of the late 70's and early 80's, much development work was done in the area of solar oven design in order to provide a cheap method of cooking food.

If a person stands in the sun, they warm up to the point where the heat being lost from their body is equal to the heating effect of the sun. If they are enclosed in a sealed car under the same conditions, the heat rises to the point where it is uncomfortable and can even be lethal. Heat is trapped within the car and builds up to a far greater level before the thermal losses become equal to the thermal gains (Millller 1997, 20).

The thermal gain process within a solar oven is effectively identical in concept to the heat gain within the car. Like the car, the oven has insulated walls and flooring and a relatively large expanse of glass exposed to direct sunlight. Short wavelength light can easily pass through the glass entering the oven. It is then absorbed by a dark surface and re-emitted as long wavelength heat. While glass will readily transmit 90% of visible light, its transmits only 10% of the heat energy, the remainder being trapped within the oven (Kerr 1991).

Unlike the "black bag" approach which utilises direct heating (the sun heats the bagged object), the solar oven heats indirectly. The oven contents are heated by warm air trapped within the oven not by sunlight. This eliminated the "shaded side / sunny side" effect and provides more even heating of the objects being treated.

Major factors affecting the heat that can be attained within a solar oven are:

- The size of the glass area
- The angle at which sunlight strikes the glass
- The effectiveness of the insulation within the walls of the oven
- Double glazing the glazed area

Due to a greater collection area/loss area ratio, shallow ovens with large windows have the most efficient heat gain. For cooking purposes where the maximum heat is required, ovens designed for the purpose are often only 100 or 200mm deep. In thermal treatment of objects, much lower heat levels are required so the oven design can be optimized around other factors such as available volume or the ability to treat unusually shaped objects.

Despite being optimized for large volume instead of cooking and therefore having a payload space of 1000mm x 500mm x 500mm (l x d x h), the prototype solar oven fabricated for the purposes of this research, has been shown to be capable of boiling water and baking a Pavlova on sunny Canberra days with ambient outside temperatures around 25^{o}C.

The prototype solar oven comprises a box 1200mm long, 700mm deep and 700mm high. The floor, ends, door and walls are 100mm thick and constructed from two 3mm plywood panels, the cavity between them being filled with shredded office waste. The roof of the oven is double-glazed and constructed from two sheets of 6mm window glass spaced 100mm apart with an air cavity between. At present, some condensation is found to build up on the glass surfaces within this cavity, thereby lowering thermal gain. Single-glazing, while having lower insulation properties should not be prone to condensation and may offer a performance improvement. Testing is currently underway to determine this.

Removing excess heat

Having developed a treatment unit with more than sufficient thermal gain, the challenge is then to develop a temperature limiting system with the following properties:

- Cheap to build
- Cheap to run
- Easy to calibrate
- Reliable in operation

After investigation, a simple electronic circuit was developed that operates from a 12 volt D.C. supply such as a car battery or similar. When triggered, the control system brings a 12 volt computer fan into operation. When running, the fan pumps cold outside air into the oven while hot air from inside the oven is forced out through an exhaust vent. By positioning the inlet fan at the top of the oven and the outlet vent at the bottom, the cooling system vents the coldest of the internal air first, maximising efficiency. This acts similarly to a fan forced oven, circulating heated air around the oven contents, thereby preventing uneven heating due to build up of pools of hot air.

The control circuit uses an electronic thermistor device encapsulated in a thin layer of epoxy resin as a temperature sensor. At a pre set temperature (determined by adjusting a knob on the control circuit), the circuit is triggered and operates a small relay switching on the fan. Once the temperature at the sensor falls below the triggering temperature, the relay switches off and fan operation ceases. The temperature sensing elements of the control circuit operate on a regulated voltage supply, ensuring that changes in battery voltage do not alter the temperature triggering point.

Calibration of the control system involves heating a vessel of water to the required triggering temperature, immersing the temperature sensor in the water and adjusting the control knob until the lamp on the circuit just turns on. As the water cools, the turn off temperature (usually within 2 degrees of the turn on temperature) can be determined and any adjustments required can be made.

By positioning the over temperature sensor in front of the cold air inlet, the envelope of temperature through which the oven operates can be further minimised by accelerating the speed at which fan switch off occurs. Not only does this result in more stable oven temperatures, it reduces the unnecessary wasting of heat from the oven.

Excluding the battery, recent retail estimates for the cost of the control system were below $60.00 Australian. A control circuit board revision is currently in progress.

Real world performance

Mid year in Canberra, Australia is far from an ideal time to be conducting solar heating experiments. Tests conducted with the same solar oven and the original first generation control circuit on an 18 ^{o}C day in October of 1998 proved the oven to be capable of reaching and maintaining a temperature of 60^{o}C +/- 2.0^{o}C for 6 hours. This temperature was recorded at the centre of a 10kg, bagged sample of radiata pine wood. (The same wood blocks have been heated and cooled from room temperature to above 60 degrees under bagged conditions over 10 times now and have thus far exhibited no signs of warping or cracking.)

Conclusions

By utilising materials that are relatively easy to obtain such as plywood, glass, shredded paper and silicone sealant; an oven can be built in house by institutions for minimal financial outlay. In order to make such a treatment unit safe for treatment of objects, the inherent limitations of such a device and treatment method need to be understood. Elevated temperature treatment is not ideal and not suitable for all materials. By following basic guidelines such as the presence of leather, wax and resin content, objects requiring treatment should be simple enough to assess.

In order to prevent excessive temperatures and greatly accelerated deterioration rates, suitable temperature limiting systems are required. Some elements of the control system such as sealed relays, thermistors and computer cooling fans may not be readily available in regional or developing areas. Once final circuit board revisions are completed and the design finalised, it is intended to make a control circuit kit available. It is hoped that cultural organisations or larger institutions might, (through means of the supply of a control kit) assist institutions that are less well off to build a solar oven in which to treat insect infestations occurring within their collections.

Given the ability of the solar oven to reach boiling point, proper maintenance (battery and fan checking) and correct operation of the unit is vital. Operators should fully understand the implications of a failure of these systems and should not regard the oven as a "set and forget" device.

The availability of solar energy is largely at the whim of nature. Scheduling treatments should take into account the possibility of less than ideal weather. Given that suitable results have been achieved as far south as Canberra, the central and northern regions of Australia and all regions closer to the equator should have little difficulty in utilising such a method for treatment.

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Attractant Pheromones of Museum Insect Pests

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ABSTRACT

Attractant pheromones have been identified for a number of insect pests which attack museum collections. They include well known pests such as, webbing clothes moth, *Tineola bisselliella*, furniture beetle *Anobium punctatum*, biscuit beetle*, Stegobium paniceum*, cigarette beetle, *Lasioderma serricorne* and the carpet beetles *Attagenus unicolor, Anthrenus verbasci* and *Anthrenus sarnicus.* There is also evidence that some other species such as the odd beetle *Thylodrias contractus* and the case bearing clothes moth *Tinea pellionella* also use pheromones although they have not yet been fully characterised. Laboratory research has clearly shown that many of these chemicals have a strong attractancy to males of the species which can be used to improve trap performance. However, successful use of the pheromones in practice is determined by lure performance and trap design. It also depends upon the commercial availability of lures at an economic rate. Trials in museums have shown that clothes moth pheromone lures can provide early warning of pest attack and detect failure of control treatments. They have also had an effect of reducing pest numbers when used at high rates. Cigarette beetle and biscuit beetle lures perform well at higher temperatures when the insects are active and flying. The performance of the pheromone of furniture beetle is not consistent and there is also a cross attraction to biscuit beetles. There are still many questions which need answering to improve the effectiveness of pheromone traps but for some species they have a valuable role for monitoring pests in museums.

Introduction

The development of insect traps for use in the public health and food industries has benefited museums because many of the traps have been successfully adapted for the detection of museum pests. The use of insect traps for monitoring is the first priority in setting up an IPM programme in historic houses or museums (Child and Pinniger 1994). Traps enable insects to be detected at very early stages of infestation and greatly improve chances of preventing damage. Over a period of time, a detailed picture of infestation problems can be built up to enable appropriate action to be taken (Pinniger *et al* 1998). They can also provide a useful check on the effectiveness of control measures.

Non-specific sticky traps should be used for basic monitoring of insects such as silverfish, booklice, carpet beetles and other crawling insects. Sticky blunder traps are good for monitoring the residual population of insects in a building to assess the risk to the collection. They can also give a useful indication of the number of crawling insects which are invading a room or building.

Although very useful, these blunder traps are very inefficient and only catch insects which actually walk on to them. Attractants, and particularly insect sex pheromones, can make traps much more effective.

Pheromones

Many insects emit chemicals to attract mates, these are called sex pheromones. In Table 1 are listed 30 of the main insect pest species of museums. The chemical structures of pheromones have been identified either definitely, or at least with reasonable certainty, for 14 of these species. For three other species (*Attagenus smirnovi, Dinoderus bifoveolatus* and *Thylodrias contractus*) there is good behavioural evidence for the existence of a pheromone but the structures have not yet been identified. For the remaining 13 species no pheromone has been reported. This might be because no one has looked for one or indeed that the species does not have a pheromone, in which case mate finding would be by means other than a semiochemical.

Within the 14 species for which pheromones have been identified, some use just one chemical while others use two or more components, each of which may make a slightly different contribution to the overall behavioural effect. Given the constraints that these chemicals must be relatively simple, in order to have sufficient volatility, they display a remarkable range of different structures. This is often achieved by subtle differences in size, functional group or shape.

Even within this small number of species there are several points of interest. For example, the male-produced sex pheromone of the dried bean weevil, *Acanthoscelides obtectus*, contains a very rare functional moiety, a carbon atom (in this case at position 5 in the chain) connected to each of its neighbouring carbon atoms by double bonds. In fact, this was the first reported example of such a linkage, an allene, produced in the animal kingdom (Horler, 1970).

Sometimes, pheromone effects may not be all that they first appear. The old house borer, or house longhorn, *Hylotrupes bajulus*, is reported to have a female-produced sex pheromone (Evans and Higgs, 1975). On the face of it, there is nothing unusual about this since the majority of sex pheromones are produced by the female and attract the male. However, in this case, all eight components are monoterpenes. They were obtained by rinsing frass from infested wood, and their activity was measured solely by electroantennography, not behavioural bioassay. It would seem more likely that these chemicals are obtained by the female directly from the wood in which it lives and signal the presence of a host plant rather than a mate. More recent investigation (Fettkoether *et al*, 1995), based on rinsing dissected prothoracic glands, has provided firmer evidence that it is the male which produces the sex pheromone in this species. It comprises three components whose structures are non-terpenoid and much more likely to be produced by the beetle than the host wood.

Pheromones are usually species-specific, to ensure that the expenditure of energy by making and releasing the pheromone result in attracting only the correct species with which mating can occur. However, there are an increasing number of examples of a single chemical structure having the same effect on more than one species. This is well represented in Table 1 by the pyralid moth pheromone, (Z,E)-tetradecadienyl acetate, but there is another, more interesting example. The drugstore beetle, *Stegobium paniceum*, has a female-produced sex pheromone of complicated structure with three asymmetric carbon atoms. At all of these the shape must be correct to elicit the desired behavioural effect. The importance of this is emphasised by the fact that the shape at one of these atoms is readily changed and that when this happens, the behavioural effect is completely lost. Nearly a decade later, colleagues of ours (White and Birch, 1987) discovered that the furniture beetle, *Anobium punctatum*, shares the same

basic structure as the pheromone of the drugstore beetle but these authors were unable to identify its exact shape. We can now announce that, by using a series of sophisticated techniques, we have proved that the shape of the chemical from *A. punctatum* is identical at all three of the asymmetric carbon atoms with the chemical produced by *S. paniceum*. This explains how it is possible to use lures for one species to attract the other but raises the question of why such a thing should occur and what would happen should the species co-exist.

With some species, such as the Phycitid moths, *Ephestia* sp and *Plodia*, only one of the components is needed in a lure to produce a very effective trap. With other species, such as clothes moth *Tineola,* both components appear to be necessary in a lure order to attract males.

Practical use of pheromones

Because of the varied behavioural response to pheromones, placement of pheromone traps is more complex than simple sticky traps. The pheromones will generally only attract the target species and may even repel other insects. The lures are also far more expensive than simple blunder traps and they are usually only used in areas where there is a high risk to valuable objects or where one of these pest species is suspected. As the lures will generally only attract flying males, it is essential to have an understanding of the pest biology and the performance of the pheromone. In warm climates, when insects are living outside museums, some insects may be attracted inside by the pheromones. In this case it is important to have traps outside to check on pest levels.

Webbing clothes moth *Tineola bisselliella* Lures containing the two pheromone components have been very successful. The flight activity of the males is stimulated by the pheromone and suspended traps provide a very useful early warning of infestation. When temperatures are high enough to promote flight, up to 20 times as many moths may be caught on traps with pheromone lures as on unbaited ones. When temperatures are below 20°C, the males are reluctant to fly and the suspended traps are relatively ineffective. In these situations, the pheromone can be placed on simple sticky traps on the floor and the traps with lures will catch more male moths. There is some evidence that the lures will have a low-level attraction to case-bearing clothes moth *Tinea pellionella.*

Furniture beetle or woodworm *Anobium punctatum* The synthetic pheromone is known as Anobinone and the results of trapping with these lures have been very varied. The pheromone-baited traps are sometimes very effective in trapping beetles, even at very low levels of infestation. However, there has sometimes been a very poor response on traps in some areas which were known to be infested. The success of the lure seems to depend upon the design of the trap, temperature, humidity and light levels. We need to know far more about the behavioural responses of beetles at low temperatures to be able to use these traps to accurately pinpoint infestation and target control treatments.

Biscuit beetle *Stegobium paniceum* Stegobinone, the pheromone of this species, is reported to be identical to that of furniture beetle *Anobium punctatum.* There is a trap marketed by Fuji containing lure which is an analogue rather than the authentic Stegobinone. Traps with these lures will catch more adults when the traps are placed in warm, well-lit areas when the adults are flying freely. At lower temperatures, the lures seem to be ineffective

As the pheromones of *Stegobium* and *Anobium* are identical, the *Anobium* pheromone lures are also effective against *Stegobium*. In trials in two small museums, more *Stegobium* adults were trapped with the Anobinone lure than on traps without the lure. In one museum, an expensive and unnecessary roof timber treatment was carried out to because the biscuit beetles on the Anobid traps were incorrectly identified as woodworm *Anobium punctatum*.

Cigarette beetle *Lasioderma serricorne.* The pheromone for this species is known as Serricornin. A number of successful traps have been designed for the tobacco industry and some of these have been used in museums. A combination of the pheromone lure and a food lure in the Serricotrap enables the trap to attract male and female beetles. As with biscuit beetles, these lures only work well when it is warm and the beetles are flying.

Case studies of trials of pheromone traps

1. **Webbing clothes moths in the Museum of the Welsh Woollen Industry (MWWI)**

The MWWI is housed in a large, former woollen mill and consists of a museum of the woollen industry with a commercial weaving operation. There are large stocks of fleeces, woollen yarns and woven woollen textiles, some of which are poorly stored in the attic spaces and other areas.

In May 1999, a large number of moths fluttering around in a number of rooms were noticed by staff. They were especially in the make-up rooms where woven material is made-up into finished items. Owing to the widespread and diffuse nature of the infestation and the large rambling building, it was impossible to locate a specific source of the infestation and to treat with insecticides in the traditional manner.
The moths were identified as *Tineola bisselliella* (webbing clothes moth) and therefore could be trapped with pheromone lures. Approximately 30 lures were placed in triple triangular blunder traps and placed at approximately 10-20ft intervals in the affected areas on the 1st and attic floors of the building. The traps were placed low down on shelves or on the floor in draft-free areas and where they were not likely to be trodden on. Within 2 weeks, the sticky surfaces of the traps were completely covered in trapped moths. The lures were transferred to new traps and replaced where they continued to catch moths for a further 1-2 months (until the end of August). No moths were seen after this time and no more were caught on the traps. Some limited spraying of affected material (bales of wool) was carried out with Constrain (a permethrin-based microemulsion insecticide).
In the following years (2000/2001) blunder traps and moth pheromone traps were placed in the formerly infested areas. Only a small number of clothes moth were caught on the pheromone traps indicating the success of the use of large numbers of pheromone traps and targeted treatment significantly reduced insect pest numbers.

2. Furniture beetle in a National Trust Store

The National Trust placed newly acquired furniture into a clean, dry store for documentation and conservation before being placed into the refurbished estate of Llanarchaeron. The furniture consisted mostly of 19th and early 20th century material and had been kept in very poor, damp conditions prior to its

acquisition by the Trust. Much of it was known to be infested with *Anobium punctatum* and, where possible, furniture was treated in April with Constrain (a permethrin water-based microemulsion insecticide). Traps baited with stegobinone (the *Anobium punctatum* sex pheromone attractant) and unbaited blunder traps were placed around the store to monitor the effectiveness of the insecticidal treatment. The traps were placed adjacent to articles known to be infested in order to check on the efficacy of the insecticidal treatment.

Emergence of *Anobium* adults occurred in the June/July period and many died within a few centimetres of the treated objects. The pheromone baited traps were no more effective in catching the emerging beetles than the unbaited blunder traps. The indications were that the pheromone lure may be ineffective on beetles already affected by the insecticide. It is worth noting that the *Anobium* pheromone was synthesised in the early 1990's and it is this batch that is still being marketed, and so the synthetic pheromone may no longer be an effective attractant. The manufacturers have no plans to synthesise further batches and the sale of the pheromone in the UK has now ceased.

3. Guernsey carpet beetle in the Natural History Museum, London

The major *Anthrenus* species in the Natural History Museum in London is now the Guernsey carpet beetle *A sarnicus*. By 1983 it had replaced the established species *Anthrenus verbasci* and it now occurs throughout the museum. It causes damage to skins, mounted mammals and birds and insect specimens. Sticky traps have been used since 1992 for monitoring adult beetles and to target cleaning and control measures. The pheromones of *A sarnicus* were identified by Finnegan and Chambers in 1993 and it was decided to carry out trials of synthetic lures in the Natural History Museum. Lures were made at CSL with decyl butyrate and decanol and preliminary trials in the Entomology Building were very promising. On 21 pairs of baited and unbaited traps, those with lures caught 35 beetles whereas those without lures caught only 6 beetles. The experiment was repeated in the following year and 22 beetles were caught on the pheromone lure baited traps and 6 on the unbaited traps. A further trial in 1995 in a large Zoology store room with 15 paired traps confirmed the efficacy of the lures. Only four beetles were found on the unbaited traps and 60 beetles were found on the traps with lures. Although these trials demonstrated that the pheromone lures were very effective, the localised distribution of the species to Southern England means that production of a commercial lure would be uneconomic. The spread of *A sarnicus* to other areas may mean that a lure may be economically viable in the future.

Conclusions

Although many insect pheromones have been identified, relatively few are available as commercial lures to use with traps, because of the cost or difficulty of synthesising the pure chemicals. Of those that are available, the most successful and consistent lure appears to be for webbing clothes moth. The lures for biscuit beetle and cigarette beetle also perform well in certain situations. The performance of the furniture beetle lure is less predictable. Successful use of any pheromone lure requires an understanding of the biology, ecology and behaviour of the pest. Pheromone traps are also far more successful when used as part of an IPM trapping programme.

Summary of general principles of insect trapping using pheromones

- Survey the site, prepare a plan and decide where to place traps
- Place sticky traps in a regular grid pattern
- Date-label traps and mark their position on a plan.
- Place blunder traps on floors in corners and near walls
- Place pheromone traps in open areas.
- Check traps at regular intervals.
- Identify and record insects caught on traps.
- Record whether insects caught are larvae or adults.
- Replace lures when the pheromone attractancy has been lost.
- Replace traps when they are dirty or if the adhesive has failed.
- Large numbers of non-pest insects may be caught on traps if they are near an outside door or window. When this happens, the traps should be replaced more frequently or the trapped insects will become food for pests.
- Over a period of time a record of catch will build up a picture of the distribution of insects.
- Additional traps should be placed in areas where pests need to be more accurately pinpointed.
- Traps should be used as a supplement to visual inspection and the information used to target preventative and remedial measures.
- Traps can also be used to check on the effectiveness of control treatments

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Table 1: Present knowledge of pheromone components of some major beetle and moth pests of museums

Insect species	Pheromone component	Reference

Acanthoscelides obtectus	Methyl (R)-(-)-(E)-2,4,5-tetradecatrienoate	Horler, 1970
Anobium punctatum	2,3-Dihydro-2,3,5-trimethyl-6-(1-methyl-2-oxobutyl)-4H-pyran-4-one	White and Birch, 1987
Anthrenus coloratus	No pheromone reported	
Anthrenus flavipes	(Z)-3-Decenoic acid	Fukui *et al.*, 1974
Anthrenus fuscus	No pheromone reported	
Anthrenus museorum	No pheromone reported	
Anthrenus oceanicus	No pheromone reported	
Anthrenus sarnicus	Decyl butyrate	Finnegan and Chambers, 1993
Anthrenus scrophulariae	No pheromone reported	
Anthrenus verbasci	(Z)- and (E)-5-Undecenoic acids	Kuwahara and Nakamura, 1985
Attagenus elongatulus	(Z,Z)-3,5-Tetradecadienoic acid	De Jarlais and Emken, 1987
Attagenus pellio	No pheromone reported	
Attagenus smirnovi	Pheromone reported but not yet identified	Morgan, 1994 Ackery et al 1997
Attagenus unicolor (formerly *Attagenus megatoma*)	(E,Z)-3,5-Tetradecadienoic acid	Silverstein *et al.*, 1967
Dinoderus bifoveolatus	Evidence for a pheromone but not identified yet	Borgemeister *et al.*, 1999
Endrosis sarcitrella	No pheromone reported	
Ephestia cautella	(Z,E)-9,12-Tetradecadienyl acetate (Z)-9-Tetradecen-1-yl acetate	Kuwahara *et al*., 1971 Brady, 1973
Ephestia elutella	(Z,E)-9,12-Tetradecadien-1-yl acetate	Brady and Nordlund, 1971
Hofmannophila pseudospretella	No pheromone reported	
Hylotrupes bajulus (from females)	*p*-Cymene-8-ol *cis*-Verbenone	Evans and Higgs, 1979
Hylotrupes bajulus (from males)	(3R)-3-Hydroxy-2-hexanone (2R,3R)-2,3-Hexanediol (2S,3R)-2,3-Hexanediol	Fettkother *et al.*, 1995
Lasioderma serricorne	(4S,6S,7S)-4,6-Dimethyl-7-hydroxynonan-3-one	Chuman *et al.*, 1979 (stereochemistry established by Mori *et al.,* 1981; 1982)

Lyctus species?	No pheromone reported	
Plodia interpunctella	(Z,E)-9,12-Tetradecadien-1-yl acetate (Z,E)-9,12-Tetradecadien-1-ol	Kuwahara *et al,* 1971; Brady *et al.*, 1971
Ptinus tectus	No pheromone reported	
Stegobium paniceum	(2S,3R,1'R)-2,3-Dihydro-2,3,5-trimethyl-6-(1-methyl-2-oxobutyl)-4*H*-pyran-4-one (2S,3R,1'S,2'S)-2,3-Dihydro-2,3,5-trimethyl-6-(1-methyl-2-hydroxybutyl)-4*H*-pyran-4-one	Kuwahara *et al.*, 1978 (stereochemistry established by Hoffmann and Ladner, 1979; Hoffmann *et al.*, 1981) Kodama *et al*, 1987 (stereochemistry at C2' established by Mori and Ebata, 1986)
Thylodrias contractus	Behavioural evidence for a pheromone but not yet identified	Pinniger 2001
Tinea pellionella	No pheromone reported	
Tineola bisselliella	(E)-2-Octadecenal (E,Z)-2,13-Octadecadienal	Yamaoka *et al.*, 1985
Trichophaga tapetzella	No pheromone reported	
Xestobium rufovillosum	No pheromone reported	

Experimental Study of Physical Effects of Freezing Method for Insect Control on Artifact Materials

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SUMMARY

We performed experiments to evaluate the physical effects of freezing methods on various artifact materials, including books made of Japanese paper and of western paper; textiles composed of silk, cotton and wool; woods of cypress, zelkova, horse chestnut and cedar; and Japanese lacquered wood board and lacquer ware. The experimental results showed that relative humidity decreased by 20% to 30% when the temperature was lowered from 25°C to -30°C. This is due to a decrease in the relative humidity of the air, which is in equilibrium with the water content of the material. Accordingly, the movement of moisture in and out of the sample is thought to be small during the process. The physical effect on the materials is considered to be relatively small. From these results, it is found to be possible to widen the application of the thermal method to cover traditional Japanese artifact materials.

1. Introduction

From 2005, the use of methyl bromide in the fumigation of insect infested materials will be banned to protect the ozone layer. One alternative method of insect eradication is the freezing method, through which affected artifact materials are sealed in a polyethylene bag and subjected to a low temperature of around -30°C for several days. Since the use of this method causes large changes in the temperature of the artifact materials, it is necessary to clarify the types of artifacts that are suited and not suited for it. Recently, this thermal method is studied extensively in European and American countries. Florian (1986) studied the mechanism how freezing process effects on insects and also on artifact materials. Strang (1992, 1993, 1995) reviewed the temperature condition for eradication of museum insects and proposed appropriate procedure for application of thermal method. This thermal method has been successfully applied to the books, textiles, leathers and herbarium. But, due to the rapid changes of temperature and humidity, there are some materials to which the thermal method is not applicable. This method is not applicable to the oil paintings and acrylic paintings, since these materials make glass transition under the temperature of –30°C. Since film, ivory and urushi artifacts are week against the relative humidity change, it is difficult to apply this method to them. It is also difficult to apply this method to composite artifact materials with paintings or lacquer on the surface, and excavated woods with high water content since there is a possibility of cracking or exfoliation during this procedure. Some of the difficulties of application are related to the moisture movement and deformation of artifacts due to the rapid temperature and relative humidity change of this procedure. This problem can be cope with enclosing the artifacts with waterproof bag. This paper reports the experimental study of temperature and humidity changes in the polyethylene bag in which various materials are contained during the freezing procedure. It is also measured the deformation of wood which are used commonly for traditional Japanese artifacts, caused by temperature and relative humidity change. From these experimental results, the applicability of freezing method for the traditional Japanese artifacts will be discussed.

2. Experimental Method

Experimental samples used here are books made of Japanese paper and of western paper; textiles composed of silk, cotton and wool; woods of cypress, zelkova, horse chestnut and cedar; and Japanese lacquered wood board and lacquer ware.

These samples were placed in a constant temperature and humidity chamber of 25°C and 60% RH for more than 24 hours. The samples were then sealed in a polyethylene bag and the temperature was lowered to -30°C over 2 hours. They were kept at this temperature for 96 hours. Then, the temperature was increased back to 25°C over 2 hours, at which the sample was kept for more than 24 hours. During this procedure, the temperature and humidity inside the bag were measured. To measure the temperature and humidity, HOBO-H8 (Onset Computer Co.) was used. Prior to the experiment, all sensors were calibrated with a constant humidity chamber of NaBr solution and $MgCl_2\_6H_2O$ solution. The temperature dependency of the humidity sensor was also obtained by lowering temperature of the humidity chamber. Figure 1 shows the results of the measured humidity with decreasing temperature by 5 _ every 12 hour. Figure 2 shows the relationship between the measured relative humidity values and temperature at steady state. In the figure the relationships between the equilibrium relative humidity with saturated solution of NaBr and MgCl2_6H2O are also shown. The measured values

and the equilibrium relative humidity are corresponded well each other above 0 _.

The strain measurements of woods, lacquered board and lacquer ware were performed using strain gage for low temperature (KFL-30-350-C1-5) and data logger (UCAM-20PC-1, Kyowa Electric Co.).

In order to obtain the quasi equilibrium relationship between the relative humidity and temperature around the materials inside the polyethylene bag, series of experiment were also carried out at slow rate of temperature change.

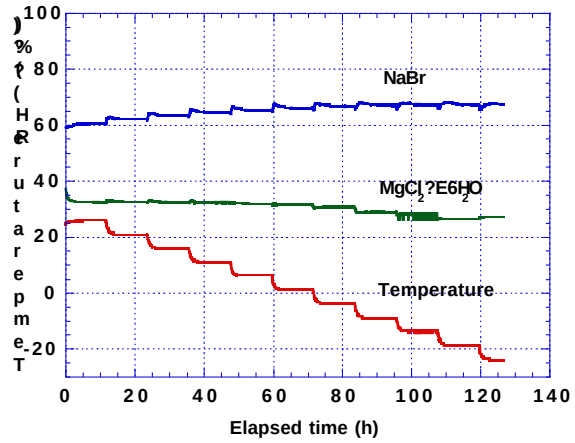


Figure1. Measured relative humidity and temperature change

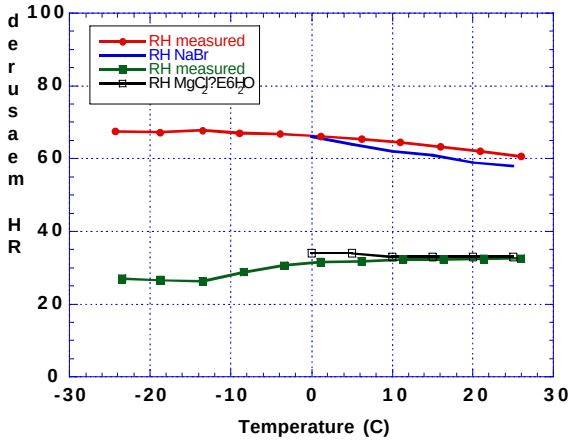


Figure 2. Relation between the measured relative humidity and temperature

3. Experimental Results

3.1 Relative Humidity Measurements

3.1.1 Books of western paper

Prior to the experiment, small space was made for humidity sensor in the center of the book by cutting and removing paper. Then the humidity sensor was installed at the center of the book. Figure 3 a) shows a

typical example of temperature and humidity change inside the book in polyethylene bag. With decreasing temperature, the humidity also decreased. And with increasing temperature, the humidity also increased. Figure 3 b) shows a relationship between the relative humidity and temperature inside the polyethylene bag. The relative humidity decreased linearly with decreasing temperature above –20°C. The value of the relative humidity is constant at the temperature below –20°C. This is due to the fact that this region is out side of measurable limit of humidity sensor. Figure 4 a) shows an example of temperature and humidity change inside the sample without polyethylene bag. When the temperature was warmed up, the relative humidity went up to 80%. Figure 4 b) shows the relationship between the relative humidity and temperature. This shows a big hysteresis. This is due to the water vapour movement to the book with the temperature gradient without polyethylene cover. In addition to these experiments, the freezing test was also carried out at very slow rate of temperature change. The rate of the temperature change was 55°C/ 48h (1.15°C/ h). Figure 5 a) shows the relative humidity and temperature change inside the polyethylene bag. The relative humidity decreased linearly with decreasing temperature. This curve corresponds to the relationship between the relative humidity and temperature of water vapor which is quasi equilibrium with water inside the material. When we compare Figure 3 b) and Figure 5 b), we can see the similarity of these curves. This shows that the water content of the material is constant and water vapour movement around the material is small.

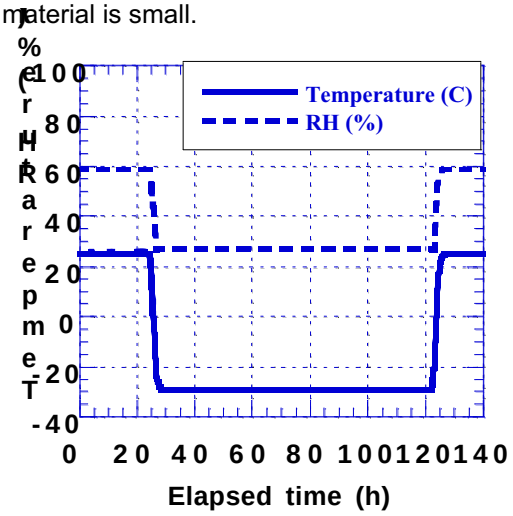
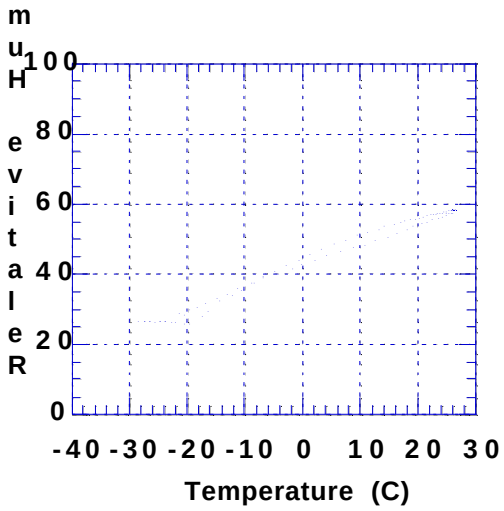


Figure 3 a) Temperature and RH change in the books of western paper in polyethylene bag.



b) Relationship between RH and temperature.

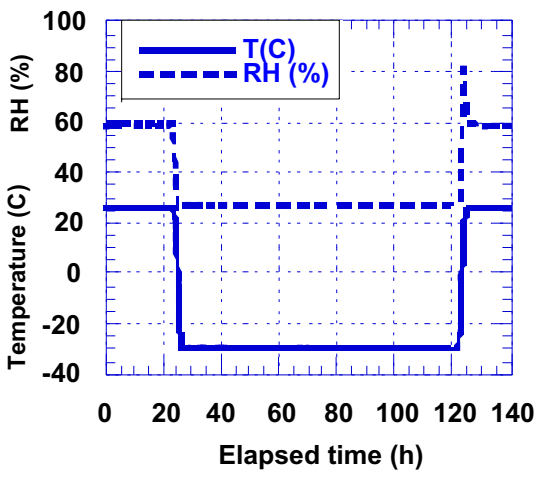
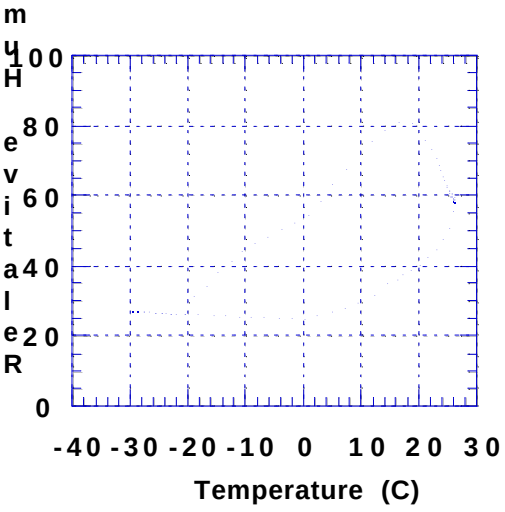


Figure 4 a) Temperature and RH change in the



b) Relationship between RH and

books of western paper without polyethylene bag.

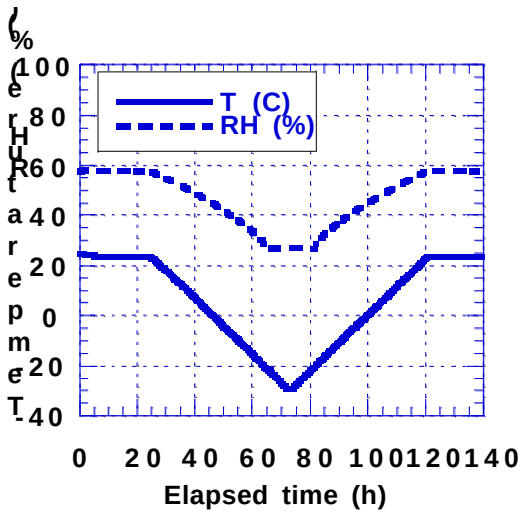
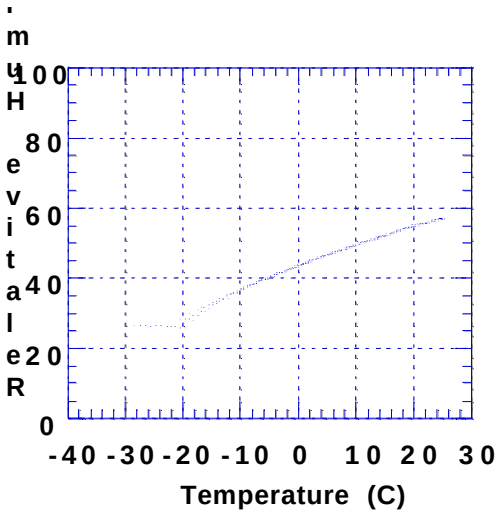


Figure 5 a) RH change in the polyethylene bag at slow temperature change.

temperature.



b) Relationship between RH and temperature.

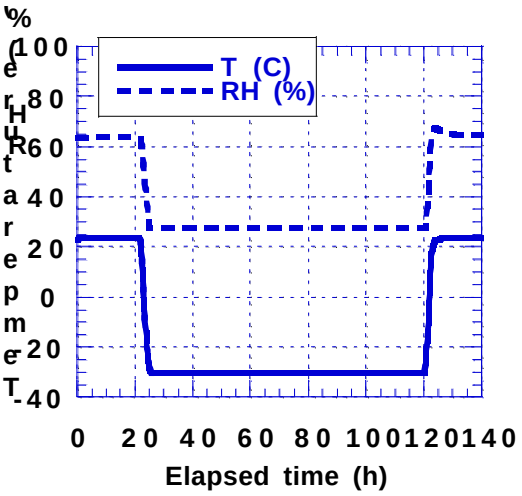
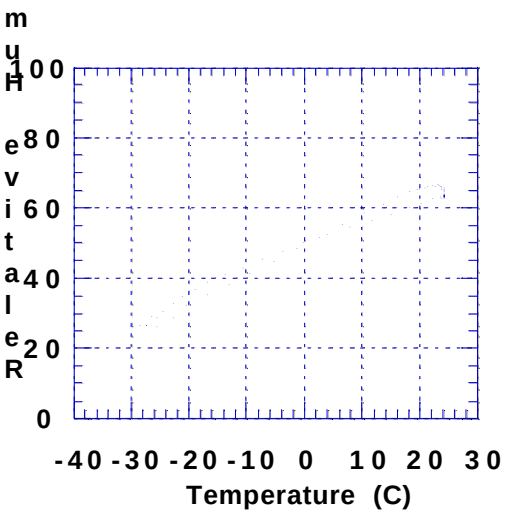


Figure 6 a) RH change in the polyethylene bag with books of Japanese paper.



b) Relationship between RH and temperature

3.2 Strain measurement

Figure 8 shows the measured strain of cypress board due to the temperature change. The dimension of the cypress board is 120mm length, 50mm width, 5mm thickness. One surface was coated with black lacquer (Kuro urushi) or with red lacquer (Shu urushi). Strain gages were attached to both surfaces in the longitudinal direction and lateral direction to the wood grain. When the temperature was lowered from 25 °C to –30 °C, the strain of black lacquer surface increased to 2650 μs in the lateral direction. The strain of red lacquer surface in the lateral direction increased to 2550 μs. The strain of the surface in the longitudinal direction increased to 250 μs. The strain in the longitudinal direction is one order smaller than that in the lateral direction. Figure 9 shows the measured strain of wood of zelkova with or without lacquer. The measured strain of lacquer surface of zelkova in lateral direction was 2500 μs. The measured strain of zelkova without lacquer in lateral direction was 2000 μs. The measured strain of wood of zelkova in the

longitudinal direction was 300 μs. From the wood and lacquer ware samples, maximum longitudinal strain and lateral strain were 450 μs and 2650μs, respectively, when temperature was lowered from 25°C to -30°C. This corresponds to 0.045% and 0.265% shrinkage of the material under the temperature change of 55 _, respectively. These values are equivalent to the deformation of the material when the water content of the material changed by only 1% (Narisawa, 1982). Their resulting dimensional changes were calculated to be 0.0008%/°C and 0.0048%/°C. This corresponds to coefficients for the thermal shrinkage of wood in the longitudinal and lateral directions. The materials returned to their original dimensions when the temperature was increased to 25_.

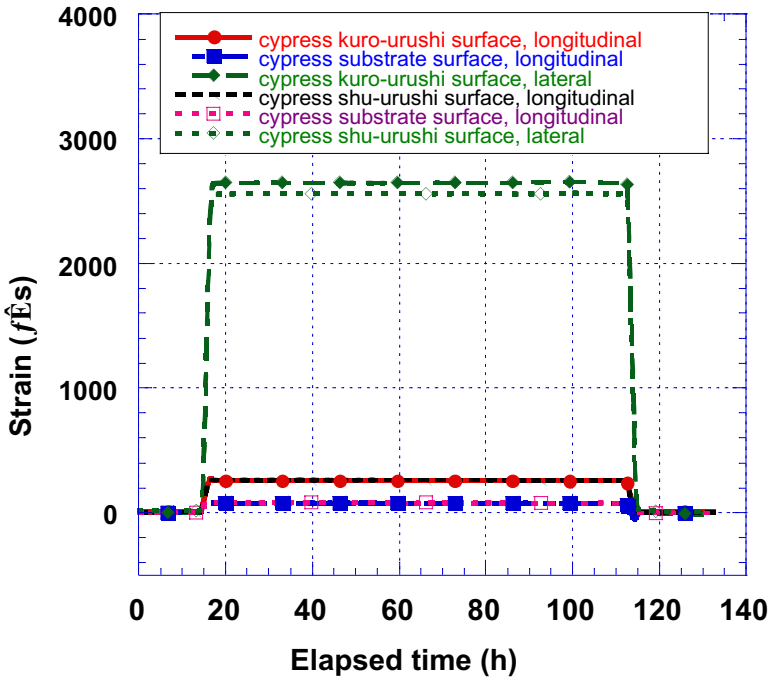


Figure 8 Strain changes of cypress board due to the temperature change

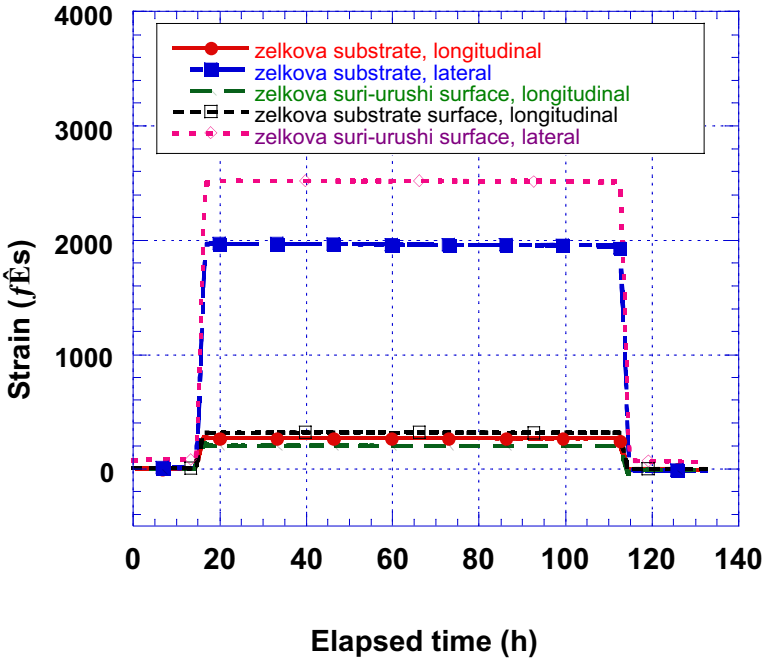


Figure 9 Strain changes of zelkova board due to the temperature change

Figure 10 shows the strain changes of the wood samples of cypress, zelkova and horse chestnut when the chamber humidity was changed from 60% RH to 50% RH. The surface of all samples were coated with Kuro-urushi on one side and samples were not sealed in the polyethylene bag during the experiment. The strain increased gradually with time even after 400 hours from the humidity change. This is due to the decrease of water content of wood sample by evaporation from the wood surface which accompanies water migration in the sample. These processes are considered to damage the wood sample and its surface. The strain changes shown in Figure 8 and 9 are quicker than that in Figure 10. The amount of strain in Figure 8 and 9 is comparable to those in Figure 10., but water does not migrate and evaporate in the former case and its physical effect on the material is considered to be relatively small.

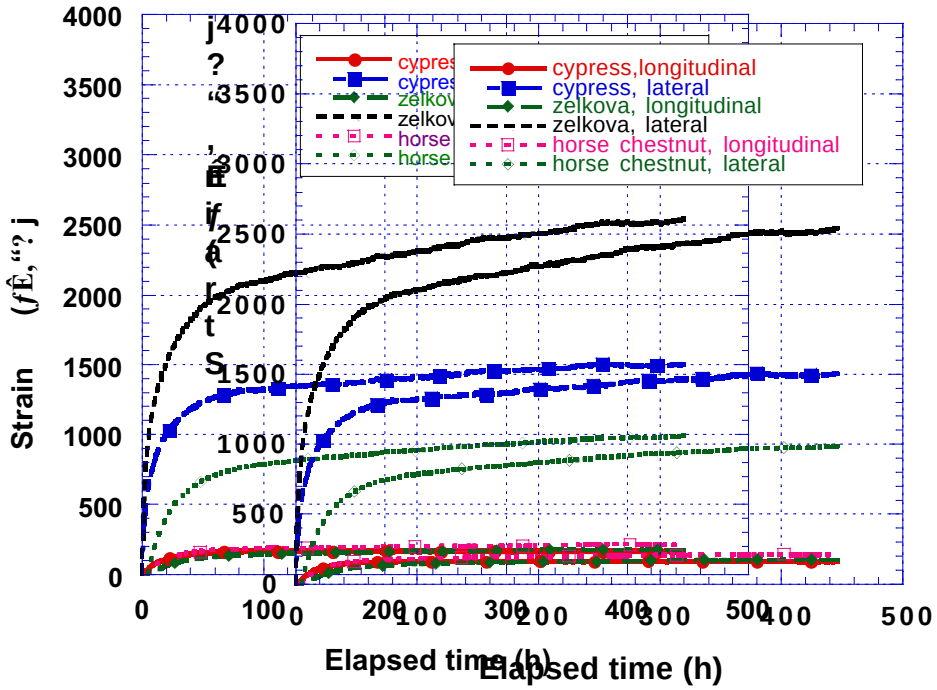


Figure 10. Strains of wood board of cypress, zelkova and horse chestnut due to the humidity change from 60% RH to 50% RH

4. Conclusions

Based on a thermodynamic calculation, the freezing points of water inside the materials are lower than -77°C at 50% RH, -57°C at 60% RH, and -39°C at 70% RH. Therefore, water inside the material will not freeze under normal museum environments. The experimental results showed that relative humidity decreased by 20% to 30% when the temperature was lowered from 25°C to -30°C. This is due to a decrease in the relative humidity of the air, which is in equilibrium with the water content of the material. Accordingly, the movement of moisture in and out of the sample is thought to be small during the process. This result is different from that when relative humidity is changed at a constant temperature, which causes the moisture content of the sample to change more radically, resulting in greater deformation of the material. During the experiment, maximum relative humidity was measured at around 70%, and no water condensation was observed in any of the samples.

From the strain measurement of the wood and lacquer ware samples, maximum longitudinal strain and lateral strain were 450 μs and 2650μs, respectively, when temperature was lowered from 25°C to -30°C. This corresponds to 0.045% and 0.265% shrinkage of the material under the temperature change of 55 °C, respectively. These values are equivalent to the deformation of the material when the water content of the material changed by only 1%. From these experimental results, the physical effect on the materials is

considered to be relatively small. It can be concluded that it is possible to widen the application of the freezing method to cover traditional Japanese artifact materials, if the treatment is carried out following appropriate procedure. We are continuing our evaluation of the physical effects of the method on composite artifact materials.

5. Acknowledgment

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Detection of Fungi and Control of Disinfections by ATP-Bioluminescence assay

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ABSTRACT

Mould contamination in indoor climate has been recognized in recent years as a major concern. Despite the fact that moulds damage works of art, they also could contribute to health hazards. Nowadays, the conventional method to detect and identify such micro-organisms is to put them in culture. Unfortunately, such method is laborious and time-consuming. Bioluminescence method and more particularly ATP methodology, has been widely applied in other domains such as medicine, food quality control, environment protection, pharmacology. Its success on bacteria and yeast has been demonstrated. Many commercial reagent kits are available and we tested some of them. Our early experiments have shown that the use of these kits did not give satisfactory results on mould. Reasons being that provided extraction buffer does not allow a proper extraction of ATP from fungi's cells. Therefore new reliable, simple and rapid extraction strategies specific to fungi are required. We compared forty types of extraction protocols. Extraction with 90% of DMSO in Tris-acetate buffer at 100°C seems to be the best. The viability of cells is estimated by the adenylate energy charge (EC), which corresponds to the equilibrium value of the metabolic energy between the three forms of adenine-containing nucleotides (ATP, ADP et AMP). We applied our method on well-known species such as Aspergillus flavus, Aspergillus niger, Neosartorya fischeri, Fusarium solani, Eurotium chevalieri, Penicillium chrysogenum and Chaetomium globosum. The results presented here are related to species taken both individually and in a mixture. In order to demonstrate and validate our method, we treated the same species with steam and ethylene oxide. The results show that the EC of viable cells is greater than 0.80, the EC of killed cells treated with steam is less than 0.50. We observed that the behaviour of cells treated with ethylene oxide is complex. For certain strains, the value of the EC varies between 0.5 and 0.8 according to the disinfection centre. So, our method can be used to monitor rapidly the efficiency of a process of disinfection with ethylene oxide. It has been also used on documents featuring suspicious stains. The results have been compared to conventional method. It showed that the ATP technique provides a very simple and fast method.

1. Introduction

Fungal contamination is a major cause of deterioration of libraries, archives and museums materials [1, 2]. The presence of biological agents are also a potential health risk because some species are highly pathogenic or are known to cause allergies, asthma and exert toxic affects [3, 4, 5, 6, 7]. Mould and fungi spores are always present in the air and will start to grow wherever conditions are favourable.

The detection of viable fungal propagules on surfaces and materials are traditionally performed using cultural techniques (inoculating, incubating and reading the plate). Conventional culture is simple but time consuming and gives wrong results when the culture medium is not adequate or when the cells sampled are viable but non-cultivable. At the opposite, the ATP based technique is sensitive, quick and reliable enough to be chosen as an alternative to conventional methods. Several applications of ATP assay for the detection of microbial and yeast contamination have been described previously [8, 9, 10, 11, 12]. Some

reagent kits are now available on the market but they do not provide convincing results for moulds [13, 14, 15, 16, 17]. The Centre de Recherches sur la Conservation des Documents Graphiques (CRCDG) has elaborated a procedure for mould. This procedure initially developed on the spores of *Aspergillus niger*, may be applied to other moulds. But we were faced to a problem: for some strains, when dosing spores treated with ethylene oxide, the measured ATP content was similar to that obtained with untreated living spores, while culturing technique revealed their inhibition. To verify if these treated strains were really dead, we considered not only the ATP itself but the all adenylic nucleotides together in order to be able to calculate energy charge (EC) i.e. the equilibrium value of the metabolic energy between the three forms of adenylic nucleotides (ATP, ADP and AMP). Some authors demonstrated that EC could be a useful indicator for cell growth and metabolic activity [18, 19, 20]. We applied the estimation of the energy charge to 10 fungal strains disinfected with ethylene oxide in different companies and we compared the results to the same untreated strains together with those sterilized by steam i.e. killed fungi. Several investigations have also been conducted on documents naturally contaminated or presenting suspect spots. In a previous paper, ATP content and EC values of some living or disinfected fungal strains were determined [21]. The results described here are a following of this study on more strains.

2. Materials and Methods

2. 1. Fungal strains

Ten strains of filamentous fungi from the mycological collection of CRCDG have been used. They are: *Aspergillus flavus, Aspergillus niger*, *Aspergillus fumigatus*, *Aspergillus versicolor*, *Eurotium chevalieri*, *Neosartorya fischeri, Penicillium chrysogenum, Fusarium solani, Chaetomium globosum* and *Ulocladium* spp. They were cultivated on paper disks placed on a malt agar substrate at 26°C until the paper samples were completely covered with fungi. Contaminated papers were placed in Petri dishes and air-dried. Fungi have been used in the form of suspension of spores (sampled with a spatula) either individually or in a mixture with other strains. Each suspension has been diluted to have a final concentration of about 1-2 10^6 spores/ml

2.2. Sampling of documents naturally contaminated

Samples were collected from different documents presenting suspect spots or true contamination using swabbing technique. They were transferred into 1 ml of distilled water. Then, the number of spores of the suspension was estimated.

2.3. Disinfection treatments

Artificially contaminated paper samples using different fungi were inserted into envelopes and treated by ethylene oxide in three different disinfection companies (EO1, EO2 and EO3) under the condition routinely used for disinfection. Another set of contaminated paper samples was sterilized by steam (20 min at 120°C). Fungi have been used in the form of suspension of spores (sampled with a spatula) like non-treated paper samples. The effectiveness of the treatment was controlled by a classical microbiological method using malt-agar medium.

2.4. Extraction of ATP

ATP was extracted from spores using dimethyl sulphoxide (DMSO) at a concentratrion of 90 % in Tris-acetate EDTA buffer at pH 7. The extractions were performed in glass vials at 100ºC during 1 minute after

adding 1 ml of spore suspension in 1ml of DMSO extractant pre-heated at 100ºC during 2 minutes. Then the extracts were rapidly cooled into an ice bath.

2.5. Main reagents

•*The luciferin-luciferase reagent - ATP Monitoring Reagent AMR* (Labsystem ref. BO1243200) was supplied as a freeze-dried powder which was reconstituted by adding 10 ml of Tris-acetate buffer (Labsystem ref. BO1243227). The solution was kept for one hour at room temperature in order to create equilibrium. The AMR stock solution was then diluted in Tris-acetate buffer (1:5) and preserved in a freezer until its use. Each lyophilized vial allows performing about one hundred tests. The reconstituted AMR is stable at 25ºC for 8 hours, at 4ºC for 5 days and at -18ºC for 2 months.

•*The ATP standard* (SIGMA, ref. FL-AAS) is also provided in a lyophilized form and has to be diluted in 10 ml of deionized water to make a final concentration of 1 µg ATP/ml. The ATP standard is used for calibration. In fact, the intensity of the measured luminescence may be reduced via turbidity or light absorption by some samples. The addition of a known quantity of ATP standard (0,01 µg) enables calibration of the measuring device and thus eliminates these bad effects. The reconstituted ATP standard solution is stable at 25ºC for 7 hours, at 4ºC for 5 days and at -18ºC for 2 months.

2.6 Nucleotides measurements

ATP was measured in a reaction mixture that contained 200 µl of extract and 300 µl of diluted luciferin-luciferase solution (AMR). The measuring cuvette was immediately placed into the luminometer (Turner Design TD-20/20) in order to get the first reading of bioluminescence value given in RLU (Relative Light Unit). A delay period of 5 seconds and an integration period of 20 seconds (time of measurement) were chosen. Then, 10 µl of ATP (0.01 µg) were added as internal standard and the number of RLU was re-measured for the second time. For each run, a blank test was performed in which the extract was replaced by the same volume of extractant. For each sample at least 5 measurements were performed. Each experiment was repeated three times on different days. Thus, the results presented are the average values obtained from15 measurements.

To calculate EC values, it is necessary to have the concentration of ATP, ADP and AMP. ATP was determined directly as presented above. Values of ADP and AMP were obtained after their enzymatic conversion to ATP by pyruvate kinase (PK in 10 mg of glycerol, Roche diagnostics, ref. 109045) and adenylate kinase (AK Sigma, ref. M-3003) respectively, and measuring ATP after each step. For each run 3 measuring cuvettes were used to perform the quantitative determination of [ATP], [ADP + ATP] and [ATP + ADP + AMP] [18]. The concentration of adenylate nucleotides (A.N) in each of the 3 cuvettes is given by the following formula: AN (pg) = [(S – T) / (I – S)] x [ATP standard (pg)] where S is the first RLU measurement of the sample, T the RLU measurement of the blank and I represents the second RLU measurement of sample after addition of the internal standard.

ATP content is given directly by measurement of Cuvette 1

ADP content = Cuvette 2 - Cuvette 1

AMP content = Cuvette 3 - Cuvette 2

The energy charge (EC) was calculated according to the following formula [18, 22]:

EC = [(ATP) + 1/2 (ADP)] / [(ATP) + (ADP) + (AMP)]

3. Results - Discussion

3.1. ATP content of living and treated strains

Table I presents the mean values of ATP content for living and treated strains. For living strains, the average ATP level ranged from 69.05 to 1226.40 pg depending on the species. Values from F. so*lani* are not to be considered because most of the figures are aberrant. We think that interactions between the pigments of this strain and the bioluminescence reagents might have occurred. On the other hand, we observed that the size of spores does not have impact on the measurements. In fact, the effectiveness of the ATP extraction depends on how the fungal spore walls react with DMSO, this characteristic being related to the species. Working with bacteria and boiled Tris for the extraction, Lundin and Thore made similar observations on the difference of behaviour depending on the strains, while using DMSO, they obtained the same ATP concentration whatever the strain of bacteria they used [23]. This confirms the difference behaviour towards DMSO between fungi and bacteria.

The ATP level of sterilized strains was relatively low (0.7 to 10.02 pg). Conventional methods showed inhibition of these steamed strains. However small amounts of ATP were found on some of the strains treated with ethylene oxide, sometimes similar to the values measured on the same living strains. The average of ATP content of these strains ranges from 2.5 to 98.2 pg, 3.2 to 104.33 pg and 3.15 to 554.48 pg for EO1, EO2 and EO3 disinfection center respectively. Culturing methods showed a growth only with the strain of *N. fischeri* and only in one location (EO3). This high quantity of ATP extracted from disinfected strains suggests that possible residual ethylene oxide might have influenced the reaction. In order to justify such a hypothesis we replaced the extract with pure liquid ethylene oxide. The result was identical to those of blank. This means that there was no interference of ethylene oxide in the reaction. So, to explain the presence of ATP in certain disinfected spores, we assume that EO prevents the degradation of residual ATP in treated strains.

Table 1. Average of ATP amount (pg) in different treated and untreated strains

Strain	Spore size (µm)	Living strains	Killed strains*	Strains treated with EO		
				EO1	EO2	EO3
Aspergillus flavus	3 – 6	190.47	7.21	12.6	8.42	112.09
Aspergillus niger	3.5 – 5	69.05	2.79	4.90	17.41	-
Aspergillus fumigatus	2.5 – 3	83.45	1.02	3.70	3.20	-
Aspergillus versicolor	2 – 3.5	78.90	0.70	2.56	5.12	3.15
Eurotium chevalieri	4.5 – 6	1226.40	7.80	98.02	95.08	94.20
Neosartorya fischeri	2 – 2.5	72.12	2.76	4.27	45.56	68.78
Penicillium chrysogenum	3 - 4 x 2.8 – 3.8	793.39	10.02	5.47	-	197.06
Fusarium solani	8 – 16 x 2 - 4	[16935]	[4.75]	2.50	104.33	554.48
Chaetomium globosum	9 – 13 x 8 – 9	321.58	9.39	30.61	38.92	209.38
Ulocladium spp.	12 – 25 x 9 - 10	466.92	3.24	-	-	333.0

* Strain sterilized with steam

3.2.Comparison of Energy Charges of living and dead strains

Energy charge of living strains was compared to sterile samples (i.e. killed by steam). Table 2 summarizes the results. They agree with the general observation that cells are dead at EC values below 0.50 – 0.60. For living spores, mean EC values are within the range of 0.70 – 0.94. The lowest values (near 0.70) are certainly due to a bad extraction or a loss of ATP during processing. However, these results confirm that the technique is appropriate for given purposes as it can clearly distinguish between living and potentially dead moulds.

As we have mentioned before, the value of *F. solani* was not considered. Indeed, the calculation of its EC gave very surprising values ranging from 0.23 to 1.5. The high standard deviation shows how spread the results are for this strain. t will be interesting to identify what element of *F. solani* is responsible for such astonishing results.

Table 2. Mean values of energy charge (EC) of living and dead strains

	Living strains	Dead strains*
A. flavus	0.9370 ± 0.1610	0.3692 ± 0.1212
A. niger	0.7176 ± 0.0995	0.3837 ± 0.1072
A. fumigatus	0.8330 ± 0.1310	0.2092 ± 0.0240
A. versicolor	0.8535 ± 0.0822	0.1970 ± 0.0296
E.chevalieri	0.7978 ± 0.0716	0.5056 ± 0.1193
N. fischeri	0.7298 ± 0.0748	0.4736 ± 0.1466
P. chrysogenum	0.8700 ± 0.0907	0.3544 ± 0.0930
F. solani	[0.7343 ± 0.5641]	0.0379 ± 0.0078
C. globosum	0.8816 ± 0.0879	0.3166 ± 0.1046
Ulocladium spp	0.9039 ± 0.0683	0.4282 ± 0.0624

* Strain sterilized with steam

3.3. Energy charge of strains treated with ethylene oxide in different locations

The growth control of the strains disinfected by ethylene oxide in different companies is shown in table 3, along with the calculated EC values. The growth of the all treated strains was negative except for *N. fischeri* treated at EO3 location where a mycelia development appeared rapidly in a malt-agar medium. The EC average values ranges from 0.39 to 0.59 and 0.31 to 0.55 for EO1 and EO2 respectively – thus indicating dead fungi, confirmed also by the conventional method. For OE3, the EC average ranges from 0.27 to 0.82. Two strains have high EC, *A. flavus* (0.79) and *N. fischeri* (0.82) – thus indicating living cells. Only *N. fischeri* grew when put in a nutritive medium. Therefore, the only problematic species seems to be *A. flavus* treated at EO3. Since it is identified as dead by cultural method and as living by EC determinations.

Table 3. EC values of strains disinfected at different locations

	Disinfection center n°1		Disinfection center n°2		Disinfection center n°3	
	EO1	Growth	EO2	Growth	EO3	Growth
A. flavus	0.4739 ± 0.0382	N	0.4077 ± 0.0626	N	0.7980 ± 0.1569	N
A. niger	0.4258 ± 0.0927	N	0.4716 ± 0.0506	N	-	N
A. fumigatus	0.3937 ± 0.0404	N	0.3533 ± 0.0233	N	-	N

A. versicolor	0.3833 ± 0.0327	N	0.5132 ± 0.0743	N	0.4321 ± 0.1000	N
E.chevalieri	0.4828 ± 0.0168	N	0.4293 ± 0.0782	N	-	N
N. fischeri	0.5915 ± 0.0667	N	0.5442 ± 0.0808	N	0.8208 ± 0.0688	P
P. chrysogenum	0.4690 ± 0.0919	N	-	N	0.5839 ± 0.0892	N
C. globosum	0.5069 ± 0.1030	N	0.5535 ± 0.0522	N	0.5503 ± 0.0523	N
Ulocladium spp	-	N	-	N	0.6446 ± 0.0558	N

N: negative growth; P: positive growth

3.4. Application of the method to mixtures of species

The method is also applicable to mixture of various species (Table 4). The EC values for living or dead strains (tests 1, 2 and 3) are similar to that of individual strain. But if only one living strain is present in the mixture the EC value is above 0.80 (test 4). Strains treated with ethylene oxide had the same behaviour (test 6). Thus we can conclude that the strain of *A. flavus* treated at EO3 is viable as the EC value of the mixture n°7 reaches 0.79 while when absent of the mixture the EC value is 0.60 (test 5). The viability of *A. flavus* and *N. fischeri* treated at EO3 is confirmed by the test 9. When the apparently viable strain treated with ethylene oxide, is re-treated with steam, the EC value is 0.51, indicating dead cells. These results show clearly that EO3 treatment is not satisfactory. Then the calculation of the energy charge can also be a good way to control the effectiveness of a disinfection process.

Table 4. Energy charge of mixtures of spores

Test on mixture of strains	Mean EC values
1. *A. flavus* [L] + *E. chevalieri* [L] + *N. fischeri* [L] + *P. chrysogenum* [L]	0.82
2. *A. flavus* [L] + E. chevalieri [L] + *N. fischeri* [L] + *P. chrysogenum* [L] + *C. globosum* [L]	0.90
3. *A. flavus* [S] + *E. chevalieri* [S] + *N. fischeri* [S] + *P. chrysogenum* [S]	0.47
4. *A. flavus* [S] + *E. chevalieri* [S] + *N. fischeri* [S] + *P. chrysogenum* [S] + *A. flavus* [L]	0.80
5. *A. flavus* [EO1] + *E. chevalieri* [EO1] + *N. fischeri* [EO1] + *P. chrysogenum* [EO1]	0.60
6. *A. flavus* [EO1] + *E. chevalieri* [EO1] + *N. fischeri* [EO1] + *P. chrysogenum* [EO1] + *A. flavus* [L]	0.82 0.79
7. *A. flavus* [EO1] + *E. chevalieri* [EO1] + *N. fischeri* [EO1] + *P. chrysogenum* [EO1] + *A. flavus* [EO3]	0.49 0.51
8. *A. flavus* [EO1, S] + *E. chevalieri* [EO1, S] + *N. fischeri* [EO1, S] + *P. chrysogenum* [EO1, S]	
9. *A. flavus* [EO3, S] + *E. chevalieri* [EO3, S] + *N. fischeri* [EO3, S] + *P. chrysogenum* [EO3, S]	

L: living strains; S: treated with steam; EO1: treated at the place 1; EO3: treated at the place 3; EO, S: treated with EO + steam

3.4. EC values of naturally contaminated documents or presenting suspect spots

As described above, samples were collected using swab techniques. The number of spores recovered from different specimens ranges from 1.4 10^4 to 1.1 10^6, that is above the limit of detection for ATP determination [21]. The average of ATP content ranges from 15.0 to 21.4 pg, that is relatively low while the cultural method shows a positive growth for four out of five species (Table 5). The calculation of EC gave

very strange values, most of them being above 1.3. It is likely that these unexpected values are due to the methodology we used. Namely bad extraction and interactions with non organic particles brought by swabs.

Table 5. ATP content and energy charge of naturally contaminated documents

Series of Sampling	Growth	Number of recovered spores (spores/ml)	ATP content* (pg)	EC value*
1	P	$3\ 10^5$	16.59 ± 1.96	2.59 ± 0.57
2	P	$1.4\ 10^4$	15.44 ± 1.13	2.50 ± 0.21
3	P	$5.7\ 10^5$	14.98 ± 1.38	2.12 ± 0.82
4	P	$5.2\ 10^5$	15.26 ± 1.34	3.71 ± 2.81
5	N	$1.1\ 10^6$	21.35 ± 0.07	1.36 ± 0.14

*Average of at least 5 measurements; P: positive growth; N: negative growth

4. Conclusion

In comparison with conventional cultural techniques, the use of the ATP assay proved to be a rapid alternative to estimate the viability of fungal strains on paper. Conventional techniques can take more than 2 weeks, whereas ATP assay gives results in approximately 1 hour or less. Although the ATP bioluminescence assay is more expensive than conventional cultural techniques, its promptness is not a negligible factor because in certain cases, it is important to proceed quickly against the extension of the contamination. The effectiveness of the method depends on the initial quantity of cells to be analysed. The initial number of spores must be greater than 1000 spores/ml otherwise the results would be false. Our tests demonstrated that it is necessary to do at least 5 measurements to calculate the average value. This method is not specific, it does not allowed the identification of strains and moreover, it does not differentiate bacteria from fungi. But, we never observed co-contamination by bacteria and fungi on documents.

The estimation of ATP concentration is a good indication for viability but accuracy can be improved by the use of the energy charge because as we saw, some species treated with ethylene oxide have a significant ATP content while the EC is < 0.5 – indicating killed cells. Given that it is not often possible to know in advance whether a document has been treated with ethylene oxide, it is wise to measure directly the energy charge.

Therefore, the calculation of EC can also be used for a rapid control of the effectiveness of the disinfection process with ethylene oxide. This control is valid only if a measurement has been performed before treatment with living strains. The estimation of the energy charge is more adequate that the conventional method to control the effectiveness of the disinfection. Indeed, we observed that the strains of *A. flavus* disinfected at EO3, despite being identified by cultural method as killed, in several tests it has been provided EC values near 0.8 – and thus indicating viable mould. We suppose that after the treatment by ethylene oxide, *A. flavus* remains viable but becomes non-cultivable.

Finally, we have got unrealistic results with *F. solani* and with strains recovered with a swab. It seems that some elements disturb the process. Although, the described method is a promising alternative to conventional technique, more experiences are required to validate and generalise our approach.

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Biological Agent in the Weathering of Sandstone Sanctuaries in Thailand

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ABSTRACT

The most obvious appearance of biodeterioration on sandstone sanctuaries in Thailand is the formation of green, yellow, brown, grey and black layers on the surface. Their presence not only darken and disfigure the exterior surfaces but also weaken the materials on which they grow. Algae, lichens and mosses are among the most important biological agent responsible for the damage.

The most common algae are cyanobacteria or blue-green algae, green algae, and diatoms. The observations of the algae-substratum interface revealed the alteration and fragmentation of micaceous and felspathic crystals. Studies of the thin section of the sandstone underweath showed higher proportion of clay minerals.

Heavy growth of lichens is very common on sandstone sanctuaries. It is obvious that the stone surfaces beneath their colonies are softer and more friable. The mojority of lichens on sandstone are classified as crustose lichens. They appear as green, grey, white, yellow velvety patches on the exposed surfaces. They are strongly attached to the surface so that they can not be readily removed. Some of them established within porous sandstone. Another group of lichens abundantly grow on sandstone are classified as foliose lichens. They look like leaves or scales attached to the surfaces .

The effects of some lichens on sandstone were studied by optical microscope, polarizing microscope, scanning electron microscope and x-ray diffractometer. The observation of the interface between the lichens and sandstone showed a deep physical disintegration and chemical alteration of the substrate.

Algae and lichens grow on sandstone and build up humus in which larger and more damaging plants can grow. It was observed that mosses, liverworts, selaginella, ferns, grasses and various dicotyledonous plants also contribted to deterioration of sandstone. Their roots and rootlets enhanced the mechanical action in the pores of the porous sandstone and between structural elements of the monuments. Optical microscopy revealed the profuse network of rhizoids in the substratum. Field experiments on the effects of various biocides and water-repellent are also discussed.

Introduction

Examination of decayed sandstone has revealed many cases where surface deterioration has been related to the presence of biological growth. Decay is most prevalent in materials in direct contact with moist soils or in locations where moisture collects and cannot readily evaporate.

A study of the sandstone samples from several stone sanctuaries in Thailand revealed that most of them are classified as arkoses, lithic arkoses, litharenites, siltstone and calcareous sandstone. The principle minerals are quartz, feldspar, and rock fragments. Most of them are typically pink and red, through the color of feldspar and haematite. Its texture is poorly-sorted to well–sorted, with very angular to sub-rounded grains. The cementing

materials are mainly calcite, silica, and haematite. Under optical microscope some accessory minerals e.g. tourmaline, mica, etc. can be observed in addition to the principal rockforming minerals. Rock fragments are mainly shale, chert, and schist.

Some sandstones are highly porous, mechanically weak, and in advanced state of deterioration. The principle causes for the deterioration of these sandstones are the inherent poor quality of the sandstones themselves and the cyclic changes in the humidity and temperature. Most of these sandstones contain large amounts of haematite and clay minerals. The exterior surfaces of the sandstone show a high degree of deterioration. Feldspars are broken and weathered. Biotite is partly chloritisated. Red siltstone on Pimai Sanctuary presents an alarming degree of deterioration especially in several zones of the exterior of the monuments.

Sandstone surfaces are commonly colonized by algae lichens, bryophytes and vascular plants. The development of algae on sandstone is often associated with places of high humidity and water retention. The most obvious appearance of biodeterioration on sandstone sanctuaries in Thailand is the formation of green, yellow, grey, and black layers on the surface. Their presence not only darken and disfigure the exterior surfaces but also weaken the materials on which they grow. Algae, lichens and mosses are among the most important biological agents responsible for the damage. The colonization is probably related to the capacity of these microorganisms for water retention during a prolonged period of time. They often grow in humid places, in holes, crevices, and depressions, where water is retained and evaporation is slow due to protection against winds or direct sunshine.
The most common algae are cyanobacteria or blue-green algae, green algae, and diatoms. The heavy growth of lichens is also common on sandstone. It is obvious that the stone surface beneath their colonies are softer and more friable. They appear as green, grey, white, yellow velvety patches on the exposed surfaces. Crustose lichens are strongly attached to the surface so that they cannot be readily removed. Some of them establish within porous sandstone and laterite. Another group of lichens abundantly grow on sandstone are foliose lichens. They look like leaves or scales attached to the surfaces Mosses, ferns and higher plants are frequently present on damp surfaces.

Materials and methods

Methods used for the study include petrographic investigation, scanning electron microscopy, x-ray diffractometry, and optical microscopy. The textural features and the mineral content of the samples of sandstone were determined by polarizing microscope. Sandstone surface samples were cut perpendicular to the surface and examined by light microscopy. Stratigraphical microscopic study of thin and opaque sections and mineralogical study by x-ray diffractometer have been carried out. The nature and composition of the mineral content of the substrate at the interface between the stone and the microorganisms have been studied.

Results

Examination of sandstone surfaces has revealed many cases where stone disintegration has been related to the presence of biological growth, particularly, algae lichens, mosses, ferns, and higher plants. The colonization is probably related to the capacity of sandstones for water retention during a prolonged period of time and the presence of clay minerals on weathered sandstone. Biological growths are common in humid places, where

water is retained and evaporation is slow. Certain growths may be due to inadequate drainage of flat areas or frequent wetting of wind-blown rain.

Algae

The most obvious appearance of microbiodeterioration on monuments is the formation of green-black layers on the surface. Their presence is more apparent on the horizontal surface of the monuments which have been damp for most of the time. Their presence not only darken and disfigure the exterior surfaces but also weaken the materials on which they grow. They activity of algae on sandstone is vigorous and obvious. They form stains which vary in color from light green to black. Sometimes they entirely mask their surface. Algae have been found to produce and secrete a variety of metabolic products, among which predominate organic acids i.e. lactic acid, oxalic acid, succinic acid, glycolic acid, pyruvic acid. etc. These organic acids can either directly dissolve sandstone or increase their solubility. The black-green crusts were investigated by direct observation under optical microscope. The most common algae are cyanobacteria or blue-green algae. Common unicellular forms are Chroococcus, Gloeocapsa, Gloeothece, Microcystis, Aphanocapsa, Synechocystis, Myxosarcina, etc. Common filamentous forms are Lyngbya, Phormidium, Anabaena, Oscillatoria, Scytonema, Hapalosiphon, Nostoc, Tolypothrix, Calothrix, etc.
Green algae were occasionally found on sandstone surface. They were identified as Chlorococcum, Trebauxia, Protococcus, Closterium.
Several species of diatoms e.g. Pinnularia spp., Nitzschia sp., Navicual sp., Cocconeis sp., Cymbella sp.,. are also common on moist area. They have an absolute requirement for silicon for cell division and frustule formation.
The surface of the algal colonized areas showed small-large cavities. This is attributable to the mucilage formed by the sheaths of algae which adhere them to the substratum and suffers deep changes in volume due to its water retention property. Sheath contraction and expansion contribute to the gradual destruction of stone surface. The shrinkage of algae in summer months has pulled away fragments of the substratum. The observation in thin sections revealed the physical and chemical alteration of stone surface underneath the algal colonies. This study also revealed that algal community can develop in a relatively short period of time compared to other organisms.

Lichens

Lichens are commonly present on sandstone sanctuaries in Thailand. Some lichens prefer surfaces with low humidity, moderate light and ventilation while others are found only on external surfaces in shady location. Some lichens prefers external surfaces which are protected from rain and drainage while others prefer horizontal surfaces and direct exposure to rain. Some lichens prefer surfaces close to soil level and in contact with vegetation, for example, Pertusaria sp. prefers damp surfaces.
Certain lichens commonly occur in fissures and on edge areas of the sandstone blocks. Foliose lichens, particularly Parmelia sp. prefers a damp exterior surfaces in the North and South. The central blister of some lichens has pulled away fragments of the substratum.

The effects of some crustose and foliose lichens on sandstone were studied by optical microscopy and scanning electron microscopy. The damaged sandstone samples were investigated in depth profiles. The samples showed considerable surface alteration with colonization of lichens. A study of the stratigraphy of the area under

investigation revealed the deep physical disintegration of the substrate. Mineral fragments are detached and incorporated in the lichen thalli. Hyphal penetration occurs chiefly through intergranular voids. SEM examination revealed that hyphae bundles occupy the voids at the lichen-stone interface, and penetrate. Some surface dissolution of feldspar grains is also apparent in areas in direct contact with lichens. The mineral existing in the interface between lichen and stone have been identified by x-ray diffractometer. It was found that the area underneath the lichen showed the presence of quartz and clay minerals with a small amount of feldspar. The study of these samples in thin section revealed an alteration of feldspar to clay minerals. The analysis revealed a decreasing content of fresh feldspars in the outer zones of the samples.

SEM and polarizing microscpy studies of sandstone surfaces immediately below crustose and foliose lichens show quite clearly corrosion, in the form of etching as well as other features of chemical alteration. It was clearly seen that feldspar grains are corroded and transformed to clay minerals. Lichen hyphae penetrate in between crystals of the sandstone and loosen them.

It was also found that some crustose lichens are involved in crust formation which hardens the surface layer and act as a protective coating from the action of wind and rain. However, lichen thalli under the crust are still active and gradually damage the stone underneath.

Higher plants

Algae and lichens grow on sandstone and build up humus in which larger and more damaging plants can grow. It was observed that mosses, selaginella, liverworts, ferns, grasses and various dicotyledonous plants also contributed to deterioration of sandstone. Their roots and rootlets enhanced the mechanical action in the pores of the porous sandstone and between structural elements of the monuments. Optical microscopy revealed the profuse network of rhizoids in the substratum which penetrate through intermineral voids. Surface area underneath moss growth had totally been altered to mixture of sand and clay. They also trapped water and retarded drying process or evaporation. The presence of mosses is therefore, always an indicator of very high levels of water.

Higher plants often grow inside fissures or cracks on the surface of the stone and widen them due to increase in the volume of the roots and root tip pressure. In some cases, the foundation and structures were affected.

Control measures

Since the monuments are outdoors, it is not possible to modify the environmental conditions. The best way is to reduce moisture absorption by both chemical and non-chemical methods. This problem can be solved by application of biocides and water repellent. Regular maintenance and the proper choice of chemicals are helpful.

Field experiment on the effects of several biocides e.g. benzalkonium chloride (quaternary ammonium compound), sodium pentachlorophenate, sodium hypochlorite, Hyvar X, Velpar K 3, etc. on sandstone was studied. Removal of biological growth was undertaken with great care by using mechanical method. Soft brushes, scalpels, and water mist were used to clean the surfaces. After drying, methyl silane was carefully applied to reduce water absorption of the stone surfaces.

The result of field experiment undertaken at Main Prang of Phanom Rung Sanctuary in 1992 showed that biological growth have been totally inhibited for more than 8 years after the application of biocides and methyl silane. The cleaned, but untreated, areas in direct contact with damp soil, were colonised by algae and certain

lichen after 5 years. The upper part of the wall showed no sign of biological growth at least 8 years after cleanning. It was also observed that the treated surfaces have not been effected by the selected biocides and water repellent.

Conclusion

Biological growth is among the many factors contributing to the deterioration of sandstone sanctuaries in Thailand. This study focuses on the roles played by algae, lichens, mosses, and higher plants. It has been found that sandstone surface layers under the biological growth have been gradually altered. These surfaces have been deteriorated by physical and chemical activities of the organisms. The mineralogical and chemical compositions of the original stone surfaces have been changed. The best way to solve this problem is to reduce moisture absorption of the stone Further study is necessary to evaluate the most appropriate method.

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The Solar Tent –
Cheap and Effective Pest Control in Museums

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ABSTRACT

Disinfestation of museum objects with heat, generated by solar radiation (solarisation), is a cheap and effective pest control alternative in regions with much sunshine. A solar tent has been developed in which objects can be heated to 55°C, at which temperature an exposure time of 1 hour is sufficient to kill all developmental stages of museum pests. The solar tent is constructed with materials that are available almost anywhere. A black inner tent of cardboard or plastic converts the solar radiation into heat. A double outer roof of clear plastic contains the heat. White stripes on the black surface improve air circulation. With this design it is possible to create temperatures inside the solar tent ranging from 75°C in the top, 66°C in the middle, to 55°C at the floor.

Tests show that small to medium size objects can be heated to 55°C within one day. Mixed cultures of carpet beetle (*Anthrenus verbasci*), cigarette beetle (*Lasioderma serricorne*), sawtoothed grain beetle (*Oryzaephilus surinamensis*) and grain weevil (*Sitophilus granarius*) showed no survival after treatment. The moisture content of objects can be kept almost constant by wrapping them in a plastic bag. The uniform temperature prevents the formation of condensation on the surface of the objects. Heat can soften materials such as waxes and resins. Nevertheless, when there are no alternatives, solarisation provides an effective and non-toxic means to save collections that would otherwise be lost to insect damage.

KEYWORDS

Solarisation, pest control, heat, solar tent, disinfestation, museum objects

Introduction

Disinfestation of museum objects has undergone a significant change in the past decade. Due to health and environmental concerns many museums have switched from chemical treatments to non-toxic pest control methods, one of which is heat disinfestation. Exposure to temperatures above 45°C effectively kills insects in all developmental stages within several hours. The higher the temperature, the faster total mortality occurs. Yet, for the sake of the objects, the temperature should not be too high. An acceptable compromise is 55°C, at which temperature an exposure time of 1 hour is required to reach total mortality in museum pests [1,2].

Conservators have always been reluctant to conduct heat treatments, but high temperatures have been used to disinfest objects and historic buildings [3]. Recently, Xavier-Rowe et al. [4] have developed a

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• *This research was carried out as a visiting scientist at and in co-operation with CSIRO Entomology, Stored Grain Research Laboratory, Canberra, Australia.*

relatively cheap heating chamber using fan heaters and the wood of the walls and the objects as moisture buffer. Strang [5] has commented that the increase of degradation of materials as a result of short exposures to raised temperatures is negligible. Also, when an object is heated in a small volume of air, no drying occurs. Physical changes to materials are also negligible. This was confirmed by experiments carried out by Xavier-Rowe et al. [4] and Mitchell [6].

For museums in areas with much sunshine, a heat treatment using the sun as an energy source, solarisation, provides a cheap but effective method to disinfest museum objects. Solarisation is the use of solar radiation to increase the temperature of (dark) materials covered by a transparent plastic film or glass. The method is based on the fact that solar radiation (280 to 2500 nm) passes through the transparent layer, reaching the dark surface where it converts into convective heat and radiant heat of lower wavelength. Because the transparent film is much less permeable to long wavelength thermal radiation, the heat is contained within the system, increasing the temperature of the enclosed air volume.

The best known application of solarisation is in disinfesting soil [7]. Solarisation for disinfesting crops and stored products has been used occasionally in tropical countries [8,9]. Other applications of solarisation are drying of stored products [10,11,12] and timber [13]. Solarisation as a means to disinfest museum objects, was introduced in the conservation field by Strang [5]. Brokerhof [14] has described a design for a solarisation box and its application for the disinfestation of wood from the support structure of an iconostasis. Meanwhile, Strang et al. [15] have presented work on the use of plastic bags, a solar envelope and a solar oven. This paper describes the development and the application of a solar tent.

The Solar Tent

The design of the solar tent is based on the 'single front pass' principle (Fig. 1). It is constructed of materials that are available almost anywhere: timber, plastic, cardboard and black paint. A black inner tent of cardboard or plastic converts the radiation into heat. Openings are left at the top and at both bottom sides. An outer roof of two layers of clear polyethylene sheet, large enough to enclose the entire tent, contains the heat. The air between inner and outer tent heats up and rises to the top, enters the tent and mixes with the cooler air. At the bottom cool air is drawn into the air layer, where it warms up and rise. A pattern of white vertical stripes on the black surface improves the circulation by differential absorption. The tent is placed on an insulated black floorboard to avoid heat loss by conduction. The frame of the tent is placed on black bricks or stones. In this way the black floorboard converts additional incident solar radiation into heat, providing extra heat at the bottom of the tent. The blocks also serve as heat storage, slowing down cooling of the tent overnight. The objects are placed on a grill, raised above the floor of the tent to allow heating from all sides. To facilitate transportation of the tent, the whole structure can be placed on a pallet on wheels, which can be rolled into the sun in the morning and back inside at the end of the day (Fig. 2-3). Detailed instructions on how to construct and use a solar tent can be found in ICN-Information No. 7 [16].

Experiments with The Solar Tent

A series of 1-day exposures was carried out to study the performance of the tent. Temperature sensors were placed throughout the tent, in between inner and outer tent and between the two layers of the outer tent. Dataloggers (Axiom Smart Reader, Atal, Purmerend, NL) were used to record temperature and

relative humidity in various places inside the tent. Blocks of pine with temperature sensors inserted into the core were used to determine whether the requirements of 1 hour 55°C in the core were met. During each test the tent was loaded with several blocks of wood of which weight and moisture content had been determined. The tent was rolled outside in the morning, the 'objects' were then placed on the grill and the tent was closed, by clipping the clear plastic to the floorboard. The tent was exposed during the entire day, starting at 10 am. During the day the sun moved from the right side of the tent to the top, being almost directly above the tent at 1 pm, and moving to the left side to disappear behind adjacent buildings at 6 pm. At the end of the day the closed tent was rolled into the shed where it was allowed to cool down overnight. The next morning the objects were taken out, their weight and moisture contents were determined again and compared with the starting values.

Insect mortality was studied with mixed cultures of various species reared at the laboratory. Species and strains tested were *Anthrenus verbasci* (CN:27), *Lasioderma serricorne* (CN:33), *Sitophilus granarius* (CN:1001-4A, CN:1001-4B and CN:1001-418B) and *Oryzaephilus surinamensis*. Culture jars with feed and insects were exposed in the solar tent. The temperature in the core of the feed/insect mass was measured with a thermocouple. After solarisation the jars were incubated at 30°C and 70% RH for 4-6 weeks before mortality was determined.

Blocks of Oregon pine cylinder of 10 cm x 10 cm diameter, and *Pinus radiata* 7x7x10 cm, an Oregon pine board of 90x24x4,5 cm and the Canberra Yellow Pages 27,6x23,5x5 cm were exposed with NTC sensors in the core to study the temperature profiles. Dummy artefacts were exposed in the solar tent to study the effects of heat on their materials. Pieces of paper glued together with gum Arabic and Scotch washable glue stick, pieces of paper painted with water colours, pieces of an oil on canvas painting, a painted and varnished wooden tray, a painted plastic plate and a small aboriginal bark painting were cut in half. One half was treated, the other half was kept as control. All the test objects were weighed and wrapped in polyethylene plastic bags before treatment and unwrapped and weighed after cooling. After exposure they were examined microscopically at 40x magnification for visual changes.

Results

Temperatures

The experiments were conducted in early summer in Canberra, Australia, on days for which the weather forecast was sunny and warm. In practice this meant there could be up to 30% cloud cover with a temperature of around 30°C. The maximum temperatures that were reached inside the tent ranged from 75°C in the top, 66°C in the middle, to 55°C at the floor. The temperature in the core of an Oregon pine cylinder (10 cm long, 10 cm diameter) reached a top of 63°C, while it remained above 55°C for 200 min. Predictions based on the heat diffusion model of Carslaw and Jaeger [17] and Crank [18] indicate that at 66°C, wooden objects with a size similar to that of a cylinder with a 15 cm diameter can be heated to 55°C in the core during a 1-day exposure (Figure 4). Paper objects comparable in size to a phone book can be treated successfully as well. The objects warm up through heat diffusion. The rate of heating depends on the difference between the temperature inside and outside the object, the thermal diffusivity of the material, and the volume of the object. Large objects require longer exposure or higher temperatures. Temperatures depend on the power of the solar radiation, the outside air temperature and the insulation of the tent. They can be increased using a plastic inner roof in stead of cardboard or using a better insulating

outer roof such as Perspex or glass. A larger tent does not necessarily generate more heat, but the tent can be made longer to fit long objects with a maximum diameter of 15 cm or a larger number of small to medium sized objects.

Insect mortality

Culture jars with feed, several hundred adults and an unknown number of other developmental stages were placed inside the tent. All four species and all their developmental stages were killed by exposure in the solar tent. In one case the temperature had not come above 53°C, yet it had been over 48°C for 200 min, which proved to be effective in killing the insects.

Observed effects on objects

The relative weight changes and the observations after solarisation of the dummy artefacts are listed in Table 1. Wrapping the objects in plastic limits the change in moisture content of wooden objects to less than 0.5%, which is acceptable. Small objects, with little mass in the bag, show a larger relative weight change. The small absolute amount of water, which can be taken up by the air in the bag, is a relatively larger part of the object. Where the Yellow Pages have a very small weight change, a single sheet of paper shows a relatively large weight change.

The pine blocks became darker after heating. The resin from the wood had moved to the surface and had formed small spots. This only happened at the radial cuts of the blocks, where the vessels have their open ends. The change was not noticed at the sides of the blocks. Whether this causes problems in artefacts depends on the type of wood and the way the wood is cut and used. A wooden tray decorated with oil paint and varnish, showed a recovery of gloss and deepening of colour. The varnish was identified by GCMS analysis as an alkyd varnish with small oil content. As a result of the exposure to heat the varnish had been regenerated. The small abrasions and craquelures, which gave the untreated surface a matte appearance, had been closed, providing a more even and thus glossier surface. The big cracks in the wood and paint layer had not increased, nor had the larger cracks in the varnish layer, but their edges had been smoothened after solarisation. A painted plastic plate and a non-stretched, varnished oil painting on canvas did not show any changes after treatment. The paper from the Yellow Pages did not show any wrinkles or buckling after treatment. There were no discolorations and no water stains. Plain paper showed no differences after treatment, neither did paper painted with watercolours. As a precaution the painted side was covered with paper to avoid direct contact of the paint with the plastic wrapping. No paint had been transferred to the cover paper. No discolorations of the watercolours were observed. Adhesives, especially gums and starch type glues, are rather sensitive to moisture. Test sheets were prepared by gluing together two sheets of paper with gum Arabic and with Scotch washable glue stick. None of the test sheets showed any loss of adhesion, or any extra buckling. Upon treating a piece of Aboriginal bark painting there were no visible changes in the colour or texture of paint or wood. However, the curvature of the bark had changed. After solarisation the bark had flattened, moving 3 mm at the far end. After one week, the bark had reassumed its original shape. It must be noticed that only visual changes were studied, no tests were performed to determine changes in mechanical strength.

Conclusions

The solar tent is the perfect design to generate enough heat for a successful treatment without having problems with condensation. With white vertical stripes on the black absorption surface, the air circulation is good enough to ensure an even distribution of heat around the objects. In the solar tent objects the size of a cylinder with 15 cm diameter can be disinfested in a 1-day exposure. The solar tent has proved to be effective in conditions with an outside temperature of 28-30°C and an instantaneous solar irradiance (ISI) of 950-1100 Wm^{-2}. The air temperature inside the tent reaches a maximum of 75°C in the top and 55°C at the bottom, which is enough to heat up the core of medium sized objects to 55°C for at least 1 hour. At these conditions mixed cultures of *Anthrenus verbasci*, *Lasioderma serricorne, Sitophilus granarius* and *Oryzaephilus surinamensis* showed complete mortality after 1-day exposure.

In colder regions replacing the cardboard inner roof by black polyethylene with white paper stripes attached to it can generate more heat. Heat is lost from the system mainly through wind chill and conduction. A thick clear outer roof, for instance glass, will provide higher temperatures.

To keep the moisture content of the materials stable during heating and cooling, the objects can be wrapped in a common household polyethylene bag. In that way the loss of moisture from wood can be reduced to less than 0.5%. When there is concern for decorative surface layers, the objects may be wrapped in tissue before wrapping them in plastic.

Any heat treatment can have side effects on the materials of museum objects. These may not be adverse per se, but changes have been observed, such as the movement of resins in pine and softening of waxes and varnishes. One should always take care with materials that have a melting point below 75°C. Yet, in those situations where there are hardly alternatives, solarisation provides the means to save collections that would otherwise be lost to insect damage.

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Table 1. Original weight (W_0), absolute weight change ($W-W_0$), relative weight change (dW) and observed changes after exposure of dummy objects in the solar tent.

Object	**W_0 (g)**	**$W-W_0$ (g)**	**dW (%)**	**Observed changes**
Oregon cylinder	365.94	-0.29	-0.08	resin moved to radial plane surface
P. radiata block	267.39	-0.45	-0.17	resin moved to radial plane surface
P. radiata block wet	244.97	-0.67	-0.27	resin moved to radial plane surface
Varnished wooden tray	214.77	-1.18	-0.55	gloss, deepening of colour, smooth edges
Plastic plate	47.46	-0.04	-0.08	none

Canvas painting	17.78	-0.27	-1.52	none
Yellow Pages	2094.6	-0.9	-0.04	none
Water colour on paper	27.89	-0.43	-1.54	none
Paper with gum Arabic	6.99	-0.12	-1.72	none
Paper with Scotch adhesive	6.78	-0.09	-1.33	none
Bark painting	56.59	-0.12	-0.21	reversible flattening

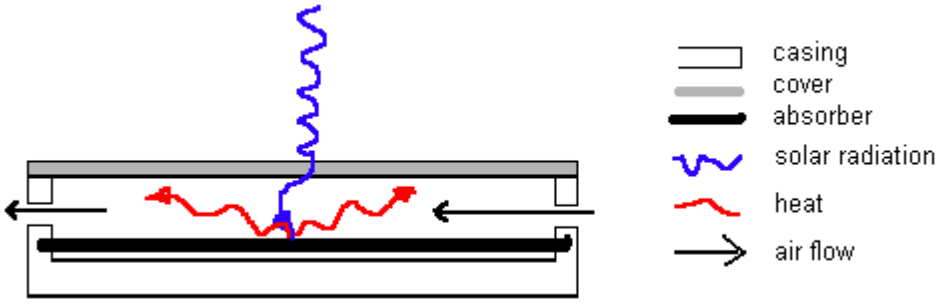

Figure 1. Principle of heating an air stream with a 'single front pass'.

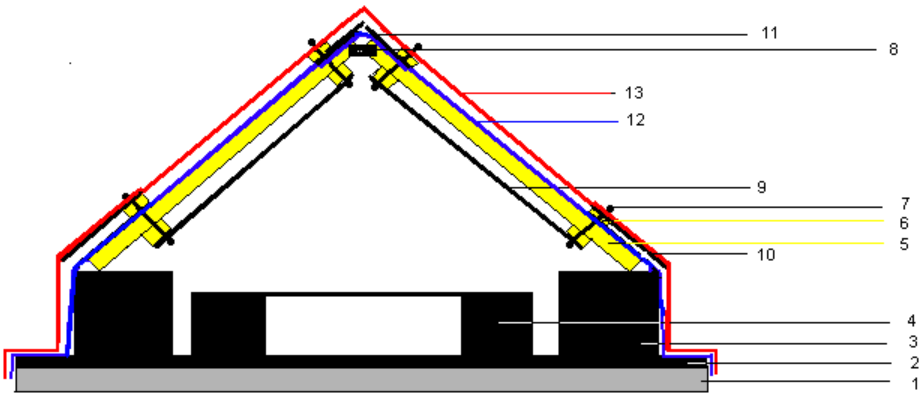

Figure 2. Solar Tent design, front view.

1: Ground insulation (120 x 120 cm): polystyrene, old newspaper, fibre, rock wool

2: Floorboard: matt black (125 x 125 cm) plywood, boards

3: Corner block (brick, stone) wrapped in black plastic, raising the frame, circa 16 cm high (4x)

4: Support block (brick, stone) wrapped in black plastic, circa 12 cm high (4x), with a metal grill, for example refrigerator shelves

5: Timber post: matt black (70 x 7 x 3.5 cm)(4x)

6: Timber cross support: matt black (100 x 4 x 2 cm)(8x)

7: Bolt and nut (8 x 100 mm) with washers, to attach cross supports to posts (8x)

8: Plate mending or hinges, to connect posts (2x)

9: Cardboard inner roof (54 x 100 cm) painted matt black, with 3 vertical white stripes of 10 cm width (2x)

10: Cardboard bottom flap (17 x 100 cm), painted matt black with 3 vertical white stripes of 10 cm width (2x)

11: Cardboard top flap (12 x 100 cm), painted matt black with 3 vertical white stripes of 10 cm width (2x)

12: Clear outer roof: first layer polyethylene sheet, 0.10 mm thick (3 x 3 m)

13: Clear outer roof: second layer polyethylene sheet, 0.10 mm thick (3 x 3 m)

Figure 3. The Solar Tent. Left: frame with black cardboard inner roof; right: with double layer clear plastic outer roof in action.

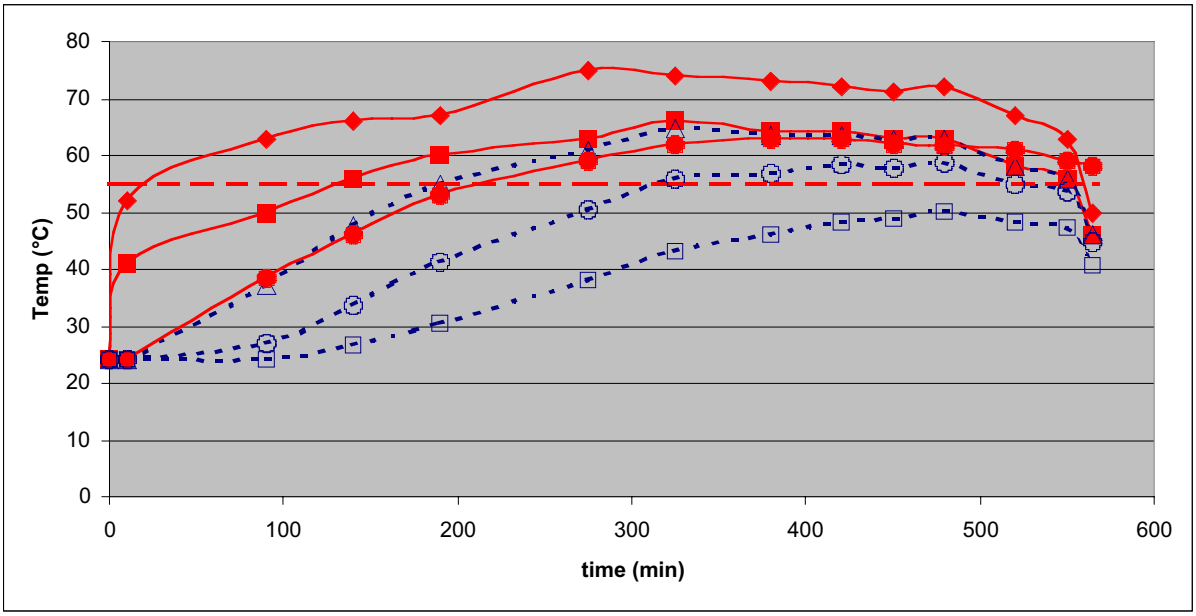

Figure 4. Temperatures during solarisation in the solar tent. Solid lines represent the measured values in the top of the tent (◆), in the middle (■) and in the core of a 10 cm diameter Oregon cylinder (●). Dotted lines represent values calculated with the model for heat diffusion postulated by Carslaw and Jaeger [17] and Crank [18] in the core of wood cylinders with diameter 10 cm (Δ), 15 cm (❍) and 20 cm (❑). The required core temperature of 55°C is marked by the horizontal dotted line.

Monitoring Insect Pests Within Buildings Using Traps – Case Studies of The Use of Traps to Monitor Activity, Spatial Distribution and Efficacy of Pest Control.

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Abstract

Traps, sometimes baited with pheromones or other attractants, are widely used to detect the presence of a range of insect pests that infest stored dried organic materials, be it cultural artefacts or food. While such traps can be effective tools to detect pests, they are often not used in a systematic way for best effect.

Data from two case studies are presented to show how data from traps have been used to monitor insect activity, spatial distribution and efficacy of pest control operations within commercial grain storage and food processing environments in Australia. Such studies are relevant to the museum environment as often the same or very similar pests are involved. Optimal pest control in any structure is achieved with a good knowledge of where the pests are and how well pest control measures are working. There is a general desire to move away from wide-scale use of pest control chemicals that make a more targeted approach more attractive, based on a rational assessment of need and pest status. Traps are a useful tool to make this a reality.

Introduction

The effective management of insect infestations of stored material of organic origin is an on-going process, be it to protect irreplaceable cultural objects or basic foodstuffs. Many of the pests that can infest such material are ubiquitous in both urban and rural environments and are a constant threat, especially in warmer climates. Effective control of such pests is made easier if good information is available as to their presence and distribution. With this knowledge, pest control can be targeted to where it is most needed. Data on presence and distribution of pests can also be used to evaluate efficacy of pest control treatments by comparing pest incidence of before and after treatment.

Targeted pest control is likely to become more and more necessary. Customers of pest control activities increasingly and understandably want an effective outcome delivered with minimal use of chemicals perceived as potentially harmful. This results from a desire to minimise risk to workers and others but potentially also to objects or materials that pest control operations are designed to protect.

Another factor requiring more targeted pest control is the increased importance of 'duty of care' in determining who is liable if anything goes wrong. Good data collection, which allows sensible and rational decisions to be made, is the foundation of quality management systems such as the ISO 9000 and 14000 series and also of HACCP systems in widespread use in the food industry. Keeping thorough records of pest infestation and the effectiveness of pest control goes a long way in demonstrating 'duty of care' in a legal and moral sense.

For many years now traps have been available to catch many insect species that attack cultural artefacts of organic origin. These vary considerably in complexity and work in many different ways. Some rely simply

on the preference that many insects have to search out and remain in crevices. Others are more sophisticated and are baited with a range of pheromones and other baits to enhance their attractiveness to particular target insects.

Traps will often detect insects at levels well below those that can be detected by visual inspection. This is especially so when traps are baited with an attractant, such as a pheromone, specific to a particular pest. Traps, being in place for days or weeks accumulate catch over time and are in position at times of the day when insects are active but people are generally not, such as during the night.

All too often however, traps are used in a non-systematic way without a clear idea about how the data obtained will be used. Indeed there is little point in spending time and money on buying, installing and servicing traps if the results obtained are not used in a systematic way. It is not the intention of this paper to discuss or review the merits of one trap or bait type against another but rather to give examples of the ways in which traps can be used to aid the process of insect control. The examples described in this paper are based on work undertaken in recent years by the Stored Grain Research Laboratory in conjunction with Australian grain storage and food processing industries. While these environments are different in some respects to those found in museums etc. many of the pests are the same and the concepts demonstrated here are equally applicable.

Use of insect traps in insect control – some case studies

Insect traps can be used to monitor both presence and distribution of target pests. In this paper, work will be described which used traps to measure or determine:

- activity of pest populations over time;
- the source or foci of infestations within buildings;
- the spatial distribution of pests within a building;
- the efficacy of control measures undertaken against a pest population.

Data obtained were used to design, target and evaluate the success of pest control activities. Results from two research projects undertaken by SGRL in conjunction with commercial partners are included to illustrate how traps have been used as tools to develop pest control procedures in an industrial environment.

1. Use of pheromone baited traps to detect and determine spatial distribution of phycitine moths infesting food-processing facilities.

Moths, in particular *Ephestia* species and *Plodia interpunctella*, are major pests of facilities handling and processing commodities such as flour and breakfast cereals. Facilities are typically treated with a space spray such as Pestigas™ or InsectigasD ™ every week or fortnight to control these pests (Nickson *et al.*1988).

In the food industry there is a general desire to minimise reliance upon chemical pest control. This is being forced upon the industry for example as a consequence of the imminent withdrawal of methyl bromide in

Australia and other developed countries for all but quarantine related uses. In addition there is a desire to minimise loss of production resulting from the down time during application of space sprays and also to minimise risk of exposure of product and personnel to pest control chemicals.

In a collaborative project between SGRL and a major food processor which produces breakfast cereals and related products, a total of 91 and 130 traps were installed in two production facilities (Plants A and B) representing a density of one trap per 68 or 106 m^2 floor area respectively (Rees and Wright 1998). Traps used were Pherocon II® units baited with a lure containing synthetic $Z_9E_{12}14ac$ ('Indian Meal Moth' lure ex. Trécé, Salinas, California, USA). These were hung from pillars, pipes and roof members 2-6 m above the floor out of the way of normal plant operations. Traps were examined every 14 days and replaced every two months

Trapping data showed that moth numbers varied over the year, especially in plant A. In this facility that was relatively open to ambient environmental conditions, moth numbers clearly peaked in summer months. Pest control effort therefore needed to be increased at this time. In the other plant, which was air conditioned, moth numbers stayed much more constant over time. However in this facility the pest problem became worse in the summer of 1995/96 (Figure 1).

Moths caught in traps were identified to species and compared with those caught in traps placed outside production facilities. Before these investigations staff were of the opinion that moths flew in from outside and were not resident in the facility. Moths caught in traps inside the facilities were identified and almost all were found to be *E. cautella.* However, in traps placed outside the facility almost all moths caught were another species - *E. kuehniella*. It was clear from this observation that moths present in the production facilities were not flying in from elsewhere but were in effect an isolated population, living and breeding within the building (Rees and Wright 1996, 1998). This observation permitted, for the first time in Australia, a successful attempt to control months by mating disruption by release of synthetic pheromone (Nickson *et al.* 1998). For such a treatment to work the target population needs to be isolated from others, a fact confirmed by the trap data.

Table 1. Proportion (%) of species caught in and around production facilities and in surrounding township.

Location	*Plodia interpunctella*	*Ephestia kuehniella*	*Ephestia cautella*	*Ephestia figulella*	*Ephestia elutella*
Plant A	10.19	6.92	79.62	0	3.27
Plant B	4.65	1.44	93.59	0	0.32
Gardens	13.27	44.25	21.24	1.77	19.47
Surrounding township	49.44	19.10	2.25	28.09	1.12

Notes

Plants A B - Total of all moths identified from traps during survey period.

Gardens - From total of 6 traps placed in gardens and other outdoor factory sites

Surrounding township - Traps off factory site, but within 2km of factory site - part of community trapping grid

Detailed knowledge of the identity of insects infesting production facilities proved to be very useful in understanding the source of insects found in product returned by consumers. Almost all of these were *Plodia interpunctella*, a species found at low frequency during survey of production facilities. Infestations returned by consumers are likely to have happened post-production - in distribution, retail or domestic environments (Nickson *et al*. 1998).

Contour maps showing the spatial distribution of moths caught in both plants were produced (Figure 2). These were produced using Surfer^{TM} – a geographical mapping program (Golden Software Inc). These clearly indicate that distribution of moths in either plant is far from uniform. Highest catches were made in traps located close to certain machinery - notably packing machines and product storage bins. In large areas of both facilities few or no moths were caught.

Trap data backed up by visual searching indicated that most infestations were confined to a few specific locations – in particular residues associated with product weighing and packing equipment. Such infestations, restricted as they were to a few foci, were highly amenable to targeted measures such as spot application of insecticide following cleaning. This is likely to be more effective than a broad-scale application of a space treatment that will not to penetrate into locations where the insects were shown to be breeding.

2. Efficacy of control measures against the psocid *Liposcelis decolor* (Psocoptera: Liposcelididae) infesting open-topped bins of cereal grain

In recent years psocids, especially *Liposcelis* species have become more important as pests in Australian grain storages. Heavy infestations of *Liposcelis* species (esp. *L. decolor* and *L. entomophila*) are detected in bulk-stored cereals with increasing frequency (Rees 1998). Control of these populations is especially problematic in grain stored in open–topped bins. Typically in Australia insect pests infesting such structures are controlled with the application of phosphine gas using the SIROFLO® process. This is a method of fumigant delivery developed specifically for such unsealed structures that nevertheless permits the establishment and maintenance of insecticidal concentrations of fumigant within the grain bulk (Winks and Ryan 1990, Schonstein *et al* 1994, Winks and Russell 1994). This treatment provides good control of most pests when applied properly. Rees (1998) however described how the behaviour of psocids allowed enough individuals to survive the fumigation to permit rapid population recovery. Large numbers of individuals were seen moving in and out of the grain mass in response to environmental conditions, which meant that some individuals remained outside the fumigated grain and hence survived. A combination treatment was proposed which involved treatment of the headspace with dichlorvos and the grain with phosphine to simultaneously control psocids both in and out of the grain (Rees *et al.* in press).

To test the efficacy of the two treatments in the field, some means to easily monitor the presence and survival of *Liposcelis* was needed, namely a trap. In this case a simple unbaited cardboard crevice trap was used, consisting of a rectangle of double-wall corrugated cardboard 150 x 100 mm with the corrugations open on the long side (Wright 1991). *Liposcelis* are highly thigmotactic and will accumulate over time in material such as corrugated cardboard.

In this trial, two cardboard crevice traps were placed on the concrete surrounds of the mouth of open topped bins, each containing about 500 t of wheat or barley (floor traps). Two traps were also lowered on string onto the grain surface of each bin. Blocks 1 and 2 contained four and six such bins respectively. Traps were replaced after about 12 hours. Trapping was commenced several days before treatment, to measure the initial population level. Trapping on the structure was continued during pest control for 16 days. Block 1 was treated with phosphine alone and block 2 was treated with phosphine and dichlorvos (Rees *et al.* in press). Immediately post treatment, trapping was resumed for 4 days to detect the presence and level of insects immediately after treatment (Figure 3). In addition, traps were also laid 2, 3 and 8 weeks after the end of treatment to monitor population recovery (Figure 4).

The trapping regime clearly detected the rapid drop in numbers of *Liposcelis* present when pest control regimes were applied. Trapping also confirmed that insects seen outside bins had come from the grain below. When phosphine alone was applied, catches dropped simultaneously in traps in and out of the grain, even though only insects in the grain bulk would have been in contact with phosphine (Figure 3). Most of the observed reduction in numbers appeared to be caused by the fumigant, however trapping demonstrated that application of the dichlorvos treatment more effectively controlled the small numbers of insects remaining at the end of the fumigation (Figure 3, Table 2).

Table 2 Assessment of *Liposcelis decolor* infesting grain held in open-topped bins immediately before and after SIROFLO® fumigation or SIROFLO® fumigation + dichlorvos treatments.

Assessment method	**Before/ after treatment**	**Mean count ±SD (n)**		**% reduction (before v after treatment) *(% survival)***	
		Phosphine alone Block 1	**Phosphine + dichlorvos Block 2**	**Phosphine alone Block 1**	**Phosphine + dichlorvos Block 2**
Floor traps	Before	244.47 ± 58.16 (n =4)	2454.31 ±213.05 (n=8)		
	After (initial 4 days)	2.98 ± 2.19 (n=8)	0.10 ± 0.07 (n=8)	98.77*(1.33)*	99.99 *(0.01)*
On-grain traps	Before	23.25 ± 12.58 (n=4)	339.96 ±135.87 (n=3)		
	After (initial 4 days)	0.11 ± 0.16 (n=8)	0.0 (n=8)	99.52 *0.48)*	100 *(0)*

n = number of 12 hour trapping periods used to calculate mean

This observation was borne out by follow-up trapping, which showed rapid population recovery in bins treated with phosphine alone (Figure 4). This grain was re-treated after 8 weeks, when visual inspection confirmed that the population had recovered. By contrast, traps showed that numbers of insects in the combined treatment remained low, leading to a longer effective treatment life.

Discussion

The examples described here show some of the ways in which traps can be used to direct and develop pest control procedures under industrial conditions. Prior to the investigations described, pest control had been applied in a fairly mechanical way with little relation to pest ecology. Understanding pest ecology is important especially when dealing with pest outbreaks infesting complex structures such as a factory or building. In addition comparative data brings with it the possibility to properly test novel methods of pest control, such as mating disruption.

It is also important that staff involved in undertaking such monitoring learn more about their pest control problems. It allowed them to get away from the culture of just blaming someone else or believing the insects were 'resistant' to the chemicals used. Having the skills to know when and why you do something and being able to determine whether measures taken have worked or not is a great deal more satisfying than just routinely applying chemicals. Use of traps can lead to a better-educated and motivated workforce, minimise use of pest control chemicals and provide data to demonstrate efficacy leading to better pest control outcomes.

Disclaimer

Mention of any preparatory product or equipment is for illustrative use only and does not imply endorsement or otherwise by CSIRO.

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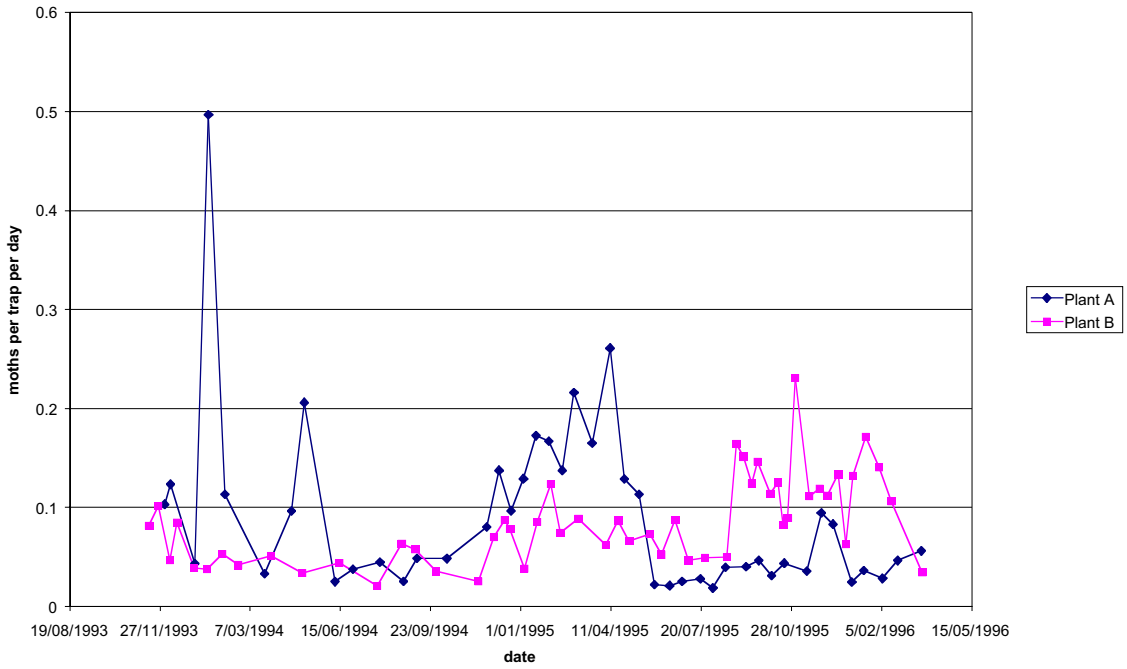


Figure 1 Incidence of *Ephestia cautella* in two facilities manufacturing breakfast cereals as detected by pheromone baited flight traps

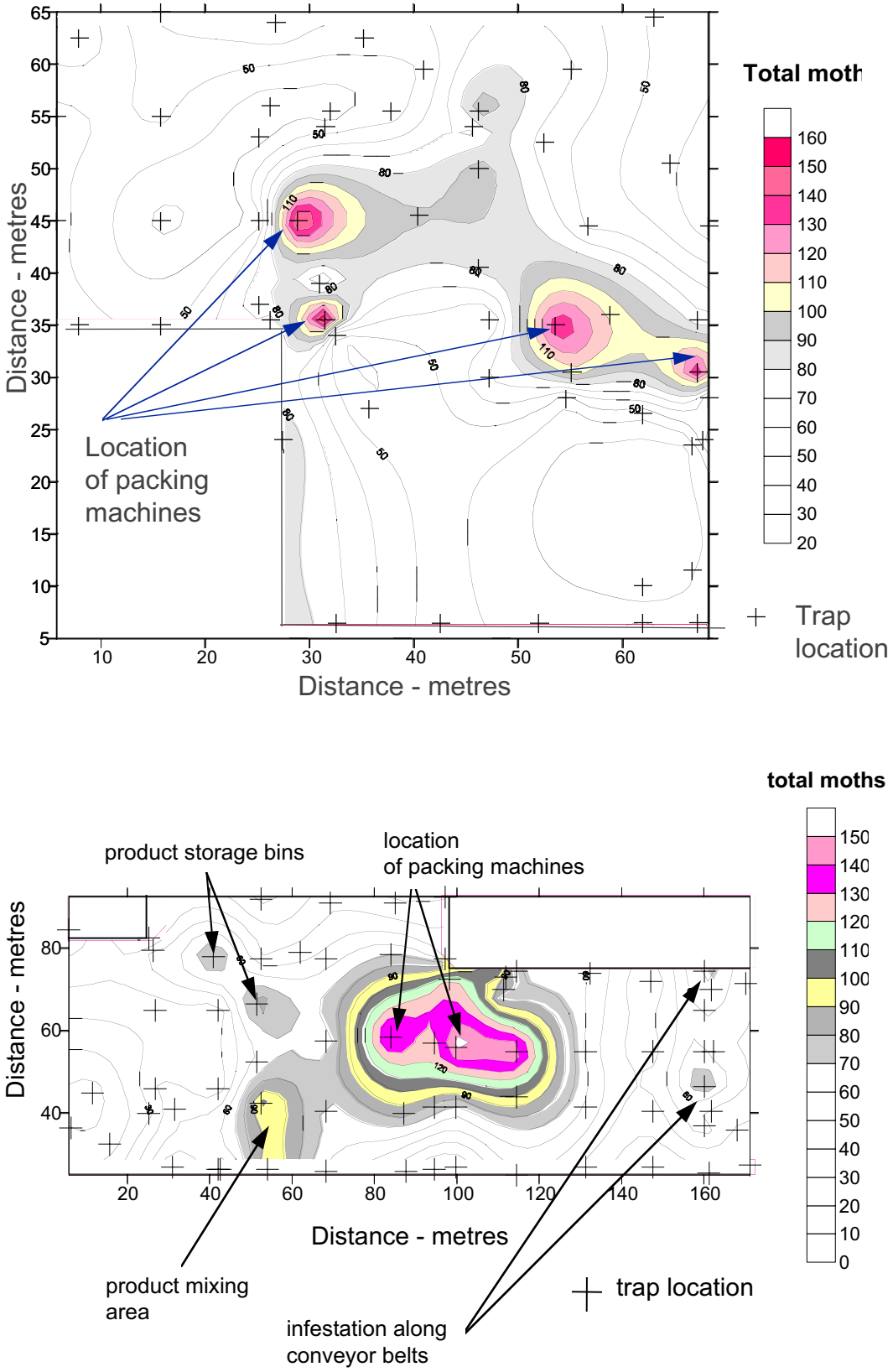
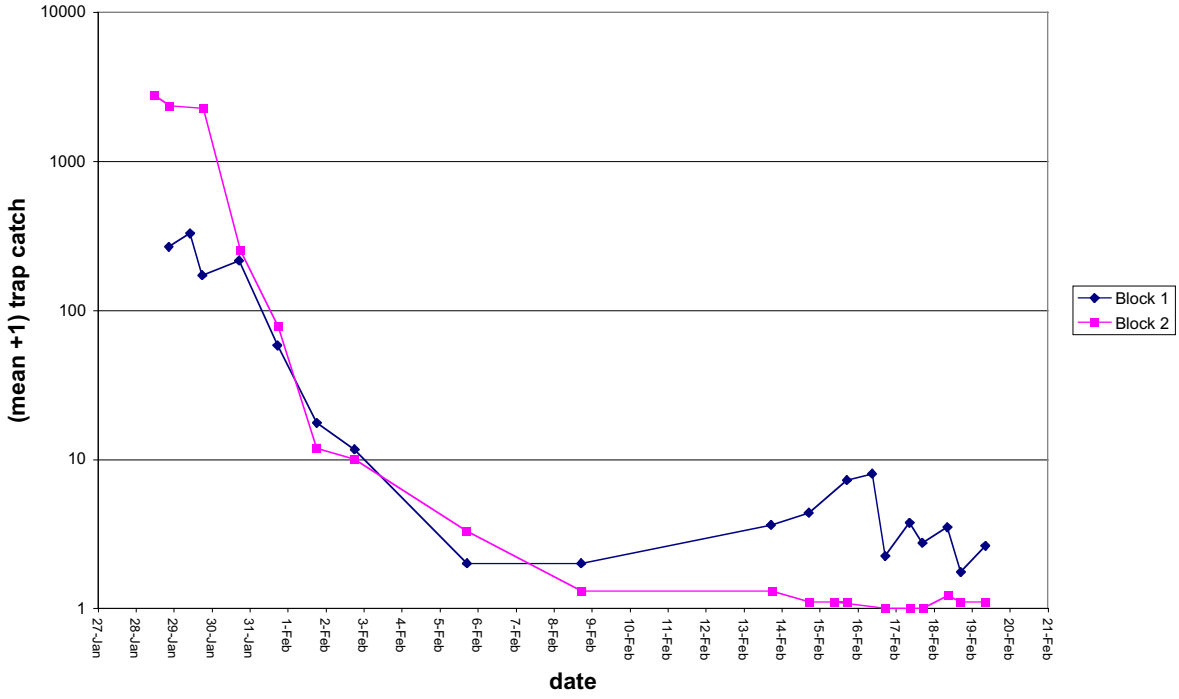
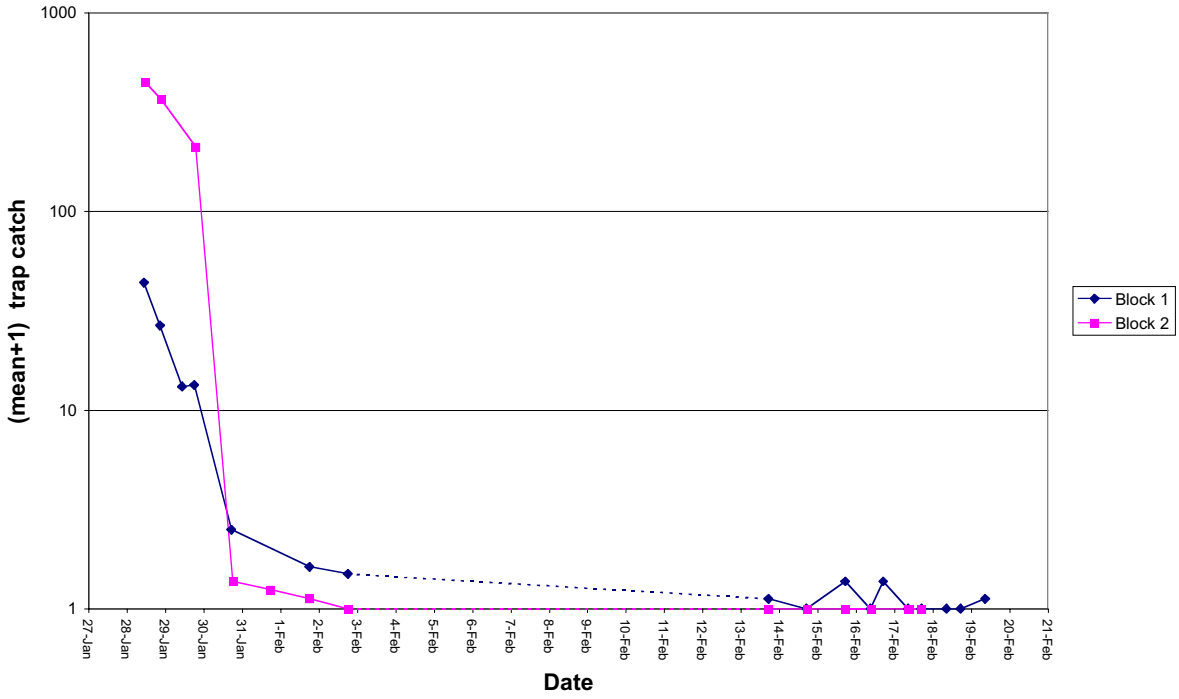


Figure 2, Distribution of *Ephestia cautella* in two food production facilities, as determined by catches from pheromone- baited flight traps.

Upper figure Plant A, Lower figure, Plant B



Traps placed on floor above grain



Traps placed on top of grain in open topped bins

Figure 3 Number of adults and nymphs of *Liposcelis decolor* extracted from traps (mean for all cells in each block +1) treated with SIROFLO® fumigation alone (Block 1) or SIROFLO® fumigation/space treatment with dichlorvos (Block 2). Treatment was commenced on 29 Jan and completed on 15 Feb 1999.

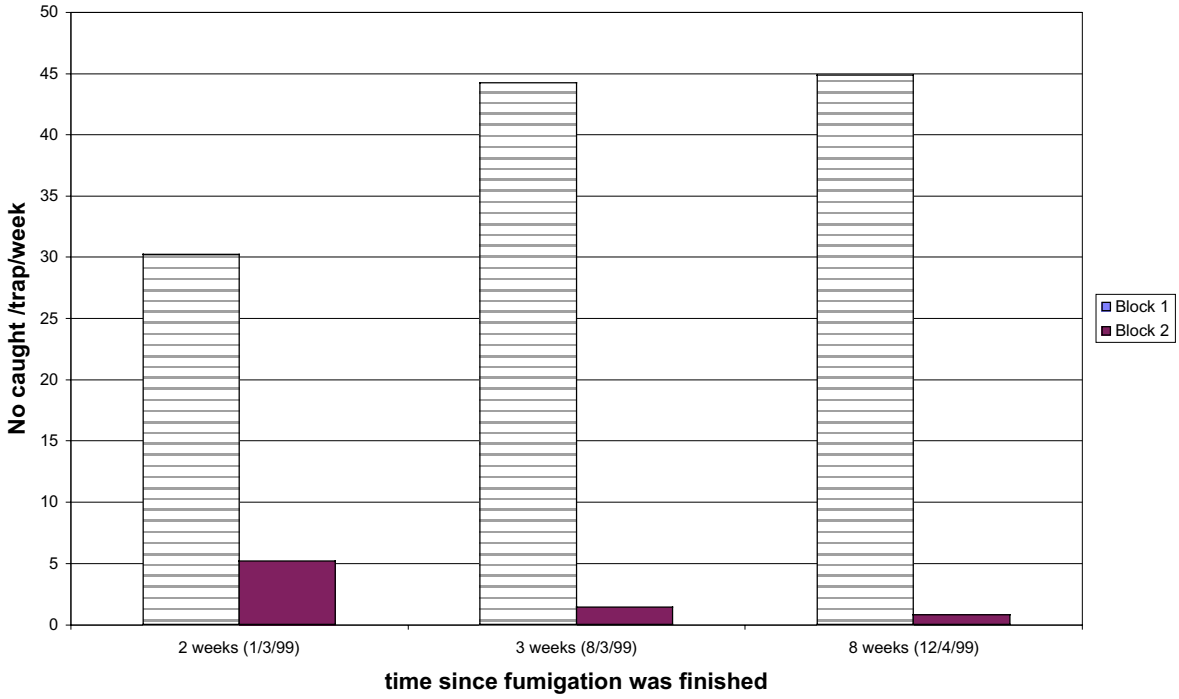


Figure 4 Recovery of populations of *Liposcelis* in the weeks after treatment

IPM x 10: Pest management on a regional level

Alice Cannon

Most conservators are now familiar with integrated pest management (IPM) strategies, thanks to previous conferences, publications and workshops. However, actually making IPM work can still be difficult.

As Tom Strang from the Canadian Conservation Institute (CCI) pointed out at a previous IPM conference[1], *change is hard*. People are busy, and it is difficult to generate the momentum to get programs started, and to structure them so that they will be maintained.

This paper will present an IPM case study, looking at the management of a multi-institution pest management program in the Adelaide region of South Australia. The paper does not contain descriptions of new technology or treatment methods, but presents some ideas about how a large-scale IPM program can be structured to work successfully.

The Situation

Artlab Australia is the state conservation centre for South Australia. Artlab provides preservation and conservation assistance for 8 government collecting institutions in the Adelaide metropolitan and country regions, as well as for a number of private and institutional clients.

This paper is concerned with the IPM programs for the government arts institutions, which are concentrated along North Terrace in the centre of Adelaide, but are as far afield as Birdwood and Port Adelaide. Off-site stores exist in different suburban areas.

These institutions are part of Arts SA, a portfolio within the Government's Department for Transport, Urban Planning and the Arts, and receive government funding to spend on conservation treatment and on preservation programs, which are provided by Artlab. Artlab itself is also part of Arts SA, and is expected to provide direction and guidelines for preventive activities, and, being a separate organisation, requires its own IPM program.

All Arts SA institutions have had pest problems in some shape or form, although we are fortunate in Adelaide that the climate is temperate, we are not on any Bogong moth migration routes and locust plagues rarely reach the city.

The existing pest situation among these institutions was as follows:

- Some institutions had existing pest management programs of varying complexity, others had no formal program.
- Some institutions had obvious pest problems, others did not.
- Some institutions had no way of knowing the extent of their problems as their collections were large and significant proportions were low-use collections with no regular inspection program in place.
- Some institutions wanted to manage their own IPM programs, others wanted Artlab to play a major role in pest control.
- Everybody was busy and reluctant to take on more work, but no one wanted to have any problems with pests.

Of course, what everyone wanted was a workable IPM program that didn't take up too much extra time or resources. So, we needed to utilise whatever resources already existed, in order to provide a basic

structure and level of control, and supplement these with various "extras" that institutions could add on as required.

We were also conscious of not wanting to "control to excess" – we didn't want to spend what we couldn't afford on problems that didn't exist.

A coordinated approach to pest management seemed sensible as the institutions involved shared similar infrastructure and all fell under the umbrella of Arts SA. However, management of the project was complicated due to the sheer amount of material needing to be preserved – 8 institutions' worth - and because so many more people and locations were involved.

The risk management approach

When designing our IPM structure, we decided to approach the problem from a risk management perspective. This is not an unusual approach to take as pests are a universally recognised risk to collections.

We found the Australian and New Zealand Standard for risk management[2] to be a very useful document for shaping our pest management program. Appendix B of the Standard recommends steps to take in developing and implementing a risk management program:

- Step 1: Gain support of senior management
- Step 2: Develop the organisational policy
- Step 3: Communicate the policy
- Step 4: Manage risks at an organisational level
- Step 5: Manage risks at the institutional level
- Step 6: Monitor and review

(NB: some phrases have been changed slightly so they are more clearly related to the IPM program in question).

Step 1: Gain support of senior management

As a first step, the Standard recommends developing a risk management philosophy and awareness of the potential risk at a senior management level, by training or briefing executive management. The Standard also recommends appointing a "champion" or team to sponsor the initiative.

These recommendations seemed like sensible advice. Previously, Artlab had offered a number of IPM training programs for the Arts institutions, with the aim of beginning formal IPM programs in each institution. While they were useful in raising awareness of the problem, it was not successful in that there was no formal structure or support for such programs – generally, it was curators and assistants who attended the sessions, and it was too hard for these staff to go away and set up programs on their own. Starting from the top instead seemed like a good idea.

We also had an easy way of achieving these recommendations – every month Arts SA holds a Senior Management Group meeting, at which the Chief Executive of the arts portfolio and the directors of each institution are present, including the director of Artlab, Ian Cook.

We promptly nominated Ian as the "Champion of the Bug-Infested and Defender of the Chewed and Stained", but he won't put it on his business card so the title isn't likely to catch on.

On the IPM support team, we included Vicki Humphrey, the Assistant Director of Paper, Training and Technical Services at Artlab, who manages Preventive Conservation, and myself, the Artlab Preventive Conservator, who was to coordinate the plan.

Together we prepared a short "philosophy" statement about pest management to present to the Senior Management Group, and an organisational policy that described how we thought the pest management program would work best in the portfolio.

Step 2: Develop the organisational policy

Step 2 in the Standard recommends developing an organisational policy for risk management. The organisation in this case consists of Arts SA collecting institutions, and the policy needed to link all the organisations together in a way that would utilise existing resources in the most effective manner. The policy also needed to identify the procedures that would make it easy for staff to identify their own role in the program and who they could contact for help and advice when necessary.

First we needed to address the formal issues: the objectives of the policy, the rationale behind the policy, the range of issues to which the policy applied, and so on.

These are fairly straightforward matters to define and will not be discussed further here. More difficult was deciding such issues as what is an acceptable level of risk, defining responsibility for the program and discovering what resources were already available. The answers to these questions allowed us to identify the procedures that would allow us to put our policy into action.

What is regarded as acceptable risk?

Conservators are trained to be people with very low risk tolerance and so this was a difficult issue to consider. We were conscious of wanting to allocate resources to the most deserving or at-risk collections. Most conservators and curators alike are unprepared to accept the possibility of permanent damage to any object, but recognise that there are often insufficient resources - time and money – to carry out ideal IPM strategies.

We decided to give risk ratings to collection types and to buildings, in order to give some guidance as to the level of vigilance required in individual circumstances. From experience gathered over the last 10 years, conservators and curators alike had a good understanding of which collections and buildings had been most affected by pests.

By and large, and not unexpectedly, most serious problems had occurred in protein-based collections, such as wool and silk textiles, ethnographic objects with feathers and in certain natural history collections – skins, entomology specimens etc - whereas we had had very few incidences of damage to cellulose-based collections, such as paper or wood. These incidences were also not as damaging as those occurring to protein-based collections. Most of our serious problems involved carpet beetles or clothes moths.

The risk standard allows risk ratings of *high*, *significant*, *moderate* and *low* risk[3], based on the likelihood of the event occurring and the expected consequences of the event. So, using the risk standard, we assigned wool, silk, feathers and certain natural history specimens a high risk rating (infestation likely to occur and major damage expected) whereas paper-based collections received a moderate rating (infestation should occur at some time, minor damage expected).

This allowed us also to quickly extrapolate these ratings to other areas and collections that had not yet experienced or seemed to experience pest problems, based on basic catalogue information. Buildings were also assigned risk ratings, based on our existing knowledge of their condition and current control mechanisms in place.

While reluctant to define any level of risk as acceptable, many collections and buildings rated as moderate or low risk do not currently experience any particular problems with pests. This does not necessarily imply

there are no pest problems within these collections, as we could simply be unaware of them, but suggested that with only a little more formal monitoring of existing controls, the current situation may be adequate.

Who is responsible for managing risks?

Each institution is responsible for its own risk management programs. Ultimate responsibility for a collection lies with the Director and Board of Trustees, but on a day-to-day basis the Collections Manager or similar is responsible for such issues. However, Artlab also has a responsibility to encourage institutions to follow the best possible risk management strategies. Another complication is that maintenance work and building pest control is carried out by contractors who are appointed via Arts SA Business Services Division. In the end we decided on the following:

- Ultimate responsibility for the IPM program of an institution lies with the Executive of that institution. Executives should include the policy as part of their risk management program and business plans.
- Staff within an institution are responsible for bringing pest problems to the attention of senior staff. An *IPM Officer* for each institution was nominated, generally the Collections Manager or equivalent who would be involved in such issues anyway. This person is the main contact for all staff and external parties involved in the IPM program and oversees communication, documentation and IPM-related activities within that institution.
- Responsibility for keeping portfolio-wide communication systems working, notifying IPM Officers of upcoming meeting dates, providing updated specialist IPM information (eg identification guides, tender specifications) and monitoring the success of the program lies with the IPM team at Artlab Australia.
- Responsibility for tender selection and management of commercial pest contractors lies with Arts SA Business Services and with the facilities management contractors for each building, with involvement from Artlab and IPM Officers.

This process was also useful in identifying the key players in the IPM strategy.

What support and expertise are available to assist those responsible for managing risks?

Here we decided to do a brief survey (via a “brainstorm”) on what resources were available to help achieve our goals.

On the surface, it appeared thus:

- Monthly or bimonthly inspections of all buildings (not collections) by commercial pest companies, who carried out the most frequent inspections of buildings and who could apply chemical treatments if necessary.
- Tender selection meetings run by Arts SA to review IPM information included in tender documents and to select the most appropriate company.
- Entomologists at the SA Museum – experts in pest identification and lifecycle information.
- Facilities for treating insect infestations at Artlab – experts in conservation treatment and IPM strategies.
- A preventive conservator who had time and special project funding available to set up and monitor the project.
- Regular facilities managers meetings with members from each institution and Arts SA Business Services.

Clearly there was expertise and information available about our pest problems, but the information was not being communicated to all interested parties. For example, the inspection reports from the commercial pest companies were generally sent to Arts SA administration and were not necessarily sent on to curators and other staff who may find them useful. Correcting situations such as this by creating an effective communication structure is one of the most important parts of the program.

What level of documentation is required?

No-one likes extra paperwork, so again we wanted to develop record-keeping systems that were simple and easy to use. Each *IPM Officer* is to be provided with a folder containing basic information about the IPM program – a copy of the Arts SA IPM policy, the IPM tender specifications that were provided to commercial pest control companies, a pest identification booklet, and a form for reporting IPM problems.

To this folder the following documentation is to be added:

- Inspection reports from commercial pest companies.
- Completed forms reporting IPM problems.
- Treatment reports for infested items treated by Artlab Australia.
- *IPM Updates* produced by Artlab Australia and sent around via e-mail.
- Inspection reports from Artlab Australia or from inspections carried out internally.
- Locations of insect traps for any additional monitoring programs.

How should we review the performance of our procedures?

Obviously we needed a way to review the performance of the program. We decided on a system to be used initially, and reviewed at the end of three years – in effect, we decided that the performance of the review mechanisms also needed to be reviewed.

- IPM Officers will fill in a simple form every time a pest infestation is discovered, and supply a copy to the Preventive Conservator. This step involves defining what is worthy of reporting – we don't necessarily want to know every time a blowfly buzzed into the building, but single sightings of other insects may be more significant.
- SA Museum entomologists will fill in a simple form every time a pest is identified for an institution, and supply a copy to the Preventive Conservator.
- Initially, the Preventive Conservator will compare the incidence of pest infestation in an institution to that of the preceding year. A reduction in the number of pest incidents will be taken as a sign the program is working. After three years this method will be reviewed and may become more useful as data is collected. Artlab has a computer database of treatments carried out; we decided we also needed a keyword search function in this database so we could easily count the number of jobs involving pest treatments.
- The Preventive Conservator will carry out a brief survey via e-mail annually to determine the satisfaction level of involved parties.

Results of both mechanisms are to be communicated through the July *IPM Update* and presented to the Arts SA Senior Management Group at the first meeting of the new financial year, for comment.

Final structure

The final basic structure of the program is outlined in *Figure 1*.

- *Staff* in institutions will report pest problems to the *IPM Officer* of that institution.

- The *IPM Officer* will liaise with Artlab's *Preventive Conservator*, *SA Museum entomologists* and *Arts SA facilities management* as required. They may also communicate directly with the *Director* of their institution and with commercial *pest control companies* as required.
- Information from commercial *pest control companies* will be disseminated to *IPM Officers* through *Arts SA facilities management*.
- Information from the *IPM Officers* will be gathered by the *Preventive Conservator* at Artlab and transmitted to the *IPM Team Leader* and the *Arts SA Senior Management Group* on a biannual basis.
- Information about IPM developments will be transmitted by the *Preventive Conservator* to the *IPM Officers*, who will then disseminate the information among *staff* as necessary.

Step 3: Communicate the policy

The biggest problem we found when we were investigating our IPM program was that communication within the Department wasn't working well – surprise.

With so many people involved, good communication was going to be the key to whether the system worked or not.

We also needed a way to let everyone know what the structure of the program was, who they could report pest problems to and what assistance was available to them.

The Senior Management Group had already been briefed, and the IPM officers and other key people (eg the entomologists and Arts SA representatives) for each institution were also called together for an introductory meeting. Subsequent updates will be provided via an e-mail mailing list.

Then we needed to figure out a way to let everyone else know about the policy. E-mail is often a curse but can also be very useful: when the procedures have been settled, the IPM Officers will send out an e-mail to all staff in their institution, letting them know who their contact person is and briefly what kinds of things to look out for.

Short 10 minutes training sessions will be made available to staff, including security and cleaning staff, to introduce them to the pest identification booklet and to let them know who their primary contact was.

Small posters will also be provided to institutions, with the name and number of the IPM Officer for that institution.

We are also investigating the possibility of placing some relevant information on the Arts SA Intranet or Artlab website, so that staff can have improved access to relevant reporting forms and pest identification guides.

Step 4: Manage risks at an organisational level

Maintaining the structure of the overall program was identified previously as the responsibility of the IPM Team at Artlab, and was essentially a management issue.

Current management models such as that outlined in Robbins et.al.[4] identify the major functions of a management role as follows:

- *Planning* – defining goals, establishing strategy, developing sub-plans to coordinate activities.
- *Organising* – determining what needs to be done, how it will be done and who is to do it.
- *Leading* – directing and motivating all involved parties, resolving conflicts.
- *Controlling* – monitoring activities to ensure that they are accomplished as planned.

These activities fitted well into the risk management approach that had already been adopted. We had already gone a long way towards planning and organising the strategy, and had developed plans on how best to lead and control the related activities.

"Motivating all involved parties" is probably the most difficult part of instigating any project of this size and we expected it to be our biggest challenge. Our best way of managing the project was to ensure that the identified communication pathways were maintained, so that the program could gradually enlarge upon the basic structure as people became more comfortable with IPM.

The Preventive Conservator was to manage the program on a day-to-day basis. To achieve these activities, the Preventive Conservator needed to act as a *liaison officer*, by maintaining networks and contacts; an *information monitor*, by keeping track of relevant information internally and externally; a *disseminator of information*, by transmitting information to others in the network; a *negotiator*, by representing the institutions at tender selection meetings and other relevant occasions; a *disturbance handler*, by taking corrective actions when processes did not work effectively; and to some degree an *entrepreneur*, by searching for "improvement projects" and other activities that could make the program more workable[5].

Step 5: Manage risks at the institutional level

We had established a basic level of pest control: a communication tree, regular pest updates and reminders via e-mail, commercial pest checks of the building, and assistance for pest identification and object treatment.

However, individualised programs were necessary for each institution. Like disaster preparedness, plans must be particular to the institution. In discussion with each IPM Officer and using the risk ratings assigned to the relevant collection types and buildings, we have started to examine the existing controls and to identify hot spots and special cases that require extra attention.

This meant we needed to provide a range of options to accommodate the different approaches of the various institutions – for example, some wanted Artlab to play a major role in their IPM programs; others preferred to carry out the bulk of activity themselves and use Artlab for advise and specialised object treatment only.

So we started to develop a range of "extras" that institutions can add on to their basic program. Some of these include:

Portable quarantine chambers (few institutions have space available for a formal quarantine area).

Quarterly or monthly inspections of high-risk collections and buildings by conservators, including sticky trap placement if required.

Use of Artlab freezer for incoming objects.

Risk assessments and maintenance reviews.

Working bees, to clean and tidy storage areas.

Special storage enclosures for high risk collections.

Training courses.

Analysis of information gathered internally.

This allows each institution to manage the program as best suits their requirements and budget. For example, an institution with low-risk collections or buildings may decide to utilise very few of these options, or use them only for one-off circumstances. An institution with high-risk collections or buildings may decide to have conservators more actively involved in inspections, cleaning and insect trapping.

This approach was used to develop a "spec sheet" for each institution, listing IPM-related activities that were to be carried out each month.

Step 6: Monitor and review

We had already determined the method of review for the program as part of our organisational structure – to assess the number of incidents involving pests and to determine the satisfaction levels of the staff involved. The first review will be carried out in July of 2002. We will attempt to link reviews to the Arts SA budget planning structure, so that recommendations for minor works can be processed more easily.

Conclusions

Although much important work has been done, we still have a way to go before being secure in our new IPM program. Most of the work carried out to date has been in the planning and organising stages, with a great deal of implementation, trouble-shooting and review still to be done.

Projects of this size and complexity require an evolutionary approach rather than a revolutionary approach, which means that conservators must exercise that patience which is apparently one of our primary character traits.

Once working and tested, we are hoping to utilise the existing framework to develop organisational policies for environmental monitoring and other preventive programs stretching across the Arts portfolio. The contacts, communication network and resources that have been developed and identified can be built upon to encompass other preventive conservation issues. In the meantime, the challenge will be to keep the IPM program going so that it develops a momentum of its own.

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Sampling and Estimate of fungal biodeteriogens of Lucknow, India

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Abstract

Biodeterioration is a biological process responsible for the destruction of materials resulting in enormous economic loss all around the globe. The degree of damage is multiplied in country like India where tropical and humid climate prevails in major parts of the subcontinent. Out of various deteriorating agents, fungi are the principal constituents of airspora responsible for the major damages of cultural properties. It is omnipresent and attacks a wide range of substrates such as textile, leather, paper, stone, wood, plastic, painting, etc.

An efficient and reliable sampling is a prerequisite for the proper identification, quantification and management of such problems. Andersen, Rotorod and Burkard air-samplers were employed over a period of one year (January – December, 1997) in both extramural and intramural environments of Lucknow. Certain predominant fungi such as *Alternaria, Aspergillus, Cladosporium Chaetomium, Curvularia, Helminthosporium, Penicillium, Paecilomyces, Torula, Trichoderma* etc. are observed to be associated with the biodeterioration of cultural properties. Both quantitative and qualitative estimates of aeromycoflora have been fruitfully utilized in prediction of various fungal biodeteriogens at particular time and place.

Key words - Air-borne fungi, Aerobiology, Biodeterioration, Cultural properties, Lucknow.

Introduction

The study of air-borne particles, including fungal spores, is important to mycologists, plant pathologists, microbiologists and as well as to aerobiologists. Aerobiology as an adjunct to micro-biodeterioration, is one such scientific approach focussed on aerial transport, dispersal, impact and interaction of microorganisms with the substrates. The proper air monitoring assess, identify, quantify biodeteriorating air-borne bioparticles in relation to their source, concentration, seasonal and annual variations. All types of art materials such as monuments, stone sculptures, bronze objects, wood articles, manuscripts, books, wall hangings, paper crafts and various types of paintings, get deteriorated due to the attack of multiple microorganisms. This problem is more grave in the tropical humid climate like that of India and can be solved to a major extent by the qualitative and quantitative measure of causal organisms in the ambient air followed by suitable control measures. In early days, the air sampling was done by flying kites / balloons which carried with them adhesive–coated slides. These methods provided only the qualitative estimates of aerobiopollutants at different heights in particular place and time. Since then, a number of sampling devices have been developed based upon some basic principles such as:

1. Settling of particles through gravimetric forces
2. Filtration
3. Impaction through suction of air
4. Impaction on rapidly moving surfaces

Though hundreds of samplers have been designed and are in use at different places by different workers but the collection efficiency varies and each has its own merits and demerits. In the recent past, the air monitoring has multiplied and diversified by employing new instruments such as automated counters,

electrostatic samplers etc. which has expanded the dimensions of aerobiological researches. The aerobiological sampling was carried out with the aim to determine the qualitative and quantitative prevalence of airborne pollen and fungal spores in the extramural and intramural environment in Lucknow city and adjoining areas and to assess their impact on the articles of cultural heritage.

Matertial and Method

The district of Lucknow (26° 30' -27° 10' N and 80° 30' -81° 13'E) is an irregular, quadrilateral area located in the Gangetic plain of Uttar Pradesh. The climate is characteristically periodic with three well-marked seasons and sub-tropical monsoon type of climate.

The sampling was carried out by three internationally recognized air samplers provided under Ministry of Environment and Forests, New Delhi, sponsored project entitled "Aeroallergens and human health: aereobiological studies". These volumetric air samplers are usually employed for aerobiological studies where both qualitative and quantitative estimates of aerobiota are required and precise identifications are the prerequisites. For effective and efficient management of the micro-biodeterioration the information on diurnal, seasonal and annual variations in the types and concentration of aerobiodeteriogens is essential, and could be procured by employing these samplers simultaneously. Long-term and short-term changes in the atmospheric load of fungal spores were also encountered and were correlated amongst the results of different sites and samplers. The earlier aeromycological studies carried out in Lucknow either in Birbal Sahni Institute of Palaeobotany or elsewhere in Lucknow only reported the qualitative estimates of aerobiota (Vishnu-Mirre & Khandelwal,1973; Khandelwal,1991, 1992). But present study has provided both qualitative and quantitative data employing the technologically advanced air-samplers.

The air samples were collected from six different places of Lucknow at different times of the year from January to December 1997. Aerobiological data was collected from six sampling sites as follows:

1. **Continuous Sampling**: It was conducted at Vikas Nagar on Kursi road, within the provenance of Kukrail Reserve Forest by employing Rotorod sampler for regular and continuous monitoring.
2. **Spot Sampling**: It was conducted by employing both Burkard and Andersen samplers at the following sites:
    - Chikan Work Place, Chowk
    - Garbage Disposal Unit, Gaughat Pumping Station
    - Military Dairy Farm, Dilkusha
    - Vegetable Market, Kaiserbagh
    - Vivekanand Polyclinic (outdoor and indoor), Nirala Nagar

The air-samplers were used whose descriptions are as follows:

1. **Andersen two-stage sampler**: It is used for the estimation of culturable fungi. It is constructed of aluminium with two stages, which are held together with three dowel pins and three teflon caps. Each stage contains 200 tapered orifices. The diameter of the orifices on the first stage is 1.5 mm and 0.4 mm on the second stage. The sampler is AC power driven and takes in 28.3 litres/min of air through the opening at the top and impinges it successively onto the petridishes containing 20 ml of media, placed below each sieve. Finally the air passes out after impacting on second petridish. The Sabouraud's nutrient agar medium (pH 5.6) was used. The disposable petridishes were poured under sterile conditions at least 2 days prior to sampling in order to check whether any contamination had occurred during pouring of media. After exposure the petridishes were covered with lid before

removing from the sampler. Petridishes were brought to the laboratory and incubated at 27°C ± 2°C in BOD incubator for 2-3 days. Petridishes were scanned on third day and noted the different fungi in each petridish. The incubation period was increased sometimes. The identification of fungi was done both by visual and microscopic studies by preparing fungal mounts in cotton blue stain. The pure culture of the encountered fungi, were maintained in glass test tubes containing Czapeck Dox Agar medium (pH 7.3).

The sample collection was done between 10 a.m. to 11 a.m. The sampler was cleaned and disinfected with 70% alcohol before and after use. The exposure time was fixed for 10 min., all throughout the investigation. The samples were collected at least 4 times a month from each site. After recording monthly data, the average colony concentration has been converted into per metric cube of air using the following conversion factor: -

Suction rate = 28.3 litres per minute

If operated for 10 min., air taken in is = 0.283 m^3

If 0.283m^3 has 1 colony, 1m^3 has 1/0.283 = 3.53 colonies

Conversion factor for 10 min. = 3.53

2. **Rotorod Sampler** : It is an impaction sampler used for continuous sampling for pollen/fungal spores employing 2 lucite rods (1.3 mm in width) whirled rapidly in a circular path (2,400 rpm), to standardize air speed and to eliminate wind direction as a variable. The particles get impacted on one face of the rod for one minute after every 10 min, rest of the time it is folded and static.

   While using the rotorod sampler, the lucite rods are coated over 3/4th (20 mm) of the surface with silicon grease. The rods are inserted in the aperture provided on the sampler with the adhesive coated surface facing outside so that when the arm moves clockwise, the impaction occurs on the greased surface. After exposing for 24 hours, the rods were stored in plastic containers provided with the sampler. The exposed rods are inserted into grooves of a lucite stage provided as accessory, for scanning under the microscope. A cover glass of appropriate width is applied over an aqueous mounting medium such as Calberla's solution.

   The site has been fixed for Rotorod sampler. The rod had been changed at a fixed time every day. After entry of monthly data the average pollen and spore concentration has been converted into per metric cube of air using the conversion factor as given below:

   Volume (V) of air sampled:

   V = (rod width) x (rod height) x (rod diameter) x π x (RPM) x time rod used = 1

   1 rod x (0.159 cm) x (2.2 cm) x (8.6 cm) x π x (2400) x 143 min = 3.24m^3 of air.

   As Rod rotates once every 10 min., therefore, machine operated for 23 hrs. 50 min or 143 min/day.

   If 1 pollen grain is encountered in 3.24m^3 of air then 1m^3 will have: 1/3.24 = 0.3086.

   The multiplication factor is 0.309.

3. **Burkard Personal Slide Sampler**: It is compact battery/ power operated, 10 cm in height and 8 cm in diameter. It has rectangular orifice at the top and a slit on the side to insert an adhesive coated microslide. The sampler sucks in air at the rate of 10 litres/min. through the orifice and the particles get impacted on the slide in the form of a band. It is used for pollen/fungal identification and estimation.

   The super deluxe microslide (75 mm x 25 mm) of 0.8 mm thickness is used. The 3/4th of the slide is smeared with safranin stained glycerine jelly in the form of a thin film. The labelled slide is inserted

through the slit in the sampler with jelly side up and the lid is twisted in order to seal the inner chamber. After exposure, the slide is removed and brought to the laboratory carefully in the small slide box. For mounting, the jelly is melted on a hot plate and a drop is placed on the exposed band of the slide. Warm 22 sq mm cover glass is placed on the drop of molten jelly. The cover glass is slightly pressed with forcep back to get an even, thin mount and thus spreading impacted material. The entire cover slip area is scanned under the light microscope for identifying and counting the different biota. The counts are expressed as number of spores/pollen per cubic metre of air sampled. The following conversion formula is used for Burkard samples:

Suction rate = 10 litres per minute

If operated for 10 min., air taken in is = 0.1 m^3

If 0.1m^3 has 1 pollen grain/fungal spores, 1m^3 has 1/0.1 for 10 minutes = 10 pollen grains/fungal spores.

Results

One year aerobiological survey revealed the existence of a rich aeromycoflora in the atmosphere at six extramural and one intramural site in Lucknow, India. In the present study, only ten dominant fungal spore types/colonies recorded over a period of one year (January-December, 1997) are considered (Tables 1, 2 & 3). The weekly, monthly and annual periodicity of total aeromycoflora have been compiled in the sponsored project entitled "Aeroallergens and human health: aereobiological studies" submitted to Ministry of Environment and Forests, New Delhi (Annon, 1994-1998).

Fungal spore types (recorded on Burkard Personal sampler)

The total components of the aeromycoflora varied from site to site recording as high as 36 types in Kaiserbagh, Chowk and Vivekanand (Outdoor) and as low as 29 types from Dilkusha. Out of ten dominant taxa *Cladosporium* , round small fungal spore, *Alternaria, Curvularia, Helminthosporium, Epicoccum* and *Nigrospora* were registered from all the sites. Other subdominant types recorded from three or four sites included *Chaetomium, Cercospora,* , *Periconia* and 2-4 celled spores. *Bispora* and Uredospores of *Puccinnia* were sporadically recorded.

Table –1: Relative contribution of dominant fungal spore types to the atmospheric load in different sites of Lucknow over a period of one year (January -December 1997) by using Burkard Personal Sampler

Sl. No	Names of fungal spore types (No. of fungal spore /m^3)	Chowk	Gaughat	Dilkusha	Kaiserbagh	Vivekanand Polyclinic	
						Outdoor	Indoor
1.	*Cladosporium*	238	254	224	224	199	241
2.	Round small fungal spore	198	220	182	238	224	209
3.	*Alternaria*	114	208	229	181	193	166
4.	*Curvularia*	63	55	65	46	64	49
5.	*Helminthosporium*	53	96	117	115	108	109
6.	*Epicoccum*	51	59	52	49	46	46
7.	2-4 celled spores	39	31	28	-	-	-
8.	*Chaetomium*	37	-	-	39	48	50
9.	**Nigrospora**	36	38	37	46	45	42
10.	*Cercospora*	28	32	38	34	38	-

11.	*Periconia*	-	43	-	24	-	26
12.	*Bispora*	-	-	27	-	-	39
13.	Uredospores of *Puccinnia*	-	-	-	-	39	

Fungal spore types (recorded on Rotorod Sampler)

The total aeromycoflora encountered in the atmosphere over the year was represented by 2545.4 fungal spores distributed amongst forty types (Khandelwal, 2001). *Alternaria* (675.8) constituted the predominant spore type. *Cladosporium* (367.1), *Helminthosporium*(363.0), round small fungal spore (264.6), *Epicoccum*(127.9), *Curvularia* (99.4) ,*Fusarium* (88.7) 2-4 celled spores (84.6), Uredospores *of Puccinnia* (59.8), and *Nigrospora* (57.1) were other subdominant types recorded in annual fungal spore calendar.

Table-2: Dominant fungal spore types to the atmospheric load in Vikas Nagar, Lucknow over a period of one year (January -December 1997) by using Rotorod sampler

Sl. No	Names of fungal spore types (No. of fungal spores /m^3)	Vikas Nagar
1.	*Alternaria*	675.8
2.	**Cladosporium**	367.1
3.	*Helminthosporium*	363.0
4.	Round small fungal spore	264.6
5.	*Epicoccum*	127.9
6.	*Curvularia*	99.4
7.	**Fusarium**	88.7
8.	2-4 celled spores	84.6
9.	Uredospores *of Puccinnia*	59.8
10.	*Nigrospora*	57.1

Fungal spore types (recorded on Andersen Sampler)

As high as 36 colonies were registered on petridishes from Dilkusha and as low as 31 types were recorded from Kaiserbagh. Some of the fungal colonies, which were present in all the sites, were *Alternaria alternata, Fusarium oxysporum, Penicillium funiculosum, Cladosporium cladosporioides, Aspergillus niger* and *A. flavus. Curvularia lunata* was recorded from all the sites except Kaiserbagh. *Mucor hiemalis* was not recorded from both Kaiserbagh and Vivekanand Polyclinic (Indoor). *A. nidulans, Aspergillus terreus, Fusarium roseum, Penicillium citrinum* were reported from three of the sites whereas *Curvularia tetramera* was reported from two and *A. candidus* from one site only.

Table-3: Relative contribution of dominant fungal colonies to the atmospheric load in different sites of Lucknow over a period of one year (January - December 1997) by using Andersen Two-Stage Sampler

Sl. No	Names of fungal colonies No.of colony forming units/m^3	Chowk	Gaughat	Dilkusha	Kaiserbagh	Vivekan and Polyclinic	
						Outdoor	Indoor
1.	*Alternaria alternata*	133	169	163	121	175	181
2.	*Fusarium oxysporum*	128	132	123	91	126	132

3.	*Penicillium funiculosum*	128	114	114	94	145	149
4.	*Curvularia lunata*	106	61	140	-	75	73
5.	*Cladosporium cladosporioides*	86	97	95	56	69	57
6.	*Aspergillus niger*	76	128	96	155	102	128
7.	*A. flavus*	57	55	74	59	68	96
8.	*A. nidulans*	42	-	-	57	45	-
9.	*A. candidus*	35	-	-	-	-	-
10.	*Mucor hiemalis*	34	81	57	-	39	-
11.	**Curvularia tetramera**	-	45	-	85	-	-
12.	*Aspergillus terreus*	-	39	42	-	-	54
13.	*Fusarium roseum*	-	-	36	36	-	43
14.	*Penicillium citrinum*	-	-	-	58	38	55

In this study, some differences were observed among the fungal spore profiles at different sites. These differences were attributed to local ecology, which is the prime factor in determining both the concentration and composition of airspora in the atmosphere at each site (Lacey, 1962). Density and type of vegetation, human interference, as well as dispersal behaviour of the fungi present at each site also govern these differences.

Discussion and Conclusion

The botanical specimens, manuscripts, books, wall hangings, wood/paper crafts, miniature paintings, lithographs in the libraries and archives are generally decomposed due to long term storage in unsuitable conditions like dust, dirt, optimum temperature and high humidity favourable for the growth of various fungi. The basic components of paper materials such as cellulose, linen, straw, wood, pulp, starches and pigments serve as nutrients for the growth of various fungi. Similarly, other art materials such as monuments, stone sculptures, bronze objects, wood articles, etc. exposed to the outdoor air get deteriorated due to the attack of multiple microorganisms. The outdoor fungal spores get easy access in indoor air through open doors and windows and on getting optimum environmental conditions and proper substrates they start growing and proliferating. Thus, from the study of aerial survey it is realized that fungal spores suspended in the air must be traced back to their sources to which much attention has not been paid by the scientist so far.

Janposri (1991) identified the fungi belonging to group *Aspergillus* and *Penicillium*, which are very commonly found on ancient famous Thai paintings of tempera technique in Thailand. Nair (1991) pointed out that in tropics under humid conditions dry preserved botanical specimens such as herbarium materials, samples of seeds, fruits,etc. are generally attacked by several species of *Aspergillus, Fusarium, Penicillium, Alternaria, Trichoderma* and *Cladosporium.*

The indoor air of library was found charged with the spores *of Aspergillus, Cladosporium, Torula, Penicillium, Trichoderma* and *Chaetomium* (Tilak and Vishwe, 1976). Many cellulose decomposing fungi such as *Alternaria, Monilia, Fusarium,Chaetomium, Myrothecium, Torula, Stachybotrys, Cladosporium, Paecilomyces, Rhizopus* and E*picoccum* were also reported from a library of Aurangabad (Tilak and Saibaba, 1984). *Alternaria alternata, Aspergillus sydowii, A. niger, Cladosporium cladosporioides, Penicillium citrinum* were found occurring on different cellulosic materials such as manuscript and books, wood craft, paintings, wall hangings, etc. in Gorakhpur (Lakhasmikant & Mathur, 1989). Tripathi (1987)

and Tilak and Pillai (1988) also reported the high prevalence of *Cladosporium, Penicillium* and *Alternaria* inside libraries. Species of *Aspergillus, Penicillium* and C*ladosporium* were in great abundance in the indoor air of library of Madras University (Nadimuthu and Vittal, 1995). The impact of microbes on different substrates such as bark of wattle used for leather taining (Kishorekumar, 1981); finished leather goods (Sharma & Sharma, 1980), wool (Sengupta, 1950) and optical instruments (Saxena, 1963) are also recorded.

In the present study, all the samplers from all the sites exhibited the predominance of fungal spores of *Cladosporium*, which is ubiquitous, and its dominance in the other parts of the world is also well documented (Cosentino, 1990; Shaheen, 1992; Dames & Cadman, 1994; Halwagy, 1994). Similarily, the occurrence of *Curvularia* was comparable to surveys conducted in India (Kumar, 1982; Singh *et al,* 1987; Vittal & Krishnamoorthi, 1989) and Malaysia (Ho *et al*., 1995).

Aspergillus flavus, A. nidulans, A. niger, A. sydowii, A. terreus, A. ustus, A versicolor, Alternaria, Chaetomium, Cladosporium, Diplodia, Epicoccum, Fusarium, Paecilomyces varioti, Penicillium citrinum, Rhizopus, Torula, Trichoderma, Trichothecium etc. recorded in the present study andin earlier reports from the Birbal Sahni Institute of Palaeobotany, Lucknow , have been identified for the considerable damage of miniature paper paintings and lithographs of the State Museum, Lucknow by Dhawan and Agarwal (1986).

Hence, aerobiological surveys need to be incorporated in order to manage the burning issues related with the micro-biodeterioration aspects of cultural properties. But the absence of a well-integrated system of monitoring of air-borne biological particles is a serious lacuna in the promotion of better management, hygiene and health. An integrated and co-ordinated national programme should be launched involving different laboratories and institutions of different parts of the country working on different aspects of environmental pollution.

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The Significance of Appropriate Sampling and Cultivation Techniques in the Effective Assessment of Biodeterioration.

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1. Introduction

In the absence of expertise and techniques of examination readily available to microbiological/molecular biological specialists, standard approaches to sampling and cultivation of organisms provide the conservation practitioner with an expedient option in the assessment of biodeterioration. Unfortunately — due to the difficulties of sampling and isolating organisms of interest from a greater population, and the inability to replicate in the laboratory the often dynamic and complex conditions under which biodeterioration occurs — this approach can have significant limitations that can lead to the misrepresentation of species and consequently the inappropriate assessment of biodeterioration [1].

An understanding of the limitations and benefits of the microbiological methods currently used for the sampling and cultivation of organisms can help to ensure that information generated is relevant to the conservation of cultural material. It will also identify ways in which informed investigations can work in conjunction with, and facilitate the appropriate employment of, microbiological and molecular-biological techniques.

1.1 A case study in biodeterioration

St. Botolph's church, Hardham retains one of the most important and complete Romanesque wall painting schemes in England. In response to the problems posed by their conservation, a comprehensive and integrated study of factors and mechanisms causing the deterioration of the wall paintings was undertaken by the Courtauld Institute of Art [2].

Investigations into the microflora of Hardham by Dr. K. Petersen identified a vivid pink and yellow staining phenomenon across wall surfaces, resulting from the activity of microorganisms [2]. By using this clearly observable form of microbial activity as an investigative marker, current research has aimed to review and assess standard methods used in the sampling and cultivation of organisms associated with the biodeterioration of cultural material.

1.2 A practical approach to microbiological assessment.

Microflora investigations can be divided into three stages, examination, sampling and cultivation. Each of these stages has the potential to provide an insight into the manifestations and mechanisms of biodeterioration. Examination of cultural material for symptoms of microbial growth/activity can reveal structural and/or aesthetic damage to the substrate. Visual examination may also provide information on the types of organisms responsible for biodeterioration, as well as evidence of other factors contributing to microbiological colonization.

Sampling of organisms can be undertaken using a range of invasive and/or non-invasive methods. However, the condition of the object under investigation is often a significant factor in the selection of sampling techniques. The technique chosen can be highly influential in determining the quality of a sample available for cultivation.

The small mixed populations of organisms collected during sampling are often unsuitable for investigations into metabolic activities, growth parameters and identification. Therefore cultivation of sampled organisms to increase cell mass and generate pure cultures is often necessary. During cultivation, changes in ambient conditions and the level and type of nutrients can exert selective influences on organisms removed from their original environment.

2. Aims

2.1 Sampling methods

A variety of sampling methods were assessed for their applicability and suitability for the *in situ* sampling from areas of pink and yellow discoloration. The range of sampling methods chosen — brushes, direct agar, fragment, loop, needle, swab, tape and velvet — varied in invasiveness, specificity, and availability to the non-microbiological specialist. The approach to assessment aimed to provide information on the working properties of each technique and its suitability as a method of organism transfer. In addition the presence of ubiquitous organisms, and the effect of transport and storage on the quality of samples was also reviewed.

2.2 Cultivation methods

In the cultivation of sampled organisms, a selection of media varying in nutrient abundance and type were incorporated into the experimental protocol. This enabled comparisons to be made between media types, and assessment of the effect of variations in media composition on the growth and activity of sampled organisms. Attempts were also made to replicate the nutritional conditions of the Hardham wall surface, by developing media based on the mineral composition determined from aqueous extract analysis of salts sampled from the wall surface.

3. Experimental procedures

3.1 Sampling of microorganisms

Three locations — the north and west walls of the chancel and the north wall of the nave — were designated for sampling. he locations chosen sustained the biodeterioration-associated phenomena of interest — pink and yellow discoloration. Moreover, the surfaces represented the varying degrees in integrity to which the wall painting had survived.

To assess and improve sampling strategies, sampling was carried out over two phases. Risks assessments were constantly made with some methods judged to be unsuitable for use on the painted surfaces due to the risk of loss or contamination.

3.1.1 Brushes

Brushes were used to dust surfaces for viable organisms. Particles suspended using this method were collected and transferred to medium containing plates or vials. Sample collection was enhanced and particle dispersion reduced by pre-wetting of brushes.

3.1.2 Direct agar

This technique transfers organisms directly from the sampled surface to a nutrient source. The nutrient agar plates used for sampling were poured so that the level of agar was above that of the petri-dish. The solidified agar could then be placed in direct contact with the surface being sampled.

3.1.3 Fragment

This invasive approach to sampling involves the removal of a small fragment of infected material. To minimize the impact of this invasive technique, samples were removed from previously damaged areas with the use of a field microscope and appropriate small tools.

3.1.4 Inoculating loops

The use of this technique is an adaptation of a laboratory-based aseptic transfer technique in which organisms present on a medium plate are collected in the eye of the loop and transferred to another location. Filling the loop with a sterile solution was found to enhance the collection of colonies.

3.1.5 Needles

Sampling using needles facilitates the investigation of painted surfaces in poor condition or those that are obscured by dirt or debris. This method involves drawing a sample into the syringe. Although best suited to the sampling of viscous micro-colonies, pre-wetting of surfaces improved the collection of samples from drier areas.

3.1.6 Swabs

The merits of swab use in sampling are similar to those that ensure their use in conservation treatments—controlled action over a localized area. Swabs can be sensitive enough to isolate individual colonies. Pre-wetting was found to facilitate sample collection.

3.1.7 Tape

Collection of samples using adhesive tape involved attaching a strip of tape to the surface being sampled. Organisms then become trapped by the tape adhesive and can be removed along with the tape and used as a source of inoculum. Initially, decorators tape was used however this proved to be unsuitable and was later replaced with 3M Post-it Indexes ™.

3.1.8 Velvet

This technique takes advantage of the brush-like qualities of velvet fabric. The fabric is placed in direct contact with the surface and organisms are collected within the fibres. The sample can then be transferred to an agar plate or other media source as an impression. The use of a replicating block accommodates the transfer of samples from the velvet, directly to a plate of solid media.

As maintenance of sterile conditions was impossible to achieve whilst working on site, all endeavours were made to minimize the potential for sample contamination. Sampling was undertaken in a rapid and fluid manner and where possible materials and equipment used were sterilized. Controls were also set-up to assess the level of ubiquitous organisms potentially contaminating samples.

Storage and transport was also assessed as part of the sampling strategy. Fragment samples were stored dry in sterile micro-centrifuge tubes. Those collected using the direct agar and velvet techniques were transported and stored on nutrient agar plates. Samples taken using other methods were initially transferred to a solution of sterile dilution buffer, which was later replaced with nitrogen containing *Pseudomonas* basal media. No special provisions were made for transport to the laboratory, and within the laboratory samples were stored at ambient conditions.

3.2 Cultivation of sampled organisms.

Media used for cultivation of organisms can range in composition and complexity. While the main purpose of media is to provide nutrients necessary for growth and biochemical activity, certain physico-chemical factors may also be provided. The influence of a nutritional environment on the successful cultivation of the pigmented organisms sampled was assessed using a range of nutritionally diverse media. The most nutrient rich medium — nutrient agar — was prepared from a dehydrated form. Both types of

Pseudomonas basal medium (with and without nitrogen) and the McClung Carbon-Free medium were prepared from recipes [3]. Microorganisms isolated from an extreme environment like the wall surface at Hardham will have adapted to nutritionally sparse, salt rich, humid conditions. Therefore a recipe for 'habitat-like' media (Hardham wall salts media) was developed based on the analysed ionic and organic composition of the wall painting surface [4]. Solid forms of all media were preferred, so that the characteristics of individual colonies— their colour, consistency, size and shape—could be observed.

Considering the environment from which the samples originate, cultivation under highly controlled conditions was considered inappropriate. To provide variations in the environment similar to those experienced in situ; plates were incubated under ambient laboratory conditions. The daily average temperature in the laboratory was around 15-20 °C and the relative humidity range 40-50 %. On average samples were inoculated 2-7 days after collection and cultivated over a period of up to two weeks.

4. Results and Discussion

4.1 Sampling methods reviewed

Assessment of sampling techniques established the velvet and 3M Post-it Index tape as the most appropriate methods for sampling of the pigmented bacteria. When cultivated on nutrient agar, samples taken using the velvet produced a dense mixed population of fungus and bacteria — including the pigmented species. Mixed populations of bacteria containing the pigmented species were observed on nitrogen containing and nitrogen free *Pseudomonas* basal media. Mixed populations of bacteria were also produced from tape samples cultivated on nutrient agar and both forms of *Pseudomonas* basal media. Only fungi were cultivated from samples taken using brushes.

Although pigmented bacteria were found on plates inoculated with samples taken using the loop, needle, fragment and swab methods, extremely low numbers of viable cells (1-2 per plate) were observed on all types of media. Modifications to sampling methodology produced no significant improvements in the ability of these techniques to produce samples with viable colony numbers above those on the ambient control plate.

4.2 Cultivation of the organisms of interest.

Although approximately two-hundred plates indicative of the different sampling and cultivation permutations were produced during this investigation, the total number of cultures containing the bacteria of interest and suitable for further investigation was eight. Depending on the method of sampling and cultivation, organisms of interest were identified in both mixed and in relatively pure populations. In all instances the viable cell count of pink bacteria was extremely low—approximately three well-defined colonies per plate. Yellow growth was more prolific, with high colony counts.

As anticipated, cultivation of a mixed population of sampled organisms containing the pigmented species was most prolific on the nutrient rich, nutrient agar plates. However due to its minimal, defined composition, *Pseudomonas* basal media sustained less virulent growth of mixed populations. This facilitated the observation and isolation of pigmented species, which were able to be successfully cultivated on both the nitrogen containing and nitrogen absent forms. Growth of pigmented organisms in the absence of a readily available source of nitrogen contrasted with the negligible growth rates observed on the McClung carbon free medium. This indicates that while organisms of interest are capable of growth without a freely available source of nitrogen, the provision of carbon compounds is essential.

The ability of the defined salt based *Pseudomonas* media to support growth can be contrasted with the inability to cultivate organisms — other than sparse fungal growth — on the Hardham wall salts media. This highlights the limitations in trying to produce a 'habitat-like' medium for cultivation. The media produced — also a defined salt-based medium — had a very high salt content and while appropriate provisions were made to facilitate growth, the nutritional environment created was extreme.

5. Conclusions

During this research attempts were made to understand and provide appropriate conditions under which the pigmented organisms associated with biodeterioration at Hardham could be cultivated. A range of sampling methods were reviewed and organisms sampled were cultivated on a variety of media. Attempts were also made to replicate — in the form of solid media — the nutritional environment of the wall. The main findings can be summarized as follows:

- The successful cultivation of pigmented organisms of interest was a product of the sampling technique employed, and the medium used for cultivation.
- In general the most effective techniques used for the sampling of organisms were the velvet and 3 M Post-it index tape. Both Methods are considered non-invasive and easily available to conservation practitioners.
- Although attempts were made to replicate the nutritional environment from which sampled organisms were collected, the most appropriate medium for cultivation was a defined salts medium — *Pseudomonas* basal medium.
- An indication of the metabolic requirements of organisms of interest was provided during cultivation. Pigmented organisms were able to be cultivated on media void of freely available nitrogen but growth in the absence of a carbon source was negligible.

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The Present Situation of Pest Control of Cultural Properties in Taiwan

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Abstract

The present study, focusing on museums, libraries, archives and wood-structured historic sites, tries to explore the biodeterioration problems and those preservation measures that these cultural properties preserving agencies have deployed and to review the various educational activities, such as seminars, workshops, etc. taking biodeterioration of cultural properties as theme held in Taiwan in the last decade.

In general, the most frequently and commonly seen pests responsible for the deterioration of cultural properties are cockroaches, cigarette beetle, booklouse, silverfish, powder post beetles, clothes moths, termites, and carpet beetles. Lately these impairments have seen apparent improvement thanks to increasingly enhanced awareness of artifact preservation. Pest control and eradication have also turned from the conventional chemical treatments to the methods to some newly developed methods such as Low oxygen atmospheres, Freezing , etc. thanks to the availability of more convenient pest control methods, gradually lowered cycle of necessary insect eradication and rise of environmental protection awareness. Now even historical site preserving parties evolved from pest chemical treatment to Sentricon Method, an ecological approach.

Should the preservation of biodeterioration of cultural properties in Taiwan be well implemented by incorporating the promotion programs in the national agencies, the R&D results achieved by the research institutions and especially the establishment of artifact management systems, the loss and threatening from the biodeterioration of cultural properties will be scaled down significantly.

Forewords

Most of cultural properties and artifacts turn vulnerable while kept under some advert environmental conditions that invite biodeterioration, typically sultry and relatively high in humidity. Cultural properties and artifacts, especially organic ones, such as those of paper, wood, bamboo, bark, fur or horn, etc. are readily ruined in any of the biodeterioration inviting environments. Taiwan, as mentioned in the present study, denotes the offshore island and adjacent islets lying from Eastern Asia continent, with an area 35,759.5Km and Central Mountain running from north to south in the main island. Geographically Taiwan is located on subtropical climate area, marine; rainy season during southwest monsoon, cloudiness persistent and extensive all year and the annual average temperature reads 23_ ranging between 17_and 32_ and the average relative humidity reads 78%. In general, most of the important cultural properties and artifacts in Taiwan are preserved in museums, libraries, archives and protected historical sites, while the rest in private collectors' facilities. The present study, based on the grounds mentioned above, tries to identify the current status and problems of biodeterioration in museums, archives, libraries and protected historical sites and the implementation of preservation at first, and then try to explore the perspective of preservation of cultural properties in Taiwan.

1. Museums:

Currently Taiwan has a museum community consisting of more than 200 museums. To the report of the recent official survey (1996 by Dr. K.N. Chen, under authorization of Council for Cultural Planning &

Development, Executive Yuan) Taiwan had about 170 museums composed of 7 national museums and other medium and small museums in different categories.

In the aforesaid survey by Chen, the questionnaire survey on the current status of collections management in museums in Taiwan revealed the follows, with the 100 returned answer sheets: among all of the museums answering the questionnaire 16% reported performing regularly the measures covering: pest control, disinfections and cleaning, 40% performing randomly the same, and 44% never performing any of the same. However, the results reveal nothing clear about the specific problematic points in biodeterioration in the museums or the measures for preservation or prevention.

Later in 1998, a survey on the current status of collections management in museums and similar institutions in Taiwan was implemented jointly by Department of Textile and Clothing, Fu-Jen Catholic University, National Palace Museum, etc. under the authorization of Project Planning Office National Center for Research and Preservation of Cultural Properties. In this survey both questionnaire and interview were used to better reflect the reality of preservation of cultural properties in the 33 artifacts preservation institutions. The researchers paid visit and made interview at the sites in person. The 33 institutions preserving essential artifacts in Taiwan consists of only one library (National Library) while thirty-two museums in contrast. The 32 institutions fall to into several groups and subgroups – 4 private museums and 28 public museums including 9 national museums and 16 local cultural centers, etc.

The latter survey gave an access to better understand the profile of problems in biodeterioration in museums in Taiwan. The survey identified the responsible pests - cockroaches, cigarette beetle(*Lasioderma serricorne*), booklouse, silverfish, powder post beetles(*Lyctus brunneus*), clothes moths, termites, and carpet beetles and collected the necessary specimens. Certainly there were still some pests remain uncertain and wanting further research to identify. The results identified some live pests, such as cigarette beetles, booklouses, powder post beetles, while identified the remainders such as carpet beetles, clothes moths and termites with the fragments or debris of their body, shell, bitten chips and frass. For better figuring out the biodeterioration, Table 1 serves as a quick reference.

Table 1. Pests Affecting Artifact and Target Artifacts Found in The Survey in The Project of *The Artifact Preservation Status in Taiwan and Collection of The Pertinent Data from Overseas & Domestic Sources*

PEST CATEGORY	**TARGET ARTIFACT SUBJECT TO AFFECTION**
Cockroach	Clothes
Cigarette	Musk, Mongolian boots
Booklouse	Deer horn
Silverfish	Clothes, paper, books
Powder post beetle	Wooden, bamboo items and barks
Clothes moth	Antelope horn
Termite	Woodenware
Carpet beetle	Turtle tongue, tiger hide

As for fungus affections, only 3 institutions, all of them were local cultural centers, reported the biodeterioration resulted from fungus. One saw fungus attacks to the surface of leather made artifacts due to its location in the basement inviting rather high humidity. Another reported fungus stain over watercolor paintings shielded under a clear acrylic sheet, resulted from lack of air-conditioning from power failure

because its location on top floor subjected to severe sunlight exposure in summer. The last one reported fungus grown on the wall surface in the exhibition rooms due to its location right above a water tank in the building.

The surveys mentioned above reveal that chances of biodeterioration reduces significantly for an institution who maintains adequate air-conditioning or implement artifact management and preservation measures on day-to-day basis.

With the exception of the institutions mentioned above, a majority of the artifact preserving institutions in Taiwan overlooks the fact that there is no permanent officer specifically authorized to deal with artifacts preservation and general clerks are rarely aware of even the existence of pest affections not mentioning about how to resolve it. Instead, any pest problem once found is normally dealt with by outsourcing the pest control companies. Fumigation was one of the more popular disposals a pest control company takes. Chemicals, such as methyl bromide or mixtures of methyl bromide and ethylene oxide were once commonly used as routines by pest control companies. Further, most of the preserving institutions were not equipped with any fumigation chamber as a result no adequate detoxification process was ever taken. Nowadays quite a number of the museums in Taiwan conduct spraying with Bunganon, a cyphenothrin aerosol containing mainly pyrethroid, of Japanese manufacture. Besides, RP-K(RP-K, oxygen scavenger), a product by Mitsubishi Chemical, Japan, is also widely used in pest control and preservation of artifacts including those made of textiles, leather, feather, bamboo, wood, and ancient books, contract forms, photos and maps, etc. Still other museums normally adopt a pest control strategy of using repellents, for examples placing natural or synthetic camphor, naphthalene or para-dichlorobenzene next to the artifact to preserve.

Currently there are totally three museums in Taiwan equipped with fumigation chamber and they are: National Palace Museum, National Taiwan Museum of Art, and Kaohsiung Museum of Fine Arts – happened to be located strategically in North, Central and South parts of Taiwan respectively. The three fumigation chambers come in three different capacities - $27m^3$_$29.4m^3$_$29m^3$, and of ambient pressure model. The fumigation chamber in National Palace Museum, built in 1992, operates fumigation 7 times per year in average, mainly for fumigating the artifacts from donation source or materials for exhibitions or storage, and the reagent used is EKIBON of Japanese manufacture. The fumigation chamber in National Taiwan Museum of Art, built in 1988, operates fumigation twice per year in average, mainly for fumigating the artifacts from donation source, and the reagent used is EKIBON of Japanese manufacture. The fumigation chamber in Kaohsiung Museum of Fine Arts , built in 1994, has operated fumigation 4 times between 1994 and 1998, mainly for fumigating the artifacts from donation source or collections of wooden sculpture and oil painting, and the reagent used is EKIBON of Japanese manufacture.

As so far, Freezing Pest Eradication has been adopted only in National Palace Museum, National Museum of Natural Science, and Textile Museum, Fu-Jen Catholic University, here in Taiwan. Further, Portable Nitrogen Pest Eradication Equipment is used merely in National Palace Museum all alone.

2. Libraries:

Taiwan boasts 5079 libraries nationwide, including 1 national library and 300 public libraries.

In general, an average library carries a large collection of books however confines its attention merely to management of rare and weighty historical books. In 1990, Hung Wang C.W. launched a survey, through questionnaires and telephone interview, on *Pest Control and Prevention for Collections of Treasured Books and Archives in Taiwan* and eight libraries responded to the survey. The survey results reveal that among all pests, cockroaches, booklouses and silverfish are responsible for major damage, and silverfish

deterioration is especially severe. However, to the impression of the writer on the vast contacts with librarians in Taiwan, a great number of books and artifacts in collection are severely damaged or threatened by cigarette beetles, drugstore beetles, etc., and they are not mentioned in the survey report. Probably the librarians are not familiar with these insects and thus overlooked and resulting no report about their attacks.

The few libraries with fumigation chambers in Taiwan are: Academica Sinica, Fu Sze-Nien Memorial Library of Historical Language Research Institute, and National Library. The latter is equipped with a Hypo-pressure Fumigation Chamber, about $8m^3$ in volume, and another room for fumigation, while Fumigation Chamber is still more frequently used taking Phostoxin as agent. In practice, books donated normally want fumigation, in average twice per year. FSN Memorial Library adopts a Hypo-pressure Fumigation Chamber, $8m^3$ in volume, and normally fumigates with EKIBON in average once per month, targeting at newly acquired or borrowed books.

A rather severe damage to a rather large number of books kept in the storages of libraries happened miserably to those storages located in the basement upon the attack of 917 (Sept. 17) Nali Typhoon bringing shower storms and causing flood. Those books soaked in flood turn swollen, stuck together, and especially invaded by fungus, and turn out to be scrapped without any remedial way available.

3. Archives :

A majority of institutions and organizations has an archives/collection conservator, however, implementing a biodeterioration survey is rarely the case. In fact reports on biodeterioration surveys have been presented only by several essential preservation organizations.

Academica Historica ranking at the top of all Archives in Taiwan is equipped with two fumigation chambers, one is a $42m^3$ volume Ambient Pressure Fumigation Chamber while another a $8m^3$ volume Hypo-Pressure Fumigation Chamber, and both started operation in 1993. In average they operate 5 – 10 times per year and the batch is about 3 or 4 thousand copies of files or achieves. EKIBON was used alone before 1999 but now Bunganon has a place, too. Another key important archive, Committee of Taiwan Heritage, performs pest eradication by spraying Bunganon directly in the site. Even the new arrivals are subjected to the same treatment. The conservatory in Ministry of Foreign Affairs currently maintains pest control over the 606 pieces of historical territory maps and atlas made in Ching Dynasty, with RP-K low oxygen atmosphere treatment.

In addition, Military History &Translation Bureau, Ministry of National Defense, has contracted Institute of Nuclear Research to perform pest control and eradication with Co60-γ irradiation at 1KGy to 20,000 copies of archives.

4. Historic Monuments and Sites:

Historical monuments and sites are assessed and fall into Class 1, Class 2 and Class 3 accordingly immediately after the enactment of The Cultural Properties Conservation Law in 1982. As of date totally 450 historical monuments and sites have been assessed and designated and they consist of Class 1 – 24 sites, Class 2 - 47 sites and Class 3 – about 220 sites, while the remainders are of either of national, municipal or county assessed sites.

Among all historic sites in Taiwan, one of the most severe biodeterioration problems falls in wood structured ones attacked by termites. The historic sites used to be maintained independently by their owners before the enactment of The Cultural Properties Conservation Law. While after that, the national

authority authorizes the maintaining or restoration projects to the contracted construction firms, and, normally the biodeterioration treatments are further subcontracted to the pest control companies. The results turn out to be far from satisfaction as many of the restored historic sites are still reported as victim of termite invasion.

According to the results of a long-range survey on 13 historic sites located in the north, central and south regions of Taiwan worked out by J. W. Wu in 1990, the terminator of life of wood-structured historic monuments or sites in Taiwan proves to be biodeterioration and among all responsible pests termites destroy or endanger them severely. The commonly seen affecting termites in Taiwan are *Odontermes Formosanus Shiraki, Reticulitermes flaviceps, and Coptotermes formosanus Shiraki and the last group has caused the major damage.*

In 2000, Y. L. Lin, a research, conducted biodeterioration problem survey over 5 historical architectures, namely: Lung Shan Temple in Lukang (Class 1 HS), Lin's Residential Garden (Class 2 HS), The Taipei Confucius Temple (Class 3 HS), Taiwan Folk Arts Museum at Peitou (Municipal site) and The Red Hall in Chien Kuo Senior High School, Taipei (Municipal site). The results demonstrate that in term of severity termite ranks at top, brown rot second, soft rot, podwer post beetle , *Cerambycidae* and plant follow in a descending order.

Conventionally Taiwan has preserved wood-based objects with CCA treatment for 60 years, as an answer to pest and microbe affection. Therefore, when coming across the necessity of replacing deteriorated wooden elements of the wood structured- historic sites, the wood materials for making replacement elements is normally treated with CCA first, next followed with sawing, plane machining, etc. Occasionally the reagent debris is eliminated. In view of the pollution and threatening to health safety that CCA derives, there are some criteria established in CNS3000 against the use and limitation of various wood preserving agents. Furthermore, the researchers and experts in the field just begin reviewing and contemplating the possibility of replacing CCA with ACQ, CuCz, BAAC and AZP.

The commonly seen conventional treatment to termites in Taiwan was either pouring or spraying the chemical agents to the target objects in the past. It was not until 1999 Sentricon was introduced into Taiwan and Tsushih Temple in Sanhsia, is the first site uses it. It is in only 7 months from the first application of Sentricon the ground termite invasion was under control effectively. Currently Sentricon is also used in The Taipei Confucius Temple to address the termite problem.

5. Training and Education:

5.1 Conference and workshop:

Every conference and workshop on cultural properties preservation receives stunning response – overbooking of reservation for participation into the event due to the shortage of biodeterioration professionals and very limit sources for the related information or literature. Among all participants most are serving or working in museum or historic sites. As a matter of fact, these activities have made considerable influence to the cultural properties biodeterioration preventive efforts.

Listed below are the major conferences and seminars held lately:

i. *1998 Conference on Cultural Properties Preservation and Fumigation Equipment* hosted by Taiwan Provincial Museum of Fine Arts (currently known as National Museum of Fine Arts of Taiwan) having made a certain positive influence to the installation of fumigation chamber in various cultural properties preservation institutions.

ii. *A New Approach to Maintain and Preserve Cultural Properties* 1994 hosted by The Cultural Properties Preservation Society, giving presentation on how to use Bunganon effectively. Soon Bunganon wins its popularity in Taiwan.
iii. *Inventory and Preservation of Cultural Properties* 1997, hosted by Hwa-Kang Museum, and on the event a guest speaker, Vinod Daniel of Australian Museum gave the lecture on Museum Pest Control and led the workshops.
iv. *Conference on Biodeterioration Strategies for Preservation of Folks Custom Cultural Properties and Historic Monuments or Sites* 1997, hosted by The Microbiology Society of Taiwan, ROC, boasting 15 papers presented and the first symposium incorporating talents and scholars from both museum and historic monument professions.
v. *On Prevention and Control Over Wood-based Materials Biodeterioration* 1997, hosted by Historic Monuments Preservation Committee, boasting the first conference focusing on biodeterioration by the historic sites professionals.
vi. *Conference of termite treatment on Historical Site and Wooden Structure* 2001, hosted The Administration Committee of The Taipei Confucius Temple, focusing on disclosing termite problems in Taiwan. Besides, Dr. N. Y. Su also demonstrates in person the research grounds of the applications of Sentricon in termites control and eradication.

5.2 School:

Currently the only programs on cultural properties preservation at higher education level available in Taiwan are Graduate Institute of Conservation of Cultural Relics, Tainan National College of The Arts, and The Graduate Program, Department of Cultural Conservation, National Yun-Lin University of Science & Technology and both were established in 1999. Only the former offers the course concentration on *Biodeterioration Preventive Dispositions*, while, other schools such as Grad. School of Arts & Aesthetics, Nan-Hwa University, Dept. of History, Suchoo University, and Dept. of Forestry, National Taiwan University put efforts on promoting the knowledge and skills of biodeterioration prevention by offering speeches or lectures by invited guest speakers.

As of the date there is no Dept. or Program of Archive available in Taiwan, as a result, the personnel in the field find it difficult to obtain any sources of the professional knowledge. Taiwan does have Library Science Dept. or Programs however offering no courses dealing with cultural properties preservation or biodeterioration strategies. It is very likely that the shortage of the teaching faculty in this specific domain contributes to the impracticability of offering the pertinent courses.

As for historic site courses, the programs dealing with restoration, repairing or maintenance of historic sites and monuments are available in colleges such as Shu-Te Technological University, Chung-Yuan Catholic University, National Cheng-Kung University and China Institute of Technology, normally in Dept. of Architecture. In addition, lecture on the related subject matters is available at Dept. of Forestry, National Taiwan University, National Chung-Hsing University, and Yi-Lan Institute of Technology, however the specific courses concentrating on biodeterioration remains unavailable.

6. Discussion:

Based on the surveys mentioned above concerning the biodeterioration ranging from the conventional pest control methods for preventing pest attacks to the cultural properties maintained in the past among private collectors, to the Integrated Pest Management implemented in today's agencies and institutions for cultural properties preservation, it is safe to conclude survey results as the follows:

(1) The pests responsible for damaging cultural properties found in Taiwan are basically the same as those found in other countries located in tropical or sub-tropical area. Being closely adjacent to the Chinese continent and southern-east Asia, Taiwan is accessible for the insects and pests from these areas. The affection to the cultural properties by the pests from these sources has been found, for instance *Falsogastrallus sauteri and Anobium punctatumn*, however if they are acclimatized remain beyond verification. A comparatively serious problem is that Bostrychidae borer have been frequently found in the wood materials used in the exhibition site or storage room. Chances are the Bostrychidae borer is found only after the wood materials are mounted and installed and there is nothing much one can do to the Bostrychidae borer. Freezing and Nitrogen Methods are normally recommended to general pests under ordinary conditions. However, the wood elements used in decoration in exhibition site are bulky in dimension and large in quantity, hence they require larger scale equipment for freezing or nitrogen gas methods. Taking National Palace Museum as example, fumigation chamber operation is started largely for decorating materials, since 3 cases were reported for the finding of Bostrychidae borer in the wood elements without being treated for pest control beforehand. Besides, major contamination by the Bostrychidae borer in the decorating materials has been found in the museum's in-house investigation.

(2) Total inhibition of using Methyl Bromide in 2005 in developed countries is already a resolution made at the 9th meeting of countries entering the Montreal Treaty. EPA of Taiwan, ROC, enacted restriction on Methyl Bromide import in 1995 and the import quotas for methyl bromide *Used in Non-quarantine and Pre-delivery Treatment* will be entirely cancelled by 2010, so it is obvious that the use any Methyl Bromide will definitely restricted in the coming future. As so far, no users of the same, with fumigation chamber, except Academica Historica, has ever taken any substituting program yet. Academica Historica has replaced Bunganon for Ekibon in fumigation. However, the effectiveness remains unevaluated. Should any method be used to replace the existing EKIBON fumigation process in the future, it is a must to find out an agent or method that makes fungus growth control possible.

(3) With the exception of the experienced researchers serving in Conservation Division, National Palace Museum who is competent to explore the biodeterioration issues and deal the problems with methods other than chemical treatments, most personnel working in cultural properties preservation are still used to and largely rely on fumigation or spraying chemicals. Furthermore, the pest control/treatment jobs in most of the cultural properties preserving agencies are still actually conducted by contracted pest control companies. This derives a challenge toward the cultural properties preserving officer's awareness and mastery of the biodeterioration prevention technology when choosing a competent pest control company among many.

Not long ago, Conservation Division of National Palace Museum was the only function that conducted repairing and rehabilitation of cultural properties and also the preservation of the same and hence played a predominant role in introduction of new technology in biodeterioration, the theoretical researches and promotions of the concept and techniques in the preservation for deterioration. Currently National Cultural Properties Preservation Research Center is still at its infantry and expect its formal commencement in the next year, therefore, the national professional organization aiming at the research of conservation should

be able to make the ever significant facilitation to the development of researches on the preservation of the cultural properties in Taiwan.

Now National Archive is undergoing the preliminary works for its formation and is trying to authorize the competent cultural properties management personnel in museums and libraries to formulate the related criteria and procedures for the administering and preserving the national archives. It is believed that the biodeterioration status of the archives preserved should be benefited from taking the tremendous experiences of the museums and libraries as reference and thus making proper development.

In addition to the surveys mentioned above, Taiwan also sees an increasing emphasis on the theoretical researches on biodeterioration, such as in the aspect of research on the fundamentals of biodeterioration phenomena and the aspect of various applications in preservation of the same. All these efforts should be integrated for effective promote the level of entire performance in biodeterioration measures for preservation of the cultural properties. In the respect of education and training, regular direction and consultation should be provided to the concerned personnel in museums, libraries, and archives in addition to encouraging the professional cultivating colleges to furnish the students with adequate disciplines and teaching of biodeterioration and its prevention. Actually it is even necessary to promote the correct recognition and concept of biodeterioration among the affection media preventing personnel. After all, dealing with cultural properties biodeterioration is indeed different from dealing with pests in general environment sanitation. A further extended consideration is that while private collections is large in quantity their preservation conditions can be far behind those of large scale collection organizations and thus in thirst of correct preservation concepts and knowledge to cope with the unavoidable biodeterioration.

At last but not the least, it is National Palace Museum, the only museum that has a technology function and a group of personnel with expertise to pay attention on how to address the issues in biodeterioration. While other cultural properties preserving organizations may not be necessary to furnish themselves with a similar preserving function, they still in desperate need of an exclusive authorized officer to take charge and the officer should be competent in biodeterioration prevention hold adequate awareness and concepts. Furthermore, the success in biodeterioration prevention for cultural properties relies largely on the management in the function.

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GASEOUS FUMIGANTS - LIMITED CHOICE OF MOLECULES

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Summary:

The need for uniform dispersion, penetration into solid materials and finally dissipation limits the choice of fumigants to predominantly volatile & low molecular weight molecules. There are only a small number of gaseous chemicals, which have the required volatility. Some candidate volatile chemicals have been eliminated because of unfavourable properties (corrosive, reactive, produce unpleasant odours, physiological active) however flammability or toxicity to humans is not usually a cause for exclusion. A practical requirement and barrier is the need for fumigants to have the toxicological & efficacy packages required for registration by statutory authorities.

Fumigation:

An encompassing definition of fumigation is the controlling or elimination of undesirable organisms, which include insects, rodents, mites, birds, microorganisms, plants and seeds. It is necessary to contain the fumigant gas while it acts on the target organism and restrict escape into areas which may be dangerous to human health.

Global fumigation legislation and regulations restrict the use of scheduled fumigant chemicals to licensed personnel. The fumigation legislation usually specifies a list of scheduled fumigant chemicals and these typically include methyl bromide, hydrogen cyanide, chloropicrin, ethylene oxide and phosphine. Choice of fumigant chemical can vary with geography because of regional preferences.

The principal of fumigation is simply defined as maintaining a **C**oncentration of fumigant in an enclosure for a minimum exposure **t**ime i.e. a product of **C**oncentration x **t**ime [**Ct**]. Concentration needs to be measured as a minimum or average fumigant level and is given in g/m^3 (mg/L) or ppm. Time is the exposure time at the minimum or average level. In practice the recommended concentration is detailed on the registered pesticide label fixed to the product container (Authorities can issue special use permits e.g. Quarantine treatments or interim approval for treatment of tolerant insects in critical uses).

Fumigants:

A fumigant is a chemical which can exist in the gaseous state in sufficient concentration to be lethal to a given pest organism. Fumigants are gases or very volatile liquids because of the requirement to permeate solids to kill internal pests. There are only a small finite number of gaseous chemicals, which have the required volatility. Some candidate volatile chemicals have been eliminated because of unfavourable properties (corrosive, reactive, high sorption, form unacceptable residues, produce unpleasant odours, physiological active) however flammability or toxicity of humans is not usually a cause for exclusion. A practical requirement and barrier is the need for fumigants to be registered by statutory authorities e.g. EPA [USA], NRA [Australia].

Existing fumigant include methyl bromide [CH_3Br], phosphine [PH_3], sulfuryl fluoride [SO_2F_2], carbon dioxide [CO_2], nitrogen [N_2], carbon disulphide [CS_2], chloropicrin [$CCl_3N\,O_2$] , dichlorvos [CCl_2=CHO.PO.$(OCH_3)_2$], ethylene oxide [$(CH_2)_2O$], hydrogen cyanide [HCN]. A potential new fumigants is

carbonyl sulphide [COS]. The listing of methyl bromide under the Montreal Protocol as an ozone-depleting chemical will ensure its demise (Year 2005 in developed & 2015 in developing countries). Existing alternatives to methyl bromide continue to be evaluated and include phosphine & sulfuryl fluoride. Use extension of the volatile ethyl formate [$C_3H_6O_2$] from a lavacide used in packaged food & sultanas to fumigation of stored product insects is an interesting development with additional potential applications.

Individual preference or choice of fumigant depends on a number of factors including:

- **market requirements** [are now being re-evaluated with the projected demise of methyl bromide. Quarantine is exempt however Quarantine Pre-Shipment (QPS) is now under scrutiny].
- **time availability** [is crucial because of commercial operational considerations and can be manipulated by forced distribution & ventilation].
- **commodity being fumigated** [may sorb high levels of the fumigant which influences choice as does phytoxicity with plants and germination with seeds].
- **cost and ease of fumigation** [is particularly important in large scale fumigation].
- **reaction with non-target material** [eliminates or puts restrictions on the fumigant in question; includes reaction of phosphine with copper & electronics; methyl bromide with natural rubber / sulfur compounds / aluminium].

The listing of methyl bromide in the Montreal Protocol with phase out of 2005 in developed countries has resulted in increased activity to find methyl bromide alternatives.

Fumigants have been used worldwide for many years. To be effective, fumigation should be carried out in gastight storage. The standard of gastightness is consistent with a decay of an excess external pressure from 500 Pa (~50mm water column) to 250 Pa in not less than 5 minutes in filled storage (SCA Technical Report 1980). Because of their volatility, fumigants dissipate from foodstuffs upon exposure to the open air with minimal chemical residues. However, after ventilation there is no ongoing protection from insect re-infestation.

Cereal grain accounts for the major use of fumigants and the dominant fumigants are methyl bromide & phosphine (generated from solid phosphide which reacts with atmospheric moisture or liquefied gas). The liquefied gas mixture, $ECO_2FUME^{®}$ (patented 2 wt% PH_3 in liquid carbon dioxide) is a non-flammable gaseous phosphine fumigant developed by BOC (Ryan & Latif, 1989) to eliminate phosphine's flammability hazard [LEL=1.6%]. $ECO_2FUME^{®}$ has been patented (British Patent 2177004, US Patent 4.889,708) and is globally marketed by CYTEC Industries Inc. with installations in Australia, New Zealand, China, Cyprus, Thailand, Indonesia, Vietnam, Bahrain, Qatar, USA and Canada. $ECO_2FUME^{®}$ will treat in excess of 12 million tonne of grain in 2001. While simple to use in sealed storage $ECO_2FUME^{®}$ is unique in being used in the SIROFLO® flow-through fumigation technique which maintains an effective low concentrations (~70 ppm) in leaky storage for the long exposure periods required (SIROFLO® is patented by the CSIRO Division of Entomology).

In Australia the quality standard for stored grain has undergone a quantum leap from the days when insects were an accepted component of stored grain. Then the only issue was whether or not the insects were “visible” [~2000 insects/tonne: Rees,1998] or if significant losses from heating or mould occurred.

The market demand for insect-free status for export grain was initially achieved using liquid insecticide ["grain protectant"] sprays, now a declining treatment because of concerns about pesticide residues contamination. There is a need to control insects in grain and foodstuffs to prevent food losses and to satisfy marketing requirements. The additional market requirement for nil pesticide residues is leading to the total replacement of liquid grain protectants by fumigants. Controlled atmospheres using nitrogen and carbon dioxide are used in specialised fumigation of "organic" grain and are potential reserve fumigants.

Pesticide residues are not an issue with gaseous phosphine [PH_3] fumigation as aeration of the grain effectively removes all traces (less than 0.001ppm) of the fumigant.

Elimination of its flammability hazard (lower explosion limit in air of 1.6 %) makes phosphine an ideal environmentally friendly fumigant. PH_3 is the most effective fumigant being some fifty (50) times more toxic to insects than methyl bromide. Phosphine is a naturally occurring gas, albeit short lived because it reacts with atmospheric air forming phosphoric acid, an acid used extensively as a food additive.

Pseudo Fumigants:

Application such as space sprays: ULV; Fog Generators etc. are sometime referred to as "fumigation" however these formulation and their active ingredient only kill external exposed insects and only achieve the surface treatment of commodities. They can be reasonably effective if used regularly as they can interrupts insect life stages. The liquid CO_2 solvent-propellant formulations [Envirosols®: Ryan et al, 1978] are such a products and they have specialised application in Quarantine etc. The small droplet size (2-20µ) of the pesticide particles formed results in the active constituent being suspended in the spaced sprayed for over two hours (Slatter et al, 1981).

BOC Fumigants

As an industrial gas company it was predictable that BOC initial fumigant involvement was using nitrogen [Ryan et al, 1994] and carbon dioxide. Nitrogen is extracted from ambient air and carbon dioxide is recovered from by-product petrochemical and fermentation industries process streams. BOC developed ECO_2FUME (2 wt% phosphine in liquid carbon dioxide) now marketed globally by CYTEC Industries Inc. The BOC fumigant portfolio includes Agrigas M (100% methyl bromide); Agrigas MC (2 wt% Chloropicrin; 98 wt% methyl bromide); Fumigas 900 (90 wt% ethylene oxide, 10 wt% carbon dioxide); Fumigas Non Flammable (9 wt% ethylene oxide; 91 wt% carbon dioxide) and the oldest fumigant known to man sulfur dioxide. An approximate comparison of concentration and exposure time for the above fumigants is given in the table below:

Fumigant Gas / Product	Typical Concentration	Exposure Time
Nitrogen	99% min.[O_2 < 1%]	15 days
Carbon Dioxide	35% min.	15 days
ECO_2FUME [2% PH_3/CO_2]	0.04% a.c.	7 days
Agrigas M [100% CH_3Br]	0.60% a.c.	1 day
Agrigas MC [98% CH_3Br]	0.60% a.c.	1 day
Fumigas 900 [90% C_2H_4O]	0.50% a.c.	1 day
Fumigas NonFlammable [9%	0.50% a.c.	1 day

C_2H_4O]		
Sulfur Dioxide	0.50%	1 hour

It should be noted that ethylene oxide (EtO) is not recommended for foodstuff because of concerns of potentially carcinogenic chemical residues formed such as chlorohydrins. There could however, be application in non-food commodities e.g. timber and soil fumigation. Sulfur dioxide is rapidly sorbed, has deleterious effect on grain and flour and is corrosion to some metals.

Conclusion:

The projected loss of methyl bromide has initiated investigation into former fumigants of choice (e.g. sulfur dioxide; acryonitrile; carbon disulfide; ethylene oxide, hydrocyanic acid) and the development of new gaseous chemicals (e.g. carbonyl sulfide, cyanogen). The realisation of the limited availability of candidate molecules has resulted in the lowering of expectations especially with short exposure times, which was a major benefit of the long established methyl bromide. The reality of long exposure times will also result in increased acceptance of "controlled atmospheres" fumigants e.g. nitrogen & carbon dioxide.

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A New Oxygen Absorber RP-System: Mortality and Use

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It is not always certain to success to avoid an insect problem at museums and libraries. Past 30 years, we have been using many kinds of chemical pesticides to kill insects. And now, the time has come that we recognized of risk of using chemical pesticides at working area, to museum objects, occurring of resistance for pesticide and effect environment and surroundings of museums and also to global environment.

Now many museums are taking good care of pest control at their museums applying the IPM program. And also “low oxygen systems” is well approaching instead of using harmful application of chemical pesticides. Low oxygen system has two ways. One is inert gas system and the other one is using oxygen absorber. New oxygen absorber, which is called the RP System Mitsubishi Gas Chemical Company, Inc., is setting an airtight packing system that makes it possible to prevent oxidation and corrosion of metal objects. The gas barrier bag stops gas penetration through the bag from outside to inside. This RP System creates no moisture, so that it is useful to kill insects in infested wooden and paper objects.

The experiments were carried out the egg, larvae, pupa and adult of Cigarette Beetles, larvae of Black Carpet Beetles, egg, larvae and adult of German Cockroach and American Cockroach. According to high powerful microscope, it was an obvious fact that the shape of egg changed under dosing oxygen absorber. The relationship between lethal time and temperature was shown how the temperature goes high, the lethal time shortening.

The relationship of lethal time and temperature

Under using RP-K System to Museum pests

Pests	Temp.___	Lethal time (day)			
		Egg	larvae	Pupa	Adult
Cigarette Beetles	30	6	4	2	2
	25	8	6	3	3
	20	15	13	5	6
German Cockroach	30	3	3	_	3
	25	4	3	_	4
	20	5.5	6	_	5.5
American Cockroach	30	2	2	_	2
	25	3	3	_	3
	20	6.5	4.5	_	5
Black Carpet Beetles	30	__	1	__	__
	25	__	1	__	__
	20	__	1.5	__	__

	15 10	— —	3 3.5	— —	— —

_ Not supplied : Cockroach is an incomplete metamorphose
(larvae metamorphoses into adult)

___ Not supplied : Carpet Beetles infest only while larvae

What Works For Us? Issues That Effect the Historic Houses Trust of NSW's Choice of Treatment for Pest Control.

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Alex Roach, Heritage Pest Management, Australia.

Introduction

The Historic Houses Trust of NSW manages 13 properties, most of which are historic buildings. Unlike many museums where the bulk of the collection is in storage and only a small percentage is on display, often in display cases, the Trust's collections are mainly on open display (less than 10% is in storage). The objects are therefore exposed to the internal environment of the building and offer an attractive source of food to insects. Materials used in, and methods of building construction, plants close to the buildings, historic fence posts, open windows and doors also encourages the presence of a range of museum pests, making treatment an ongoing necessity.

Conventional pest treatments that have been developed for modern buildings are not always appropriate for historic structures. Even treatment options like low oxygen and nitrogen can be quite a trial when applied to large objects in situ, especially where they are located in small, furnished rooms.

This paper looks at a range of options proposed and trialed by the Historic Houses Trust of NSW for the treatment of museum pests and describes what does (and what doesn't) work for us.

Museum pests

The Trust's collection covers a wide range of objects, including furniture, books, paintings, carpets, wallpapers, curtains and replicas, providing a wide range of materials that can be destroyed by pests. The level of risk from pest attack varies from property to property. For example, the Museum of Sydney is a modern, air-conditioned structure with purpose built storage and prep areas. Conditions within this style of building are much less conducive to pest attack from termite or borer than properties such as Rouse Hill Estate or Meroogal, which have no environmental controls. However it does suffer from bogong moths and hide beetle, due to the proximity of Governor Phillip Tower and the night lights that attract the migrating moths.

When the current pest contractor, Alex Roach[1], first inspected the properties, varying levels of pest problems were noted. Housekeeping, which tended to focus on presentation of the house for the public, did not always address the areas where pests are to be found, or that many pests utilise as egg-laying sites (i.e. wall edges, behind or under furniture, the top of canopies over beds). This problem was dealt with in a number of ways:

- Staff training sessions were conducted at a number of properties covering such topics as cleaning, common museum pests, and recognising signs of insect attack.

[1] Alex Roach was selected as the contractor for pest control for the Trust in 1997.

- More rigorous conservation-cleaning programmes were introduced.
- Programmed 'spring-cleans' were introduced at some properties (eg: to remove the bogon moth bodies at the Museum of Sydney).

Conventional chemical treatments were also unsuitable for many properties. The risk that water-based chemical sprays would 'run' (i.e. drip from one floor to another) and leave stains on the building meant that other suitable chemical formulations had to be used (insecticidal dusts). Insect growth regulators and insecticidal baits are routinely used, rather than more toxic chemicals.

Termite attacks

Having discussed general museum pests, we're going to look in more detail at some specific pest problems.

One property in particular, Rouse Hill estate, has a long history of termite attack. Rouse Hill estate was a working property that had been owned by 7 generations of the one family until 1987 when it became one of the historic houses under the Historic Houses Trust of NSW's management.

Over the years a range of treatments have been applied. It is arguable whether they have been effective or not. Some would say that they were, given the fact that the house and outbuildings are still standing. Given the ongoing level of activity, one could also argue that the treatments have been inadequate. All arguments aside, let's review the treatments in the recent past.

The last owner of the property routinely applied sump oil, arsenic dust, or 'chemical cocktails' to areas where termite activity was detected. It is also believed that the Public Works Department established an organochlorine barrier in the early 1980's. This barrier, if applied properly, should have lasted 20-50 years. Recent activity in the area suggests that this barrier was either not applied, or had been breached.

Given the ongoing problem, the Trust's current pest contractor, Alex Roach, recommended a one-off solution and identified a number of new options for treatment. The options available fell into three categories: chemicals, physical barriers, and baits. The pros and cons of each were discussed and as a result a list of criteria for assessing the suitability of treatments was prepared:

- *Safety* - Visitor and staff safety was of paramount concern. It was also desirable that the selected treatment should only affect termites, and not impact on other animals or plants within the local ecosystem. Chemicals available for treatments included several organophosphates (moderate to high toxicity), synthetic pyrethroids (low to moderate toxicity), and a new chemical developed by Bayer, Amidachloropid (low toxicity).

- *Residual nature* - Some chemicals required frequent re-application (eg: Amidachloropid – every two years), while others could provide longer protection (organophosphates – 10+ years).

- *Level of intervention* - The various methods were reviewed for the level of intervention each required. Possible impact on the site varied with each method, from applying chemicals directly to the soil or in trenches dug around structures, to drilling holes in buildings and trees, to lifting structures.

- *Affect on building fabric* – Changes to the building were not desirable (eg: colour change or accelerated deterioration). Some options may have introduced a visual distraction (eg: Termimesh "socks" on fence posts have to protrude several centimetres above ground level).

- *Ability to work with other contractors* – As it was unlikely that no single treatment would be suitable for all parts of the property, it was imperative that the contractors were prepared to work with other companies.

- *Cost* - While not as important a consideration as the above criteria, it needed to be taken into account. It was also important to consider ongoing costs for monitoring, re-applications of barriers, etc.

Using these selection criteria, a number of proposed treatments were reviewed. Comments on the application of each option to Rouse Hill estate are summarised below.

Colony location/destruction

It was proposed that an effort be made to locate the termite nest(s) and destroy them by either chemical or physical means. This would involve the use of a trained termite sniffer dog to try to locate termite entry points, and the test drilling of trees to establish the presence of termite colonies.

Termimesh™

This is a physical stainless-steel barrier. As this material is commonly applied to new buildings, it would be necessary to retrofit this barrier into existing walls (i.e. cut the wall and insert the mesh). This idea was considered to be too invasive for historic building structures, but could possibly have application if installed as a barrier (like an ant cap) for posts and wood in contact with the ground (eg. wood piles).

Termguard™

This is a reticulation system. Retrofitting would involve lifting flagging stones and/or boring a hole underneath the building and inserting a series of hoses alongside each wall. There was a risk of boring through archaeological material. Its advantage is that once in place, the preferred chemical treatment is easily applied via the hoses.

While its use may be limited around the house, it may be a viable option for areas where the ground soil is more accessible (i.e. the Barn or the Slaughterhouse). It may have application under the Arcade flagging, where termite nests have routinely been found. It could also be applied underneath the floors of the School Room where evidence of termite attack is so visible. This would involve raising some of the floorboards, risking disturbance of original fabric.

Premise

The chemical company Bayer presented a new chemical option, Premise, which, unlike other termiticides, is insect specific. The method of application involves a "trench and flood approach", which is not desirable for many areas of Rouse Hill estate due to archaeological sensitivity, impermeable soil across most of the site and resistance to using poisons around the property. It may have application however around posts

where the soil is soft enough to bore a hole for flooding (eg. around the Potting Shed and Chicken Shed), or for flooding post-holes when replacing fence posts.
It is worth considering if a reticulation system is installed (see comments on Termguard).

Termite™

This borate-based chemical treatment is applied directly to wood. While the company that registered the product gives glowing accounts of its many virtues, no evidence of research results were available at the time (1997). Curatorial concern was expressed over potential staining and introduction of salts that could wick up moisture. If further research can show that the long-term effects of this product on historic structures are not damaging, this chemical may have potential application. Until then it is proposed for use on fence posts only.

Sentricon™

This is a sophisticated bait station system. Plastic bait stations containing wooden bait stakes are installed at approximately 3-metre intervals around the building. Bait stations are inspected at regular intervals (monthly or bi-monthly). When termite attack is noted to a bait stake, it is replaced with an insect growth regulator (IGR) impregnated bait. The bait is distributed throughout the colony and the termites are eliminated. One of the disadvantages of this system is that it is slow to control termites (several months to eliminate termites). Another is that, as far as historic interpretation is concerned, the green caps of the bait stations are visually distracting.

Given all the above considerations, it was decided to:

1. Drill all trees and fence posts that were identified as having termite activity and apply termiticide emulsion into the holes.
2. Trial the Sentricon system around the house.

The Sentricon system has been in place since - October 2000. Termite 'hits' (signs of termite activity) are still being found to some bait stakes and baiting is ongoing. The system can take 12-18 months before termite activity is controlled. Its use at Rouse Hill will be reviewed at the end of 2001. There are now a number of alternative bait systems available that use growth regulators and some of these are being trialed at other Trust properties.

Low oxygen/nitrogen treatment

In this section we're going to look at the evolution of a technique for bagging large objects for low oxygen treatment.

In November 1998 the Trust had its first experience of using low oxygen/nitrogen for treatment of active borer *in situ*. The object in question was a piano at Meroogal, a Trust property in Nowra. Having identified fresh borer activity, we first considered moving the piano to Sydney for treatment in the Powerhouse Museums' nitrogen chamber. However, having weighed the risk of damage from handling/transport and listening to some persuasive arguments (by my enthusiastic colleague, Alex Roach), we opted for low oxygen treatment *in situ.*

The exercise highlighted a few problems:

1. We were rather limited in availability of a large clean floor space for laying out the 4 lengths of Cryovac needed for making the bag. (The bag size was 4 x 5 metres)
2. Moving a 4 x 5m plastic bag around a small house without bending it and without knocking over other objects is not easy.
3. Getting a piano into a large plastic bag is nerve-wracking.
4. Moving a piano in a plastic bag back to its original location is a challenge!

Having sworn never to repeat this exercise, we found ourselves doing just that in April 1999. This time it was an escritoire at Lyndhurst. My first instinct was to put it in the Powerhouse's nitrogen chamber but I was thwarted this time by the size of the object, which at 1.82m was slightly too high for the 1.8 m high chamber. As there was no escape, we proceeded to bag the object on April 22.

This time, in order to reduce the stress caused by trying to manoeuvre a large heavy objects into a bag, we decided to try constructing the bag around the object. To do this we joined the sheets of Cryovac together, then put the large sheet in the final resting place for the object, moved the escritoire onto the sheet, wrapped the sheet over, and did the final join with the object in place.

Imagine my reaction when, having done the welding of the sheets and commenced moving the object, we discovered that the escritoire did in fact come apart and would have fitted in the Powerhouse chamber when disassembled! However, this time the experience was a lot less stressful and the high visibility of the bag (in place for several months due to the cooler temperature range) was a useful tool to advertise the new approach to pest control.

Having fine-tuned the process to a large extent, I <u>almost</u> looked forward to our third experience, another piano, this time in a small sitting room on the second floor of Vaucluse House. Like the piano before, the risk of damage from moving the object to a chamber was considered too high. The curator of the property was keen to keep the object in its location (not lose it for 4-6 weeks) and to promote the non-toxic approach to pest treatment in the guided tours. We were feeling so confident that this time we opted to perform the operation without the able assistance of Alex. As with the escritoire, we opted to assemble the bag to a stage where it could be put in place, the piano moved onto it, then wrapped and sealed.

The first problem we experienced was the need for careful calculation of the size of the bag (we had to make a second, larger one). The other problems related to the logistics of trying to make and move a large bag in a small space that as crowded with other objects, much like with the first piano. The excess plastic at the sides also intruded into the room space, which is an issue in an historic interior space.

Needless to stay that while I like a challenge, the mere mention of low oxygen treatment of a large object makes me shiver and I'll try anything to avoid it. Because of the difficulty of moving large objects around and the fact that most of our collections are on permanent display, the Trust will continue to rely on *in situ* treatment. I believe that much of the stress could be removed by the development of a portable structure that could be neatly and easily assembled around the object and welcome any suggestions from the audience.

Alex has however since modified the procedure with a project that he has recently completed for the National Trust.

Heat treatment

Our third and final topic for consideration is that of controlled heat treatment for treatment of borer infested buildings. Going back to Rouse Hill, we have identified active borer in a number of buildings, including the Cottage and Stables, and to a car housed in the Stables. The discussion of heat treatment resulted from my adamant refusal to have anything to do with bagging the car for low oxygen treatment. Despite assurances that it would be easy to move the car into/onto a bag, my recent experience with the Vaucluse House piano suggested that this procedure needed serious modification before I'd consider it acceptable for another large object. In defending my seemingly irrational stance, I won some support for my cause by pointing out that there was no point in going to all the trouble of treating the car for borer and then putting it back in the same borer-infested area.

This led to a review of suitable methods for treating entire buildings. On reviewing the current literature, there seemed to be only two options available for treatment: chemical fumigants (i.e. methyl bromide) or heat treatment. While the costs were similar, comparison of the two options in terms of safety, level of intervention and affect on the building fabric, together with a preference for avoiding the application of more potentially toxic chemicals at the site, made us lean towards heat treatment.

- *Safety* – In terms of safety we needed to consider staff who live and work on site, animals (cows, geese, ducks, dogs, frogs and migratory birds), plants in the vicinity (including a climbing rose on the cottage) and visitors. Methyl bromide poses serious health risks, and would sterilize the soil around the buildings treated resulting in the death of the historic plants in the area. Heat treatment would not have any lasting affect on the animals or plants in the vicinity.
- *Level of intervention* – There was concern that the weight of the tarpaulin required for methyl bromide treatment (estimated to be approximately 300 kilograms) would be too much for the fragile cottage structure.

- *Affect on building structure* – Methyl bromide is reported to have a deleterious effect on leather and other proteinaceous material, examples of which are found in both borer affected buildings. However, there is little information on the potential long-term effect of heat on original (flaking) paint, aged wallpaper, oilcloth and linoleum.

Conclusion

While the Trust looks to wider museum community for guidance in methods of pest control, we find that some methods that are straightforward for a state museum are fraught with difficulty in our own environment. For the most part, our properties are 100-200 years old, presenting a range of conditions conducive to periodic (or regular) attack from termites, borers and other museum pests.

PRESERVATION OF CULTURAL PROPERTY USING A NON-FLAMMABLE ETHYLENE OXIDE FUMIGANT

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ABSTRACT

Fumigators have used ethylene oxide [EO] for three-quarters of a century. This paper reviews how EO is the most effective pesticide against insects, fungi and other vegetative matter. Its two main hazards – flammability and toxicity – are managed by using non-flammable mixes in controlled environments where good engineering practices and strong industrial hygiene procedures protect those who must perform the fumigation or who must handle the property later However, EO in methyl bromide, the most popular EO fumigant, will be phased out over the next several years; because, methyl bromide can deplete stratospheric ozone. This paper presents the non-flammable EO/fluorocarbon mixes that will replace EO in methyl bromide, summarizes their features and shows how they meet the criteria for good fumigation of cultural property.

One promising new blend for long-term use is EO in hydrofluorocarbons HFC-125 and HFC-227ea. It is a non-flammable, non-ozone depleting mix that inactivates insects, fungi and microorganisms at ambient temperature in the same time that fumigators now use. Testing compatible with property materials, the new mix will cost more than the one it replaces. But, being no more difficult to handle, [perhaps easier to handle, because it is non-flammable under all conditions of fumigation and fumigant handling] the non-flammable EO/HFC mix should not increase total fumigation costs significantly.

Introduction:

Ethylene oxide [EO] has proven to be the most effective fumigant against insects, algae, fungi, and all microorganisms. EO has:

> "..an unparalleled track record of effectiveness against insects as well as microorganisms and also the absence of any significant ill effects on archival as well as museum collections themselves, although it is not entirely unblemished."[1]

> "[EO] fumigation of museum objects is applicable to all types of objects and, when properly conducted, is 100 per cent effective in destroying infestations of insects, insect eggs, and fungi. No other alternative fumigants are as widely applicable and as effective for fumigation of museum objects."[2]

EO fumigation properties were first reported in 1928. Techniques to make the highly flammable EO non-flammable were patented one year later. [See Table 1, Timeline of EO Use in Fumigation.] US museums and libraries began to fumigate on premises with EO in the 1970s. [About 1 in 6 US museums surveyed in 1980 were using non-flammable mixes of EO.]

The concern by both the suppliers of EO, their customers and the government was how to use EO safely. In 1978, it was found to cause cancer in and reproductive harm to laboratory animals. In 1984, the US Occupational Safety and Health Administration published guidelines limiting worker exposure to 1 ppm EO averaged over an 8-hour workday; and, in 1988, they added an excursion limit of 5 ppm (max) EO in any 15 minutes period.

Hospitals and the makers of medical devices had installed equipment to help use EO safely. [They could sterilize only in controlled chambers using processes approved by the US FDA]. But, much fumigation was conducted by untrained workers in uncontrolled spaces – open fields, warehouses, museum and library rooms, airplanes, buses, trains and ships. There was no clear way to ensure that all workers in all these spaces were properly protected. In 1989, major US EO suppliers withdrew their products from uses in uncontrolled spaces and US EO pesticide registrations were changed accordingly.

In other countries where licensed fumigators performed the work, conservators continued to fumigate using EO. And, in the US, a few spice fumigators and cosmetic makers, who use closed chambers, continue to fumigate with EO. US preservation researchers did continue to develop technology for the safe use of EO.

The most common fumigation blend containing EO is one of 14 wt. % EO in methyl bromide; and, the largest supplier of this blend is Ekika Carbon Dioxide Co. Ltd. of Japan, who markets it under the brand name, “Ekibon.” But, methyl bromide is implicated in stratospheric ozone depletion and will be phased out over the next several years, depending on the country in which it is used.

This paper describes new non-flammable mixes that contain EO and presents data to show how they meet the requirement to fumigate cultural properties safely and effectively.

Criteria for a good fumigant of cultural properties.

1. Effectively reduce insect infestation [“Effectively reduce” means: “Use the least gas in the least time to inactivate the most organisms.”]
2. Effectively reduce vegetative matter – fungi, yeast, and mould
3. [Sometimes] Effectively reduce microorganisms
4. Do no harm to the materials or construction of fumigated objects
5. Can be handled safely by fumigators and museum workers using simple equipment and procedures
6. Safe for viewers and future workers who might be exposed to the fumigated object.
7. Complies with environmental rules.
8. Cost effective

Epoxides

Table 2 shows the properties of the two epoxides that have been used as fumigants – ethylene oxide [EO] and propylene oxide [PO]. Both epoxides inactivate organisms by alkylating DNA. And, both are very toxic. Like all other fumigants, both are acutely toxic. Short-term exposure to high concentrations can cause burns, or injure the eyes and lungs. Both are chronic toxins. Long-term exposure to low concentrations can cause adverse health effects. As might be expected from the interaction with DNA,

both are reproductive hazards and both are carcinogenic. IARC [International Agency for Research on Cancer] classifies EO as Group 1, *Carcinogenic to Humans*, and PO as Group 2B, *Possibly Carcinogenic to Humans*.[3] These cancer ratings are based mostly on cellular and animal toxicology studies. IARC also considered several studies of human EO exposure, the largest of which was conducted by US NIOSH [National Institute for Occupational Safety and Health] part of the CDC. NIOSH findings, after studying 18,254 sterilization workers:

" A small increase in leukemia and lymphoma [2 types of cancer]. However, it was unclear if they were related to [EO]. The death rates from all other diseases were within the expected [normal] range. [EO] may be linked to leukemia and lymphoma, but we still don't know for sure. We will continue to study this issue."[4]

Both epoxides are also highly flammable. However, epoxide flammability may be controlled by mixing with flame retardants. EO flammability is easier to suppress in mixtures. It has a higher LFL [Lower Flame Limit]. Approximately 50% more flame retardant is needed to suppress PO.

Given the hazards of epoxides, one may well ask why they have been used for so long as fumigants, especially EO, which has been used for three quarters of a century. Three key reasons are:

Epoxides are very effective at inactivating all organisms that may infest an object. Of the two epoxides, EO by far is the most effective. It deactivates organisms in 1/20th of the time that it takes PO to do so. [See the D-values in Table 2.] And, it has been shown more effective for more organisms in more materials than PO.[4]

1. Non-acidic, non-oxidizing, used at ambient temperatures in the gaseous phase, epoxide fumigants have proven compatible with all materials normally tested to assure maintenance of artifact integrity.

2. The small, non-reactive epoxide molecules penetrate most materials and constructions easily. More than surface fumigation is achieved.

Most fumigators have chosen to use EO. It inactivates pests twenty times faster. It's the smaller molecule, thus it ensures higher penetrability. EO should leave less residue, because it has a lower boiling point. But, as with any potent fumigant, the EO operator must take precautions against toxic hazards to protect workers, visitors and neighbors. Controlled fumigating spaces and strong industrial hygiene practices will protect workers. Allowing time for the EO to off-gas from the fumigated property, while in a controlled space, will help protect those who must handle it at a later time.

EO flammability is most easily controlled when mixed with a flame retardant.

Non-flammable EO mixes – fumigant properties

See Table 3.

Users control EO flammability in one of two ways:[1] Process control in the fumigating chamber [i.e., deep vacuum or inert gases] or [2] fumigant control whereby the EO is blended with an inert flame retardant.

Process controls work; but, they require more expensive explosion-proof equipment and construction, higher risk operating processes, staff skilled in managing fire hazards, and the ability to ship, store and handle flammable fumigants. Also, fumigators may not be able to use deep vacuum, a key process technique to control pure EO flammability, because it can harm cultural property. Non-flammable mixes avoid all these problems. [However, it is necessary to ensure that the flame retardants meet government regulations and are compatible with the materials of cultural properties.]

Potential EO flame retardants are CO_2 and certain fluorocarbons.

Non-flammable EO/CO_2 Fumigants:

CO_2 was the first flame retardant used with EO [in 1929]; but over the years it has been replaced by fluorocarbons. There are several reasons:

A non-flammable mix with CO_2 flame retardant contains relatively little EO; therefore, the fumigator, in order to get the same efficacy as with a fluorocarbon mix, must operate at up to three times the pressure needed for non-flammable fluorocarbon mixes.

The fumigating environment tends to be an acidifying one, which when maintained for 24 to 72 hours, can harm cultural property. To be effective, EO fumigation must be performed at high relative humidity [>33%] [6]. At high RH, water vapor condenses in micro-pores within the property being fumigated. CO_2, in turn, dissolves in water to form carbonic acid with a pH between 4 and 5.

The fumigator must use the entire contents of any fumigant container in a single fumigation. Because the vapor pressure of CO_2 is 40 times that of EO, EO/CO_2 mixes tend to separate on withdrawal from the container. After 60% withdrawal, the remaining mix will be too dilute in EO to be effective. Of equal concern, the initial withdrawn mix will be very rich in EO and more flammable than planned.

Non-flammable EO/Fluorocarbon Fumigants:

Fumigators may use two non-flammable fluorocarbon mixes. They contain high concentrations of EO, are non-acidic and have very little tendency to separate on withdrawal. One, Oxyfume® 2002, contains hydrochlorofluorocarbons [HCFCs]. While in common use, and very effective, this mix has an ozone depletion potential [ODP] of 0.027 – and, under the Montreal Protocol, HCFC will be phased out over the next ten to twenty years, depending on the country. The other mix is one of the new non-flammable mixes that contain hydrofluorocarbons [HFCs]. These materials have zero ODP and are not scheduled for phase-out.

Both mixes contain specific HCFCs or HFCs that are proven excellent flame retardants. They contain enough EO so that they may be used, for normal fumigation at less than one atmosphere pressure. They pose no fire hazard at typical operating conditions, both inside and outside the fumigation area.

Fumigation Performance of EO Mixes

Table 4 compares fumigating properties of four EO fumigant blends:

1. Ekibon® mix, EO/Methyl Bromide, Lower Flammability Limit: 275gms./m^3
2. Nonflammable HFC [NF HFC/EO mix], EO/HFC-125/HFC-227ea.
3. EO/HFC-134a, Lower Flammable Limit: 250gm./m^3
4. Non-flammable HCFC, [Oxyfume® 2002 mix], EO/HCFC-22/ HCFC-124.

Ekika Carbon Dioxide Co. Ltd., of Japan, has performed extensive tests with the non-flammable HFC mix [10.4 weight % EO in HFC-125 and HFC-227ea].
Tests have shown:

- NF HFC/EO mix inactivates insects in 24 hours at 20°C and fumigant concentrations of 200 gms/m^3.
- NF HFC/EO mix inactivates fungi in 24 hours at 20°C and fumigant concentrations of 350 gms/m^3.
- At higher temperatures, concentrations may be reduced; at lower temperatures, either exposure time or concentration must be increased.
- NF HFC/EO mix is compatible with all materials that the Tokyo National Research Institute of Cultural Properties requires be tested.

Ekika offers NF HFC/EO mix as one replacement for Ekibon® fumigant, the gas now used to fumigate cultural properties in Japan. Ekibon contains 14 weight % EO in methyl bromide. But, methyl bromide has a high ODP [0.6] and is scheduled for phase-out over the next several years.

While Ekibon is flammable, its Lower Flame Limit [LFL] is higher than the concentration at which fumigators must use the fumigant. The LFL of Ekibon is 275 gms./m^3. Ekibon is used at 100 gms./m^3. Thus, property may be fumigated below the concentration at which Ekibon will burn.

Less Ekibon is required than is NF HFC/EO mix. First, methyl bromide is a fumigant as well as the EO; hence it enhances the inactivating effect of EO. Second, Ekibon contains 14 weight % EO, 35% more than is contained in NF HFC/EO mix.

Ekika has also formulated a flammable blend of EO in HFC-134a. Containing 14 weight % EO, this blend is more economic than NF HFC/EO mix; but, not as economic as Ekibon. More importantly, it is more flammable than Ekibon. Its LFL of 250 gms/m^3 can be lower than the concentration at which it may be used to fumigate. [200 to 400 gms/m^3]. Unlike Ekibon, it can burn in the fumigation chamber.

CESCO, Korea is investigating the fumigation performance of non-flammable Oxyfume 2002 [EO in HCFCs]. While it is expected to inactivate organisms as effectively as Oxyfume HFC, test results are not yet back on its material compatibility. The HCFC mix costs a little less than does the HFC mix; but, sometime in the next 10 years HCFCs will phase out – and, even today, their use is not permitted in some countries.

NF HFC/EO mix costs more to use than does Ekibon. EO/HFC –134a and EO/HCFCs are intermediate in costs. But, fumigant cost is a small fraction of total fumigating cost or of the value of the objects being fumigated. It is not the driving factor in choice of fumigants.

Table 5 shows how the EO/fluorocarbon mixes and EO/methyl bromide meet the criteria for good fumigation. While there are trade-offs for each choice, the fact remains that EO/methyl bromide will be phased out for environmental reasons; and, the mix that offers the most benefits for the longest term – at a small cost penalty – is the NF HFC/EO mix [EO in HFC 125 and HFC 227ea] that Ekika is now testing.

Oxyfume in the field

As of the writing of this paper, Ekika is testing NF HFC/EO mix in the field. CESCO is conducting further laboratory tests of Oxyfume 2002. Results should be available before the end of the year 2001.

The authors wish to thank the staff of Ekika Carbon Dioxide Co. Ltd. for their support in drafting this paper and for their energetic and knowledgeable development of NF HFC/EO mix fumigant applications.

We also wish to thank the staff of CESCO for their efforts in establishing the information needed to use Oxyfume 2002 fumigant.

NOTES

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2. Canellos, Georgia, *Preliminary Impact Analysis of Ethylene Oxide as a Fumigant in Museums,* Report sponsored by US Environmental Protection Agency, Contract No. 68-01-3965, Mitre Corporation, March, 1980.

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Table 1: Dates in the Use of Ethylene Oxide [EO] Fumigants

YEAR	EVENT
1928	***Union Carbide Corporation reports use as a fumigant***
1929	Non-flammable EO/CO_2 mix patented
1930-1949	Multiple fumigation uses developed: Food, Tobacco, Spices, Warehouses, Trains, Buses

1949	U.S. Army Chemical Corps reports: EO most effective low temperature fumigant: Used as medical device sterilant
1950s	Non-flammable EO/fluorocarbon mixes replace EO/CO_2 mixes. Effective at 1/3rd the operating pressure.
1970s	Use in museums and libraries expanded
1978	Shown to cause cancer in rats
1984-1988	US Occupational Safety and Health Administration [OSHA] promulgates rule to manage EO in workplace.
1989	◆ US EO suppliers withdraw from open space fumigation, where unlicensed fumigators use EO. Too many untrained, unidentifiable users. Inadequate engineering controls. Could not comply with OSHA EO Rule. ◆ No longer used in US museums, libraries, airplanes, trains, buses, warehouses and fields. ◆ EO open space fumigation continues to be used other nations, where fumigation is conducted by professional fumigators with engineering controls.
1995	Preservation Directorate, Library of Congress publishes paper showing how to reduce EO residues on library materials
2000	Ekika Carbon Dioxide Co. Ltd. and Honeywell International develop non-flammable EO-fluorocarbon fumigant to replace EO-methyl bromide.
2001	◆ Ekika and Japanese fumigators field test new fumigant ◆ CESCO [Korea] lab tests a different non-flammable fumigant

Table 2: Epoxides – Properties

PROPERTY	ETHYLENE OXIDE [EO]	PROPYLENE OXIDE [PO]
Molecular Weight	44	58
Chemical Formula	C_2H_4O	C_3H_6O
Boiling Point, at 1 atm, ^{o}C.	10.4	34.2
Vapor Pressure at 20 ^{o}C, atm	1.4	0.6
Liquid Specific Gravity at 20 ^{o}C	0.87	0.83
Lower Flame Limit, vol % in air	2.6	1.7
Upper Flame Limit, vol % in air	100	36.5
Auto Ignition Temperature, ^{o}C	429	465
Liters CO_2 /Needed to Retard Flammability of 1 Liter Epoxide	10.8	15.2
Efficacy [5]: D-value *, min.	7.6	144.0

* D-value = time to inactivate 90% of *Bacillus subtilis, var. niger*, on paper at 38^{o}C, 40 to 80% RH

Table 3: Properties of Non-flammable EO Fumigant Blends

Property	EO IN HCFC 22 AND HCFC 124	EO IN HFC 125 AND HFC 227EA	EO/CO_2
Flame Retardants % by weight	HCFC –22 – 27% HCFC-124 – 63%	HFC –125 – 81.9% HFC-227ea – 7.7%	CO_2 – 91.4%
EO % by weight	10.0 %	10.4%	8.6%
EO, mg/l, at 1 atm, 25°C	445	480	170
20°C Fumigant Tank Pressure, atm.	3.3	8.2	51.0
Needed fumigation pressure at 25°C, 35 gms EO/m^3, mmHg	130	120	340
pH dissolved gas	7	7	4.4
Ozone Depletion Potential per lb.	.027	0.0	0.0

Table 4: EO/Halocarbon Fumigants

PROPERTY	EO IN METHYL BROMIDE	EO IN HFC 125 AND HFC 227EA	EO IN HFC 134A	EO IN HCFC 22 AND HCFC 124
EO, weight %	14.0	10.4	14.0	10.0
ODP	0.5	0.0	0.0	0.027
Lower Flame Limit, LFL, gm/m^3	275	0	250	0
Needed Fumigant, gm/m^3	100	250 to 500	200 to 400	250 to 500
Material Compatibility	Good	Very Good	Very Good	Unknown
Fumigant Cost, estimated % of total fumigating cost	1%	3-6%	2-3%	3-5%

Table 5: Ranking EO Fumigants by Fumigant Criteria

CRITERION	EO IN METHYL BROMIDE	EO IN HFC 125 AND HFC 227EA	EO IN H F C 134A	EO IN HCFC 22 AND HCFC 124
Kills Insects	+++	++	++	++
Kills fungi	+++	++	++	++
No harm to	+	+	--	Under test

property				
Safe handling	0	+	-	+
Safe for future handlers and viewers	0	0	0	0
Environmental Compliance	---	+	+	-
Cost	+++	0	++	+

All fumigants are compared as to whether their properties are good [+] or bad [-].

Novel methods of termite management: application to cultural properties

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Introduction to termites

Termites are hemimetabolous insects that are related to cockroaches. All species are 'social', meaning that they live in family groups that are called 'colonies'. These colonies range in size from dozens to millions of individuals. There are over 3000 described species of termites in the world; the majority of these occur in the tropics and sub-tropics. They are important to conservators of cultural artefacts because their primary food is cellulose, a structural molecule in all plant products, such as leaf litter, grasses and, of course, wood.

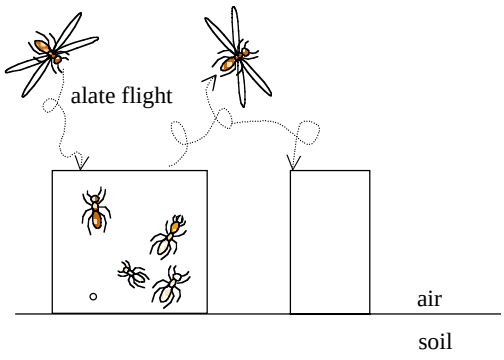
There are many species of grass harvesting termites, distributed primarily in tropical and subtropical areas. Grass-harvesting termites are usually considered to be benign; some species are pests of crops (especially sugar cane), or in some areas of Africa are competitors of cattle and goats for forage. Therefore these species might not normally be considered of importance to conservators. However, pests attack grass and bamboo huts and thatched roofing, which might be of particular concern of conservations of culturally important buildings in Africa and Asia.

Termite species that eat wood are perhaps of the greatest concern to conservators; they can infest wooden artefacts and buildings that are constructed from wood, they can also attack wood based products, such as paper, and so can damage books and other paper based cultural artefacts. The building activities of some species can damage other cultural property, such as rock paintings. Wood eating termites have the widest geographical distribution, from the tropics into temperate regions. They are occasionally found in cool temperate cities, kept alive in buildings during the cold winters by central heating (e.g. *Reticulitermes* in Hamburg, Germany).

Not all wood from all tree species is equally susceptible to termite attack. Sapwood (the younger wood near the bark) is usually more susceptible than heartwood (the old wood in the centre of a tree), and softwoods (coniferous species) are typically more susceptible than hardwoods (the flowering or broad-leaved species). Termites and trees are in an evolutionary arms race between predator and prey, so in general, tree species that evolved in areas with many termites have evolved defences whereas those that did not, have not. Defences may not be effective everywhere, so resistance species may become susceptible once growth or transported to different geographic locations.

Wood will become more susceptible to termite attack after it has been attacked by fungi (i.e. rotted). There are some termite species that only eat rotted wood, the 'dampwood' termites, and others that will attack sound wood. This latter category can be divided into two groups with quite different biology: drywood and subterranean termites. Both of these groups of termites are of great concern to conservators and most attention of this paper has been focused on these termites.

Drywood termites



Drywood termites eat sound, dry wood that is typically isolated from the soil or other sources of moisture; they meet their water requirements largely from the metabolising of their food. This strategy is effective in areas of high atmospheric humidity, consequently most drywood termites are found in coastal areas. The natural habitat of drywood termites is in the dead branches or branch stubs of trees.

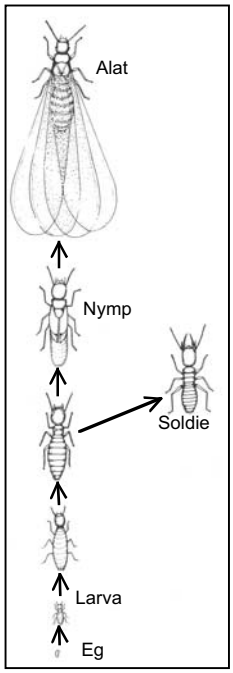
Fig. 1. Schematic of drywood termite nesting behaviour.

Drywood termites are called 'single site nesters' because they are incapable of tunnelling through soil in order to reach another piece of wood and so live their entire lives inside a single piece of wood. Colonization of a piece of timber occurs only by flight, which is dispersal behaviour of the adult winged reproductive caste (alates) (Fig. 1).

Colony size is usually limited by food resource size, so colonies are often small; from several hundred to a few thousand individuals. If the food resource is large, then some alates will not fly when they mature but remain in the natal colony and breed there, or additional alates may colonize from outside. As a result a single large piece of wood or cultural object may contain a number of colonies, with the total population reaching many thousands. Their habitats in urban areas mimic their natural situation. Drywood termites can infest, for example, roofing timber, furniture, picture frames and wooden objects in collections. N.b. They can tunnel from one piece of wood into another if the two pieces of wood are touching (such as floorboards and supporting joists).

Fig. 2.

The developmental pathway of Drywood termites. This is the normal or 'winged' pathway, common to all hemimetabolous insects. N.b. all individuals, except for soldiers, might mature into winged reproductives.



Single site nesters, such as drywood termites, have a single developmental pathway: egg – larvae – nymph – alate. This developmental line is the normal hemimetabolous path, and is also called the 'winged' line. Thus almost all individuals can eventually mature into alates (the winged reproductives). Those that do not are the soldiers; this caste develops from nymphal instars, and is the only sterile caste (Fig. 2). There are few soldiers in a drywood termite colony (usually less than 5%).

Drywood termites grow very slowly, in fact they are the slowest growing termites. Queens increase little in size, and so produce relatively few eggs at any given time. This is a consequence of their very low quality food (in terms of nitrogen content, necessary for protein production). Colonies also take a long time to reach their maximum size.

Cryptotermes and *Kalotermes* are the largest and most important genera of drywood termites.

Subterranean Termites

Subterranean termites are named after their ability to tunnel through soil. Subterranean termites use their ability to tunnel through soil to find new food sources. They dig tunnels or construct surface galleries from soil, saliva and faeces, to connect their nests to their food sources. A colony can feed on different food sources simultaneously – up to 20 trees in some cases. Subterranean termites can tunnel down to the watertable to get water, so they are capable of living anywhere with suitably warm climate and food. Subterranean termites nest underground, on top of the soil surface (epigeal mounds), inside of tree trunks, or even on a branch of a tree or cliff face.

The alates of subterranean species colonize new areas by flying, as with drywood termites. The colonies develop similarly, but as a rule much more rapidly than those of drywood termites. Colonies are also often much larger, as they are not constrained by food resource size – they can simply tunnel through the soil to find another food resource. How subterranean termite colonies develop from this point depends on a number of factors, notably reproductive biology and nesting behaviour (NB. 'nesting' refers specifically to breeding sites). There are two groups of subterranean termites: multi site nesters or central site nesters. There are differences between these two groups that have implications on how infestations occur and develop, and how they are controlled.

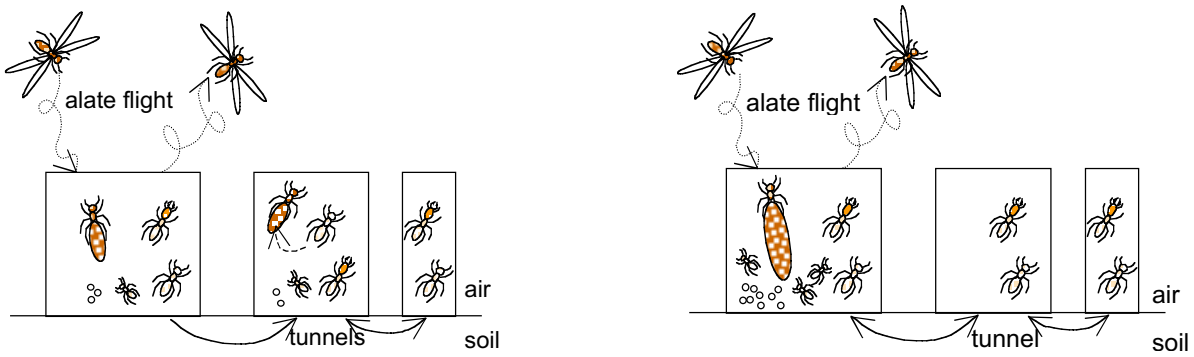


Fig. 3. Schematic of subterranean termite nesting behaviour.
Left panel: multi-site nesting species. NB. the larger size of the queen (top left in left block), the secondary nest in the larger food source and potential of secondary reproductives in secondary nests (centre block).
Right panel: central site nesting species. NB. the very large size of the queen and the lack of both secondary nests and secondary reproductives.

Multi site nester species may build new nests in the new food sources, especially if the food source is large. Eggs and larvae will be transferred to these new nests, thus a colony may have multiple nesting sites. Queens will increase in size by growing their ovaries and extending their abdomens (called 'physogastry'). They can lay more eggs at a faster rate compared with drywood termites, and colony sizes are commensurately larger (tens of thousands to hundreds of thousands of termites) (see Fig. 3, left panel).

Species with central site nests always transport the food from new food sources back to their original nest. They do not build nests in new food sources and do not transfer the young to food sources, instead the reproductives and their progeny always remain in the original nest. Thus the original nest is the only nest and is 'central' to the life and foraging activity of the colony. Central nests can be large and complex, and

often have the capacity to maintain constant temperature and humidity levels. Queens can be enormous, with greater levels of physogastry than in multi site nester species. The higher egg production results in very large colony sizes (hundreds of thousands to millions of termites) (see Fig. 3, right panel).

Individuals of both multi site and central site nesters have two developmental pathway options. There is a split after the youngest instars into the normal, winged developmental path and a second 'wingless' developmental path. The wingless path produces the 'worker' and soldier castes: egg – larvae – worker – soldier. The crucial difference between the two types is that the multi site nester termites can change between these two developmental paths and that reproductive potential is not restricted to the winged developmental pathway (i.e. individual termites can have flexible growth and reproduction options) (see Fig. 4, left panel); whereas central site nester termites cannot change between the two developmental pathways and workers and soldiers are sterile (individual termites have fixed growth) (see Fig. 4, right panel).

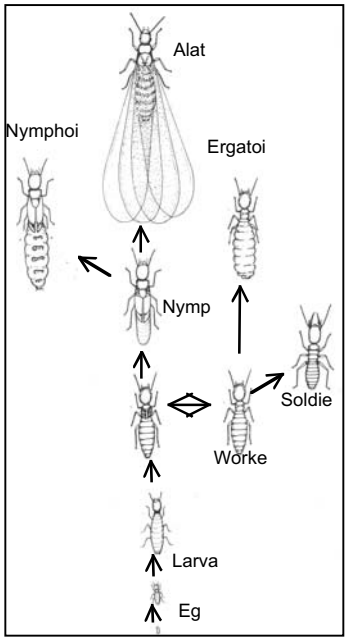
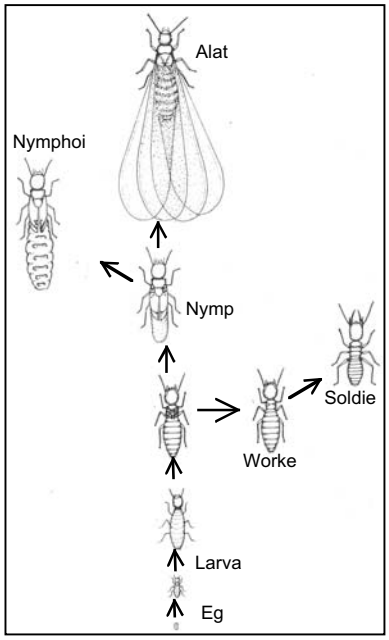


Fig. 4.
The two developmental pathways of subterranean termites, the normal or 'winged' and the secondary or 'wingless' pathways.

Left: multi-site nesting species. N.b. the flexibility in this group, with inter-changeability between winged (nymph) and wingless (worker) pathways, and the potential of wingless workers to develop into ergatoid reproductives.

Right: central site nesting species. NB. the fixed development in this group, and reproduction restricted to the winged pathway.



It is perhaps convenient to remember that the more flexible nesting habits of multi site nesters are reflected in the more flexible developmental pathways seen in this group. It is possible for wingless 'workers' to change into winged nymphs and eventually grow into alates; it is possible for nymphs and even wingless 'workers' to mature their gonads and become wingless neotenic reproductives (called 'nymphoid' or 'ergatoid' secondary reproductives respectively). These secondary reproductives often disperse to and reproduce in the larger foraging sites. The more fixed developmental pathways of the central site nesters prevent such flexibility. Dispersal of reproductives follows the pattern of the drywood termites: only by alate flight; wingless workers remain wingless and sterile (see Fig. 4).

Subterranean termite colonies can grow very fast from colony foundation, and therefore can damage wood more rapidly compared with drywood termites. Furthermore, the larger sizes of subterranean termite colonies, measured in the millions, can result in severe destruction in short periods of time, especially in warmer latitudes or at warmer times of the year.

Multi site nester termites generally have smaller colonies than central site nesters due the smaller queens found in the former species compared with the latter species. However, because these colonies can increase the number of reproductives and the number of nesting sites, it is possible for some colonies of

multi site nester termites to grow very large. Connections between parts of such 'colony clusters' can eventually break up, resulting in more than one independent colony.

Important multi site nester termite genera include: *Mastotermes*, *Reticulitermes*, *Heterotermes* and *Schedorhinotermes*. Important central site nester termite genera include: *Coptotermes*, *Nasutitermes*, *Amitermes*, *Macrotermes*, *Microtermes* and *Odontotermes*.

Detection

Termites are well known for their secretive ways. That does not mean that they cannot be found. There are visual signs that termites are active in an object or structure, as well as other signs, and recently electronic inspection methods have been devised. These are used for both drywood and subterranean termites, and so are discussed together.

Termites themselves can be found. Both drywood and subterranean termites produce alates seasonally, typically once during the hot or wetter season. The alates fly en mass – a large colony might produce hundreds of thousands of alates. Therefore the presence of alates, or their discarded wings, is indicative of the presence of a termite colony. Subterranean worker and soldier termites will appear outside of mud tubes to reconnoitre where to build. This often happens at night in larger buildings when air-conditioning is not functioning. Once the air-conditioning is operating again, such as in the morning, termites can be blown out of ducts. It is possible to view into wood, in fact there are several visual inspection devices for probing into structures. These are typically thin, lens-filled tubes equipped with powerful light sources that can be linked to cameras or video monitors (e.g. BugEye Technoscopes of Australia). These devices will usually penetrate 50 cm or more, and termites can be seen clearly.

Fig. 5. Drywood termite holes and frass.

Visual signs of drywood termites are few as they are the most cryptic of termites; timber attacked by drywood termites shows only one outward sign of termite presence: entrance holes. There are few such entrance holes to drywood termite galleries; these are round or nearly so, and are sealed when not in use. It is the excrement of these termites that is typically the first sign of attack. The excrement, called frass or 'poppy seed', is in the form of dry pellets. These are usually stored in hollowed out chambers in the wood, but can also be ejected by the termites from the entrance holes. In the latter case, the frass will accumulate underneath and point towards the entrance holes. Frass often has a distinctive shape for different species (Fig. 5).

Fig. 6. Subterranean termite mudding.

Subterranean termites do not have entrance holes and do not produce dry frass like drywood termites. Instead they use their excretion in their construction activities, usually referred to as 'mudding', which makes the detection of subterranean termites easier. Subterranean termites construct mud galleries over inedible materials and mud walls over cracks in attacked wood (Fig. 6). Some species also build special narrow mud tubes or platforms from which alates emerge for their flight.

Most termite species hollow out the wood that they inhabit, excavating very close to the surface without ever breaching it, so a reduction in the weight of a wooden item might also indicate infestation.

There are three forms of electronic detection that might be useful in detecting all termites: movement detection using microwaves, acoustic emission detection and infrared heat detection.

Movement of individual termites can be detected using microwaves. A microwave beam is transmitted into the object or structure and termites are detected as they pass through the beam as they have a higher water content than the surrounding wood and air in the galleries. A system has been developed by Termatrac, Brisbane Australia, which is used by licensed and trained pest control operators only.

Acoustic emission (AE) detection involves electronic detection of wood fibres snapping as termites bite; these are easily distinguishable at a frequency of 150 kHz. AEs are discernable for 50 cm along the wood grain and 20 cm across the wood grain. Two models have been built, one by Maruwa Biochemical Co. Japan, the other by Dow AgroSciences USA.

Infrared heat detection has been suggested as a possible detection method. Living organisms emit infrared heat rays; these can be detected using an appropriate camera system (this idea is used in other applications, e.g. night vision). Several companies that use infra red cameras offer termite detection services; for example TermiCam, Melbourne, Australia.

There is a forth electronic method that can be used for subterranean termites: moisture metres. Moisture metres are useful for subterranean termite detection because these termites will bring water to a feeding site if it is not already damp. Therefore, a moisture metre can indict the possible presence of termites if an area is very damp when it would otherwise be dry (such as wall cavities, attics etc).

Finally, some termite species have a distinct odour. Dogs can be trained to identify these termite smells, and used in a similar fashion as those trained to detect drugs and explosives.

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Control Methods

Drywood termites

Considerations

It is important to remember that drywood termites are single site nesters, which means that they live inside a single piece of wood – unless this wood is constantly sitting on or resting against another piece of wood. It is also important to remember that

all individuals in the colony are capable of maturing into reproductives. This is illustrated in Figure 7, in which four secondary reproductives are pictured. These are all neotenics, which means that they matured sexually before they matured physically (i.e. before their wings and eyes had finished growing). This often occurs when the primary reproductives die. Therefore all termites must be killed to destroy the capacity of the colony to regenerate.

Prevention

There are two methods by which drywood termites enter a wooden object. The first and more common is when two alates chew into a piece of wood to establish a new colony. If the wooden object has a painted or varnished surface that is unbroken, then it has a greater chance that alates cannot enter it. If the object has cracks or splits in the surface that break an otherwise completely painted exterior, then these should be covered to lower risk of attack. It should be noted that de-alate drywood termites can chew through some paints, but most infestations of painted wood occur through a breech in the paint. The second method by which drywood termites enter a wooden object is when the object is placed on or against another wooden object that is already infested, and the nymphs chew from one to the other. As there are more termites available to chew, paints and varnishes are not as effective at preventing attack as with just two alates.

Wood preservative formulations can be applied to the surface of some wooden objects, and provide an alternative protection method, or after termites have been found as a remedial treatment. Most formulations are applied by vacuum pressure impregnation (in order to have greater penetration of the formulation into the wood), but there are several that can be applied with a brush. In the latter case, highly penetrating solvents are desirable, but these might affect paint or varnish. Most solvents will penetrate the less dense sapwood of softwood tree species easily, but are less successful with heartwood of softwoods, and even less penetrating with hardwood species.

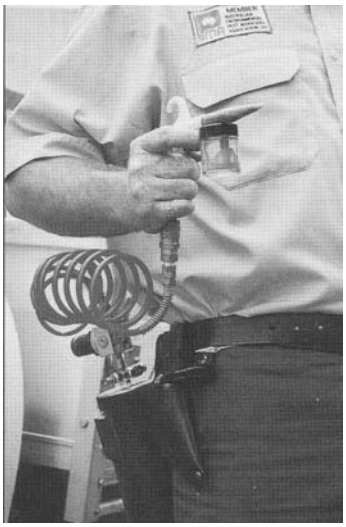
The older wood preservatives are oil-based (e.g. coal-tar cresosote, diesel fuel, copper naphthenate, pentachlorophenol). These are less desirably now as they have a strong odour and a darker colour that is obvious after application. One benefit is that they have good persistence in the wood after application and resist fungal attack well.

Liquid chemical insecticides are applied as a wood preservative, but these have little or no fungal resistance. Organochlorines were used commonly until recently; organophosphates and synthetic pyrethroids are used in their place (complete information on liquid chemical use is placed under the subterranean termite control section below).

Water based wood preservatives are of more interest for conservators, as these generally have low odour and low colour after application. One exception are the older Copper-chromium-arsenic salts (CCA, ACA, ACZA, CCB etc.) that have a green colour. The newer ammoniacal copper quaternaries (ACQ's), copper azoles, copper dimethyldithiodicarbamate (CDDC), copper HDO, and borates (e.g. Bora-Care, Boracol) are perhaps more desirable from the colour perspective, but these can leach – this is especially true of borates.

An emerging trend is to use several formulations simultaneously, as they can have synergistic effect. This will increase the protective effect, usually at lower concentrations of the forumulations.

Remediation



Poisonous dusts have a long history in termite control: the earliest known treatments with dusts were in the 19th century in east Asia. Large quantities of arsenic dusts were blown into wall cavities and attics of buildings, a practice that was extremely dangerous for the human occupants. In the early 20th century a variety of hand held puffers and dust guns were developed. These can be powered with compressed air to achieve greater spread of the dust (Fig. 8). The dusts are blown into termite galleries in the wood through holes drilled for the purpose. The active ingredient is usually arsenic, such as copper acetoarsenite (Paris green), lead arsenate, and calcium arsenate; or fluorine, such as sodium fluoride and sodium aluminium fluoride (cryolite); currently arsenic trioxide is used in Australia (see also dust formulations in subterranean termite control below). Dusts must be ground finely to achieve maximum impact; 100 _m particle size or smaller. Such fine dust will travel further in the termite galleries and may be imbibed more readily during grooming. Dusts might have residual effect, i.e. continue to be lethal after application, but this will depend on the stability of the active ingredient, the temperature, the quantity injected and how much of the gallery system was reached by the dust.

Fig. 8. A modern dust gun. The compressed air tank is on the belt and the reservoir for dust is the jar on the gun.

Fumigation gas is the most common method for treating drywood termites infestations in buildings. The building or other large structure is enclosed within 'tarpaulins'; gas-proof sheets, usually made of nylon coated with PVC, neoprene or rugger. Fans are often placed inside the building, near the nozzle outlet of the fumigant, and near inner doorways, to ensure even application of the fumigant. There is a single application of fumigant gas; only three types are used against termites: sulfuryl fluride (Vikane, Dow AgroSciences), methyl bromide and hydrogen cyanide. Carbon dioxide is sometimes mixed with the fumigant as it can increase the fumigant's toxicity. The application procedure typically requires 24 hours, with the fumigant venting slowly to the atmosphere. The extreme toxicity of hydrogen cyanide to humans, and the toxicity and ozone depleting nature of methyl bromide, has reduced the use of these gases considerably in recent times.

Heat or cold treatments are more common for smaller objects, such as furniture and sculpture. These objects are heated in ovens or cooled in refrigerated rooms for minutes to hours at temperatures either too hot (>50 ºC) or too cold (<0 ºC) for termites too endure. Although this approach is usually applied for smaller objects, however, small buildings have been treated with hot air, in a similar fashion as fumigants. The success of this method in buildings will be variable, as the complex shape of buildings creates a range of temperatures; of particular concern are the areas near the ground or concrete that often do not reach lethal levels. Liquid nitrogen has been used as a 'spot treatment', i.e. at very localised sites, in buildings also (nitrogen is liquid at -273 ºC). It is important to remember that there is no residual control effect of heat, cold or fumigation treatments.

Subterranean termites

Considerations

Fig. 9. *Coptotermes*, a subterranean termite. Top. The primary queen is the very large individual, the primary king is the dark individual near the centre, a soldier is above the queen with the darker head, the remainder are workers. **Bottom**. Secondary reproductives. The male is the small individual towards the centre.

Although killing the individual subterranean termites that are attacking an object is desirable, it is important to remember that these are workers, and so their deaths will not eliminate the colony's potential to cause further damage. To destroy this potential completely, the reproductives must be killed also. NB., that in multi site nester termites, there is a greater flexibility of development, especially with the 'wingless' line that produces workers. This means that a greater proportion of the colony might become replacement reproductives, and in a greater number of potential nesting sites. Central site nesters might produce secondary reproductives, but only from maturing nymphs (Fig. 9). Also remember that in central site nesting species, there is only one nest. These species might build bivouacs, or temporary resting and food storage sites that resemble nests often in walls or under buildings, but these contain neither eggs nor young nor reproductives, so destroying these bivouacs does not equate to destruction of the colony.

Control

Subterranean termites threaten buildings and outdoor structure to a much greater extent than drywood termites, and consequently this section is focussed on this possibility. Subterranean termite pest control is a dynamic research area, with many changes in both the approaches to control and the application of specific methods. The primary reason for this dynamism is the decline in use of persistent chemical soil barriers for health and environmental reasons.

Chemical soil barriers employing persistent organochlorines such as aldrin, dieldrin, chlordane and heptachlor, were used extensively worldwide from their invention in the 1940s until the 1980s. Organochlorines were the key products in managing active infestations until only recently. These compounds act by disrupting ion transport in nerves. Chemical soil barriers have also been made with organophosphate insecticides. These act by inhibiting cholinesterases, enzymes involved in transmitting nerve impulses.

Organochlorine compounds are highly toxic to many insects but are considered less toxic to mammals than organophosphate insecticides, and have long persistence in the environment. However, many types of organochlorine insecticides are readily absorbed through the skin and can cause damage (such as neurological symptoms, respiratory depression, clotting inhibition, birth defects, cancer and death) in mammals (including humans), birds and fish. Such health and environmental concerns have lead to banning their use in many countries, beginning with Saudi Arabia in 1984. It occurred in 1995 in Australia when a number of alternative termite management options demonstrated their reliability and accessibility to consumers (see below). Organophosphates are related to the nerve gases and are among the most toxic of all pesticides to vertebrates, including humans. Therefore the use of organophosphate insecticides is under review in some countries.

A diversity of termite management systems (TMS) for impairing attack or eliminating infestations by subterranean termites has been developed in more recent years in many countries, but especially so in Australia, as alternatives to the past reliance on applications of organochlorines.

The main focus for Australian termite management can be categorised as: physical barriers, which are non-chemical, preventive measures; chemical barriers, which are either safer in their application or have environmentally acceptable insecticides; or 'trap-and-treat' control of infestations, which includes dusts and bait systems, and can use either chemical or biological control.

Physical barriers

In Australia, the concept of 'building-out' termites forms an integral part of strategies to reduce the incidence of termite damage. This comprises several measures such as: ensuring that the building site is less attractive to termites; the design of buildings, keeping ease of inspection of their structural elements as well as termite biology in mind; and on-going site maintenance. The Australian Standard AS3660 (1996 and 2000 editions) was written as the most comprehensive 'building-out' termite guide available in the world. Physical barrier systems comprised about 40% of the Australian market in 2000.

Obviously 'building-out' termites is not applicable to existing buildings, such as culturally significant buildings, museums or warehouses used for storage. However, if a new museum or storage building is to be designed, then the concept is worthwhile, and the Australian Standard AS3660 – 2000 is an excellent guide to put this into practice.

The oldest form of a physical barrier is the 'ant cap' or 'ant shield'. The floor of a building is raised on posts or piers (preferably not wood) to a height greater than the height to which termites can build their free-standing mud galleries. The ant caps, usually made of metal sheeting, are placed between posts, piers or lower parts of walls and the superstructure under the building. It is important to remember that ant caps do not prevent termites from gaining egress to the structure; termites can build their mud galleries against the posts or piers, and can build their galleries out and over the ant cap. The ant caps force the termites to build their galleries into the open (on the surface of the ant cap), where they are more easily discovered during (regular) inspections.

Concrete slabs are more common substructures in modern construction ('on-ground constructions'). A concrete slab, designed and produced to certain specifications (called an 'engineered slab' in Australia) can minimise cracking and so prevent the primary route by which termites can attack buildings erected upon such slabs. The Australian Standard AS3660 – 2000 on termite management has given recognition that the concrete slab, if constructed according to certain standards, may form part of the barrier system allowed in new on-ground constructions.

Structures built on concrete slabs are not impenetrable to subterranean termites. There are two main areas of vulnerability. The first is joints between two or more

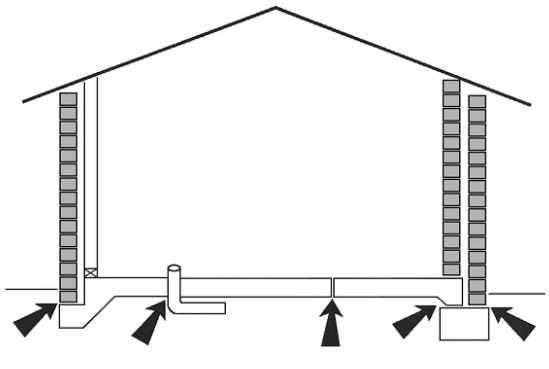


Fig 7. Schematic of building on concrete slab showing areas vulnerable to termite egress.

concrete slabs: after concrete is poured, it shrinks during drying, leaving a gap between the slabs. The second is the service penetrations through which plumbing pipes, electrical conduit and other pipes are inserted. Specific physical barriers are available to impede termite entry at joints and penetrations in concrete slabs. Plumbers might be able to install some penetration protection methods, but usually only trained and accredited installers can place any component of the more comprehensive physical barrier systems. These include:

Perforated or solid sheet material barriers:

1. Smartbuild (Termimesh) (stainless steel mesh)
2. Alterm (marine aluminium sheets)
3. Termite Tite (flexible stainless steel sheets)
4. many other 'collars' made of PVC or metal or both just for service penetrations

Graded particle barriers

1. Granitgard (crushed granite and other stones)
2. Basalt barrier (crushed basalt)

NB. that novel physical barriers systems and new materials are being investigated, so this list is likely to change in the future.

Some physical TMS are better adapted for different construction methods or situations. Furthermore, the complex nature of construction of large buildings, together with renovation and extension of existing buildings, typically requires that the installation of these physical TMS as part of a combination system (rather than complete, stand alone, barrier). An example of this might be an engineered concrete slab with perimeter graded stone and penetrations protected with stainless steel sheet.

Chemical soil barriers

Chemicals that are persistent in the environment are not desirable in the post-organochlorine world. Therefore, modern chemicals used for soil barriers will breakdown with time (faster in hotter climates), and are generally less toxic to vertebrates then insects (with the exception of organophosphates), and so are considered to be more environmentally benign. Although application of these formulations can be done as a hand spray preconstruction, their ephemeral nature requires eventual retreatment. Several reticulation systems have been designed for this purpose (e.g. Altis, Termguard and SlabSet in Australia all have systems).

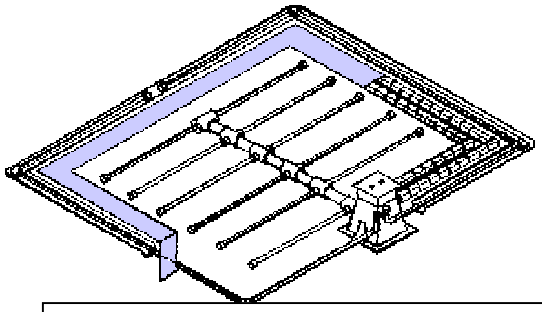


Fig 8. Schematic of a reticulation system for retreatment of insecticide under a concrete slab (Term~Guard).

There are two general approaches for chemical soil barriers, those that can be detected by termites (more or less repellent), and those that cannot (more or less non-repellent). Repellent chemical soil barriers will kill termites, but part of the strategy behind repellency is to encourage termites to tunnel elsewhere. In comparison, termites will tunnel through non-repellent barriers and die from doing so.

Repellent barriers:

1. Organophosphate insecticides: Chlorpyrifos ('Dursban', Dow AgroSciences) and generics (number of sources)
2. Synthetic pyrethroids: Bifenthrin ('Biflex', FMC); α-cypermethrin (BASF).

Non-repellent barriers:

3. Imidacloprid ('Premise', Bayer)
4. Fipronil (Aventis) under development
5. Chlorphenapyr (BASF) under development
6. Thiomethoxam (Syngenta) under development

Chemical barriers not in soil

A new strategy is to isolate the chemical barrier into a medium other than soil. This will prevent dispersal of the chemical into the environment and slow breakdown; thus increasing the effective life of the barrier. It also allows safer application of the chemical under controlled conditions. It is important to emphasise that the effectiveness of the product relies on the chemical's toxicity or repellency or both, not the medium in which it is placed. There is only one product currently available on the Australia market, but others are being developed.

1. Synthetic pyrethroids: Deltamethrin applied in a fibrous blanket, laminated between two sheets of plastic ('Kordon TMB', Aventis). Kordon TMB has a dual role; it also serves as the protective moisture membrane below the structure.
2. Products under development (Cecil, Syngenta, Nufarm and others).

'Trap-and-treat' control of infestations

'Trap-and-treat' control methods are a recent development in termite pest management, introduced in the 1990s. It involves aggregating subterranean termite foragers, usually into some form of bait box or station – the 'trap' stage, and then applying a toxicant chemical – the 'treat' stage. There are two major approaches: dusting and baiting. This approach is considered to be a more environmentally benign approach than chemical soil barriers, because although the 'trap-and-treat' method does use a chemical toxicant, it is a small amount applied to a localized area, compared with the large amount over a wide area in a chemical soil barrier.

'Trap-and-treat' control introduced the concept of 'colony elimination', i.e. killing the entire population of termites in the colony, reproductives and brood included, to termite pest control. Prior to this, colony elimination required the discovery of the nest for the destruction of the reproductives and their brood; not surprisingly, this was a rare event. 'Trap-and-treat' allowed for the possibility of colony elimination remotely and without knowing the location of the nest. Colony elimination has been demonstrated experimentally under favourable dusting or baiting conditions, and there is evidence that it can happen under normal, urban conditions. However, in the 10 years of development of 'trap-and-treat' methods, there is a growing perception that these methods, in combination with other termite pest control methods, will reduce rather than eliminate populations. This highlights TMS as the combination of many methods, depending on circumstances, instead of any one complete solution.

Dusting

Dusts can be applied to subterranean termites as described for drywood termites above ('stand-alone'). They are also used with the 'trap-and treat' method. Typically, a box that has been filled with wood, cardboard or paper, or a combination thereof, is buried in the ground next to a building with an infestation, or placed inside the building adjacent to an infestation with some form of connection. A period of time is allowed for the termites to infest the box and once this has happened, one of two treatment methods follows. The first is when dust is simply blown into the box (similar to 'stand alone'), whereas the second requires that the termites be removed from the box and shaken with dust, and then being returned to the box. The latter method ensures a more complete cover of dust and highly disturbed termites. These disturbed termites will return quickly back to their nests without grooming themselves, and so carry sufficient quantity of toxicant on their bodies to eliminate the colony in its nest. Active ingredients used include:

1. Arsenic trioxide (in some countries, including Australia, but not available in many other countries).
2. Synthetic pyrethroid: permethrin ('Permethrin D', Barmac Industries, Australia).
3. Insect moult inhibitors: Triflumuron ('Intrigue', Bayer). This is a chitin synthesis inhibitor (CSI); it prevents synthesis of chiton, the building material of insect exoskeleton. The insect dies when it attempts to moult without a new exoskeleton.
4. Insect pathogenic fungi, *Metarhizium anisopliae* and *Beauveria bassiana* (Deuteromycotina: Hyphomycetes). (Several research teams investigating this possibility, including CSIRO Entomology and various companies).
5. Insect pathogenic nematodes, *Heterorhabditis* and *Steinernema* have symbiont bacteria *Photorhabdus* and *Xenorhabdus*. The bacteria kill and digest insects, which gives nutrients for the nematodes that carry the bacteria around. Considerable research has been conducted on these nematode worms as biological control agents for a range of insect pests (CSIRO and other agencies).

Baiting

In general, baiting systems comprise many bait stations that contain a food material (wood, cardboard, paper, or cellulose), which are buried in the ground around or placed inside (against walls etc) an infested building. A period of time is allowed for the termites to find and infest the bait stations. Some baiting systems have untreated wood as 'monitoring devices'; these are replaced with paper or cellulose treated with the toxicant once termites are active in the stations. Other baiting systems are set up with the (non-repellent) insecticide already in the bait stations. The termites remove the bait and return to the colony and feed the bait to their nestmates, thereby killing them. If sufficient insecticide is consumed, the colony will be eliminated. All of the insecticides used in baiting systems are slow acting, so that the foraging individuals that carry the bait do not succumb to the effects of the bait before delivering their colony.

There are several types of insecticide. These include:

1. Organochlorines: Mirex can be used in baits against *Mastotermes darwiniensis* for specific circumstances in geographically restricted areas of tropical Australia.
2. Insect growth regulators: 'Hexaflumuron', a CSI, in 'Sentricon' (DowAgroSciences); 'diflubenzuron', a CSI in 'Exterra' (Ensystex)

3. Fluorinated sulfonamide, Sulfluramid (N-ethylperfluoro-octane-1-sulfonamide) (described as a 'slow-acting stomach poison', it affects mitochondrial respiration) (FMC) under development in Australia, available in USA
4. Phenylpyrazole: 'Fipronil' a, disrupts the chloride channel in nervous tissue, (Aventis) under development.
5. Insect pathogenic fungus *Metarhizium anisopliae* (CSIRO Entomology) under development.

There are also various devices for termite aggregation and monitoring that do not include an active bait insecticide.

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Insecticides - what they don't tell you

Robert E. Child

Introduction

Insecticides are chemicals that kill insects by a toxic action. Until relatively recently the insecticide was the active ingredient and was used on its own; such as sulphur, arsenic, methyl bromide etc. However, in recent years the development of the insecticide industry has led to newer, better, safer insecticides that are effective in much lower dosages than their predecessors. Also, they have to be applied in various ways to make them accessible to the insects in their various growth states. Now, the active insecticidal ingredient of a commercial insecticide may be a fraction of a percent of the total product. The rest may be solvents, carriers, emulsifiers, dilutents etc., which may have their own problems from a conservation point of view, in their effect on artefacts.

Insecticidal Formulations

Modern insecticides are formulated so that they can be applied in the most efficient (and economic) fashion for the intended application. Applying more than the required insect killing agents is unnecessary, uneconomic and increases potential risks. Therefore, it makes full sense to only apply that which is necessary to carry out the job of eliminating the insect pests.

The new generation of insecticides are very effective at very low dosage rates and therefore have to be diluted in various ways in order that they can be spread evenly over an area. Unfortunately, simple dilution in an appropriate carrier is rarely possibly on its own and a variety of other chemical agents to be added. Many of these can be toxic in themselves or are not applicable for conservation reasons.

Typical liquid insecticide formulations include the following:

Aerosols : solid or liquid insecticide dissolved in a solvent and atomised into the air with a propellant. Early propellants were C.F.C.'s now a typical can aerosol might contain a mixture of insecticide, methylene chloride, petroleum distillate, water, emulsifier and butane.

Solutions : insecticide dissolved in a solvent usually petroleum distillate, but occasionally water.

Emulsions : insecticide dissolved in organic solvents and/or light oils mixed with an emulsifier and diluted with water.

Micro-emulsions: insecticide and surfactants diluted with water. Early versions also had solvents and emulsifiers.

Wettable powders: dry powders and a wetting agent, diluted with water to form a sprayable suspension.

Typical solid insecticide formulations include:

Dusts : powders of solid insecticide with an inert carrier.

Smoke generators: a burning mixture that products an insecticidal smoke. The burning mixture is often based on potassium chlorate, oxidising agents and glycol esters.

Baits : insecticides mixed with good or other attractant carrier.

Lacquers : insecticides formulated into clear lacquers that dry to a hard surface.

Resin strips : volatile liquid insecticides impregnated onto cardboard or resin strips, to give a slow-release insecticidal vapour.

Conservation problems with carriers

The conservation problems associated with certain insecticide formulations often caused by the carriers. Obvious problems such as smell, toxicity, inflammability etc., are often due to the solvents such as xylene and petroleum distillate. However, conservation problems can be caused by the following carriers:

Solvents : petroleum distillates, halogenated organic solvents etc., commonly used can soften plastics, damage painted surfaces, and some polished surfaces.

Water, as a solvent, can cause dirt 'tidemarks', staining and raise relative humidity levels.

Oils : technical white oils, kerosene derivations etc., often found in emulsion-type formulations can leave sticky residues and cause soiling.

Emulsifiers and surfactants : often associated with oils, solvents and with water. They Frequently have a very high pH or are acidic and can damage accordingly.

Wetting agents and Micro-encapsulated polymeric materials : including 'stickers', 'spreaders' etc.

Anti-blooming agents: used in some organic solvent formulations to stop white blooms forming on the surfaces.

Carrier powders: evaporates off surfaces. Can leave sticky residues in insecticidal dusts. They are often inorganic powders or ground-up nutshells

Mixtures: in insecticidal smokes, contain chlorates nitroles etc., which, on burning, can reduce to corrosive chlorides and nitriles.

Food and other attractants: can go mouldy.

Vinyl and cardboard corner strips : DDVP (Vapona) strips can drip sticky, acidic residues.

Precautions

When using insecticides it is important to choose the correct formulation for the treatment, and to apply it correctly. The choice of insecticide will be affected by such conditions as :

- the insect pest to be treated
- the affected material being treated
- the surface type, e.g. absorbent, non-absorbent easily stained etc.
- the most appropriate treatment, e.g. spray, fogging, powder application etc.

It is essential that the total formulation of the insecticide be known in order to make a considered judgement on the best treatment, and some manufacturers will not divulge the information. Where possible, it is best only to use materials that have been conservation tested, e.g. by the Oddy test, pH measurement etc.

Gases for insect control: factors that influence their effectiveness.

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Gases, which include, fumigants and controlled atmospheres, have a range of physical properties that make them ideal for controlling insects in relatively dry porous matrices. There is a range of insecticidal gases ranging from reactive gases with high toxicity to inert atmospheres with lower toxicity. The concentration of the gas and the duration of exposure are usually the primary factors that determine the efficacy of a gas as a means of killing any insect species. These two factors sometimes have an equal effect and sometimes one or the other is more important. Other factors that will influence the efficacy of the treatment include temperature, relative humidity, the presence of other gases, and the age and developmental stage of the target insects. This paper draws on the published literature, and some recent laboratory studies, to show that there are many factors that need consideration before deciding on a "fumigation" strategy for an object. These may go well beyond the obvious one of the safety of the object that needs treatment.

Introduction

Fumigation is a widely used process to disinfest/disinfect materials in a range of circumstances from the sterile closed environment of operating theatres to the wide-open spaces of strawberry paddocks. Somewhere in the middle comes my field — stored agricultural commodities and your field — a wide range of stored and possibly valuable objects.

In the context of this paper fumigation is the treatment of any object with a chemical, where the chemical acts in the gaseous/vaporous phase. There are two important properties of any fumigation treatment.

- The ability to kill any potentially or actually damaging organisms: commonly insects and other invertebrates, but also a whole range of other biota including fungi and bacteria may also be targets.
- The materials should not cause "collateral damage". For the grain fumigator this means not damaging germination, quality, and the consumer. For the conservator it means, as far as I can tell, not changing the treated material at all.

How a gas kills an organism

Any biocidal treatment must cause enough damage to a critical site, or a set of critical sites, before the treatment can kill the target organism. The amount of damage is likely to be primarily a combination of both the intensity of the treatment and the duration of the exposure. In fumigation the intensity of the treatment is related directly to the concentration of toxic gas. Duration is the amount of time that the treatment lasts.

Intensity and duration of treatment are primary factors but their joint action may be moderated a range of other factors:

Environmental — such as the chemical nature of the toxicant, temperature, water activity, the presence or absence of food, and concentrations of oxygen and carbon dioxide;

Physiological — the nature of the critical damage done by the toxicant to the target organism, detoxification, damage repair, metabolic slow down and gaseous exclusion;

Behavioural — responses such as avoidance through refuge seeking and reduced activity.

Differences in *physiological* and *behavioural* responses are likely to occur between species, developmental stages and ages, and different strains within a species due to genetic difference selected through previous treatments and other aspects of strain history.

The scientist trying to establish reliable treatment regimes for new fumigants or optimising regimes for existing materials is faced with a large and multi-factorial problem. In the case of durable commodity fumigation, Annis (1987) suggested that it requires approximately 10^6 individual treatments involving 10^9 insects to systematically establish a dosage regime for a new fumigant. This number makes allowance for major species, developmental stages/instars, temperature, water activity (relative humidity), time, concentration, replication and controls but only a single strain of each species

These difficulties tend to cause people to stay with what is known to work. This is even more so when one considers that the above is only about killing the organism and not about harm to the object treated. There are two further hurdles for any new or modified gaseous treatments. Firstly there is finding a commercial partner to make, transport, supply and more importantly register the treatment — all pesticides require some form of registration to be used legally. Secondly there is the act of registration itself.
Finding biocidal gases and vapours is not hard, but identifying those which will make acceptable fumigants is much more complex. The following list shows those that have at some stage been used, are now used, or are seriously contemplated as fumigants.

Main materials in use (all applications):	Nitrogen (Low oxygen)
Phosphine	Sulfuryl fluoride
Methyl bromide	**Under development**
Carbon dioxide	Carbonyl sulphide
Minor/restricted use	Cyanogen
Carbon disulphide	Methyl isothiocyanates
Dichlorvos	Methyl phosphine
Hydrogen cyanide	Ozone
Ethyl formate	Propylene oxide
Ethylene dibromide	**No longer used**
Ethylene oxide	Acryl nitrile
Methyl allyl chloride	Carbon tetrachloride
Methyl formate	

To a large extent what follows will be appropriate to all of the above but of course exceptions may occur. During nearly 30 years of personal research on the interaction of gases and insects, one thing has become very obvious. That is, that very few generalization hold 100% true. This paper illustrates this point on six of the above gases: phosphine, carbonyl sulphide, carbon dioxide, carbon disulphide, nitrogen and ethyl formate.

Concentration time regimes

A widely used simplification in thinking about effective dosage rates for gases is to combine the two dosage metameters (concentration *C* and time *t*) to a single value the *Ct* product. Haber's rule (Haber 1924) states that a defined value of *Ct* will give the same level of kill no matter what the value of *C* or *t* (equation 1). This relationship implies that *C* and *t* have an equal influence on mortality, put more simply half the concentration requires double the time to get the same effect.

Equation 1 $Ct=k$

However useful Haber's rule is in reducing the amount of data required and in simplifying description of dosage response, it is of little use where it does not apply. The following example (Annis 1998) uses the dosage of carbon disulphide to give 99% mortality in *Sitophilus oryzae* adults to illustrate the type of problems that exist with *Ct* as a measurement of dosage (Fig. 1).

It is clear in this specific case that below a concentration of about 13 g m^{-3} a rapidly increasing *Ct* product is required to give the same level of response. There is approximately a four times decrease in required *Ct* between 2 to 25 g m^{-3}. This is but one example of very many (Annis 1998) that show that Haber's rule does not apply universally.

Concentration and developmental stage

The developmental stage of an insect is widely documented to influence the dosage/mortality response for fumigants. Pupae are frequently the most tolerant stage, however this is not always the case, nor is it the case that one particular stage is the most tolerant at all concentrations of a single fumigant. This is particularly well illustrated in the response of *S. oryzae* to carbon dioxide (Annis and Morton 1997) (Fig. 2). This response shows two important features.

a) The response varies significantly between stages, not just in magnitude but also in pattern with respect to concentration and time.

b) There is a general reduction of efficacy at high concentration.

Numbers treated and survival

A treatment under a given set of exposure conditions should always result in the same the proportion of an insect population being killed. This means that the risk of a single survivor under those conditions is dependent on the number of insects present. While this is obvious from the mathematics the implications are not so obvious. Morton et al (2000) conducted a series of experiments on a wide range of different numbers of adult *S. oryzae* treated with 100% nitrogen. Their results showed a variation of about 4 days to give effective fumigation in numbers varying from 10s to 10s of thousands (Fig. 3).

Temperature and concentration

It is usually expected that fumigations work more effectively at higher temperatures. This effectively means shorter exposure periods at high temperatures, conversely at low temperatures longer exposure periods required. This is by no means always the case. There are examples where the trend goes in the opposite direction, especially at relatively low concentrations. This is especially well illustrated in the work of Hyne and Winks (1997) where the response of whole cultures of *Rhyzopertha dominica* to phosphine at three concentrations tested at 15, 25 and 35ºC show the shortest exposure for disinfestation occur at 25ºC for the two lowest concentration tested (Fig. 4).

Gas composition, temperature and completeness of kill

When variations of temperature, gas composition, and the required degree of kill occur concurrently the picture becomes more complex but some patterns may become obvious. For example a comparison of the temperature effect on treatment of *S. oryzae* with 60% carbon dioxide and 0% oxygen (100% nitrogen)

show a similar temperature coefficient (i.e. similar slopes on log response vs temperature plot) in three quite different treatment scenarios (Fig. 5).

Relative humidity

Relative humidity is one of the environmental variables that is likely to change or to be controlled for reasons other than pest control. Its influence on the effectiveness of a treatment is hard to predict. Pearman and Jay (1970) and others since have shown that lowered RH can assist carbon dioxide treatments (Fig. 6). More recently (Damcevski and Annis, unpublished data) have shown that at least in some circumstances increasing RH can markedly increase the effectiveness of ethyl formate treatments (Fig. 7).

Strain variation

Differences in the pattern of response can occur when individual strains within a single species show a wide range of responses to a fixed challenge under constant environmental conditions. This range of responses may be either naturally occurring or as a result of selection by repeated treatments. The varied response to CO_2 treatments in strains of *S. oryzae* is an example of unselected variation (Fig. 8). CO_2 is very rarely used for fumigation and as far as can be determined had never been used on the strains investigated.

In the case of phosphine treatment very significant variation can be selected by repeated sub optimal treatments (Fig. 9). Ten repeated selections cause the change from the normal pattern of tolerance (RD2 and RD316 in Fig 9) to the significantly resistant strain (RD235 with 10 selections in Fig 9).

Conclusions

It is superficially attractive when establishing dosage regimes for insecticidal gases to make assumptions based on other known, but often widely different, scenarios. The data presented here show several cases where using this kind of assumption can be totally misleading. The only reliable method of deciding effective dosage regimes for new or significantly modified treatments is to carry out test exposures, under relevant environmental conditions, over a suitably graded range of concentrations and exposure times. This is especially important where the outcome of the fumigation is critical to the safe long term storage of an object.

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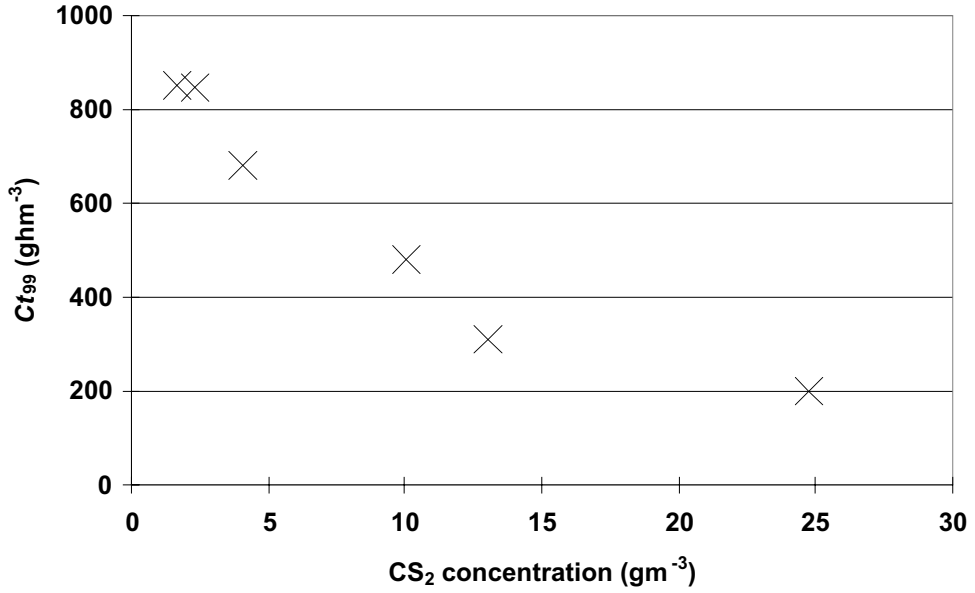

Figure 1. *Ct* product for carbon disulphide to kill 99% of *Sitophilus oryzae* adults at various initial concentrations and at 25ºC.

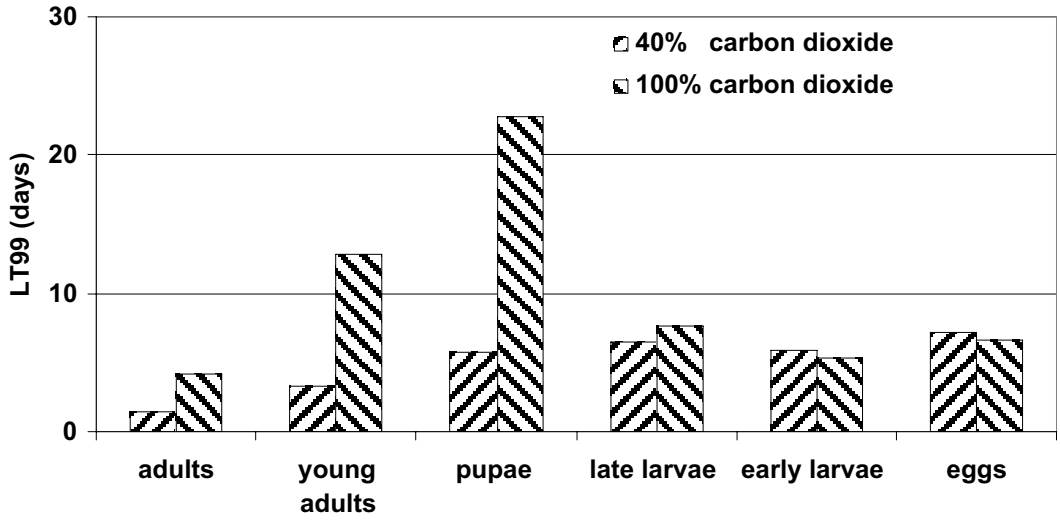

Figure 2. The time to obtain a 99% kill of various developmental stages of *Sitophilus oryzae* at two concentrations of carbon dioxide treated at 25ºC.

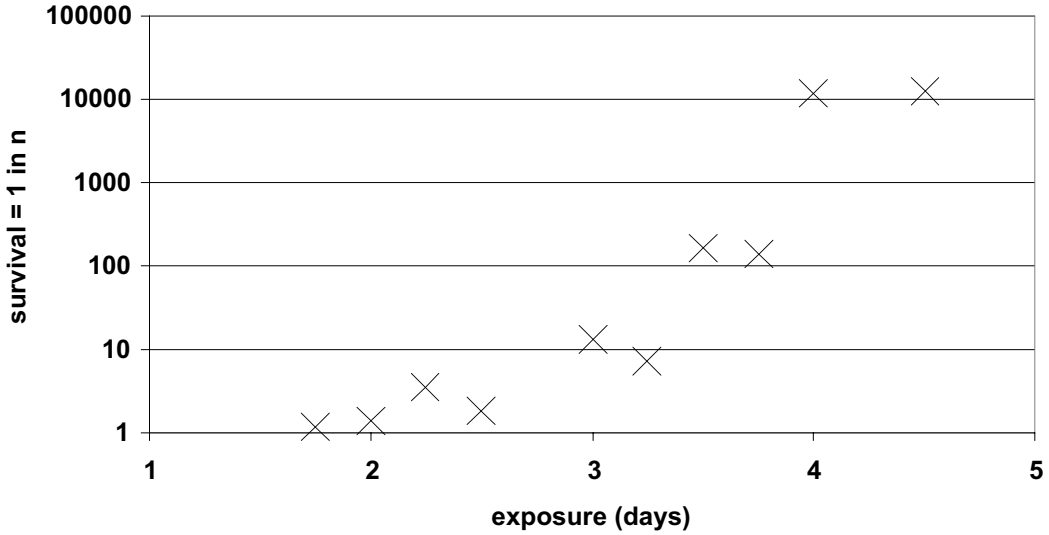

Figure 3. Exposure time for 100% nitrogen (0% oxygen) allowing one surviving adult *Sitophilus oryzae* in the number treated (*n*).

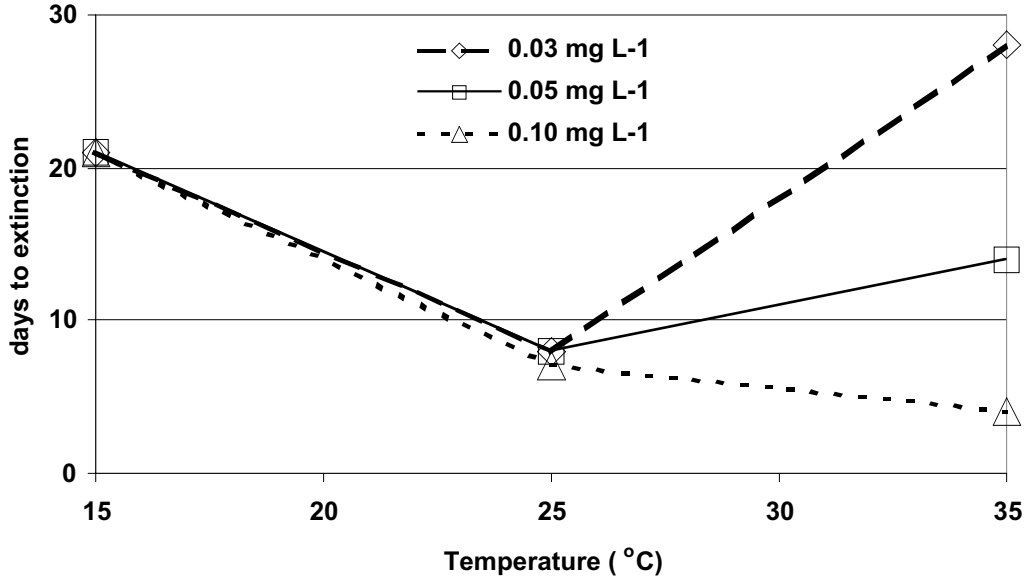

Figure 4. Exposure time required for three concentrations of phosphine to kill a culture of *Rhyzopertha dominica* containing all developmental stages at 15, 25 and 35ºC.

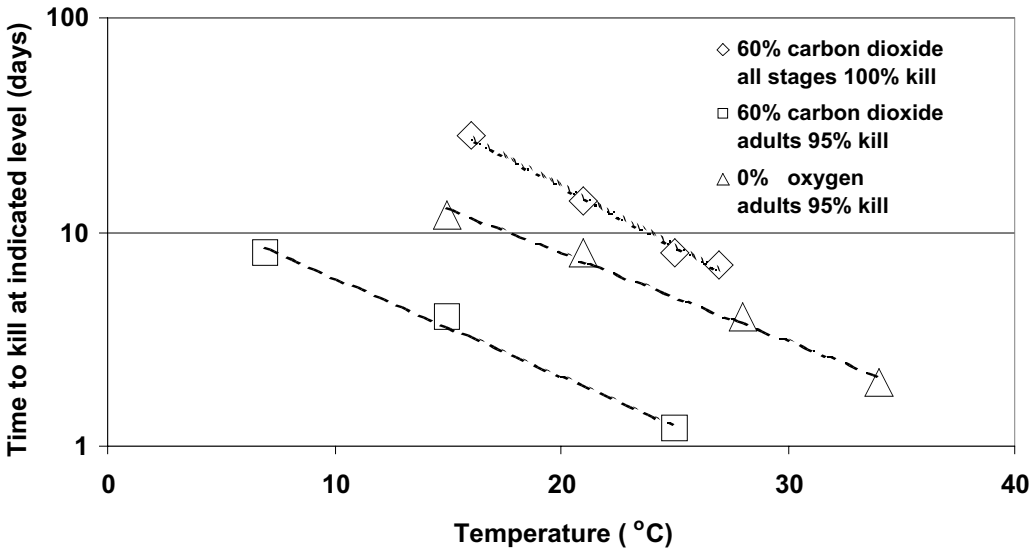

Figure 5. Effects of two controlled atmosphere treatments on *Sitophilus oryzae,* showing the difference in times required to achieve different targets over a range of temperatures (replotted data from Banks and Annis 1990).

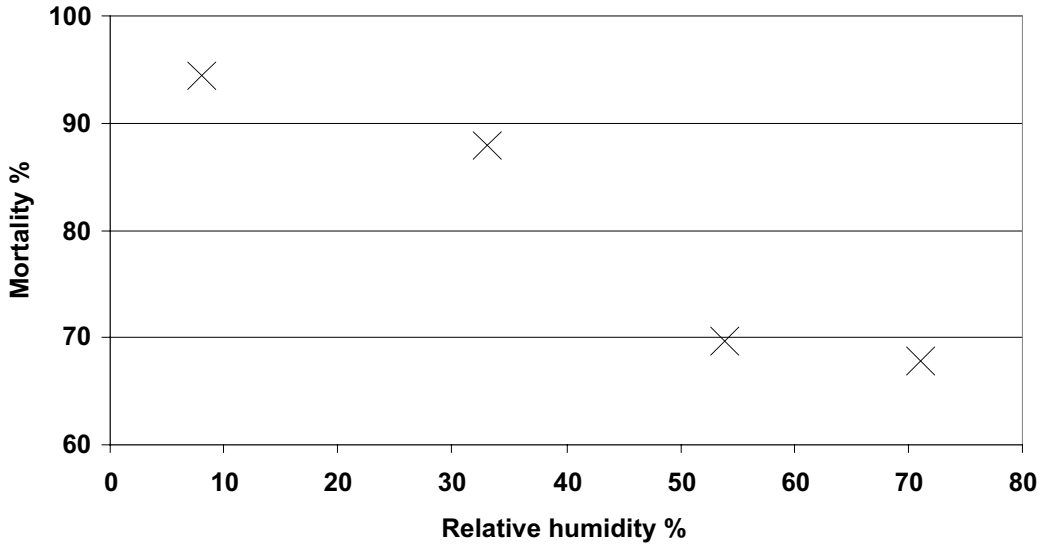

Figure 6. The effect of relative humidity on mortality due to an exposure to 46% CO_2 for 7 days for mixed stage cultures of *Tribolium castaneum* (Pearman and Jay, 1970).

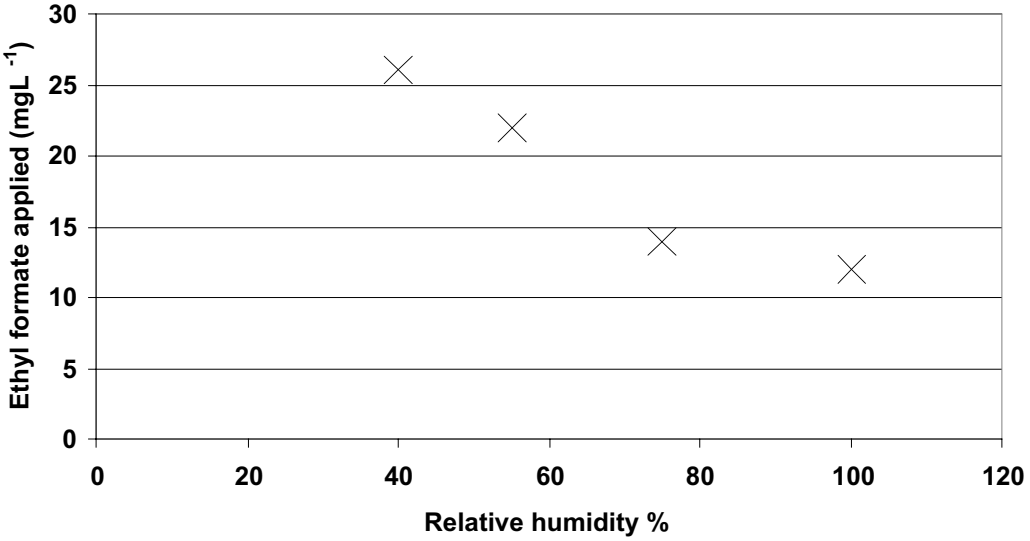

Figure 7. Effect of relative humidity on the dose of ethyl formate required to give 99% mortality of adult *Sitophilus oryzae* with a 24 hour exposure.

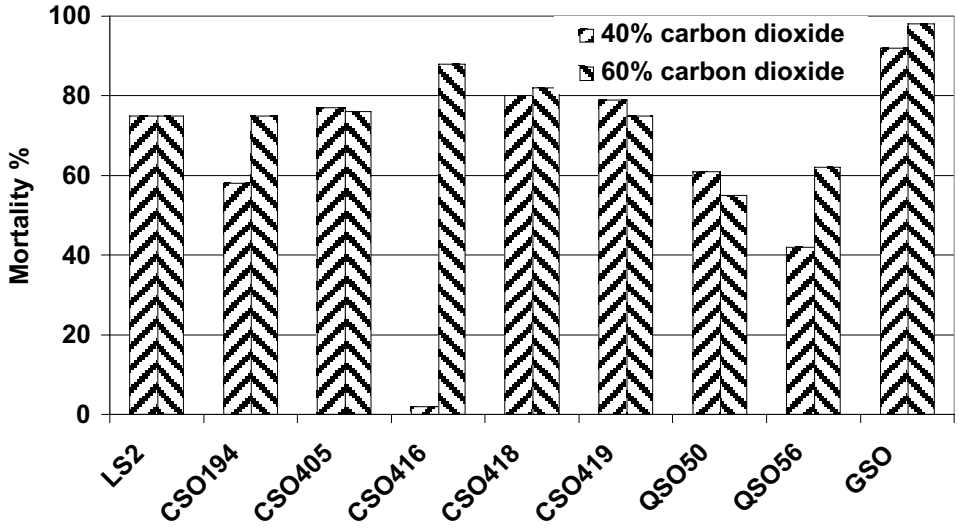

Figure 8. Response of a variety of strains of *Sitophilus oryzae* to exposures of two concentration of CO_2 that give 75% mortality in adults of the reference strain LS2 (Annis, 1990).

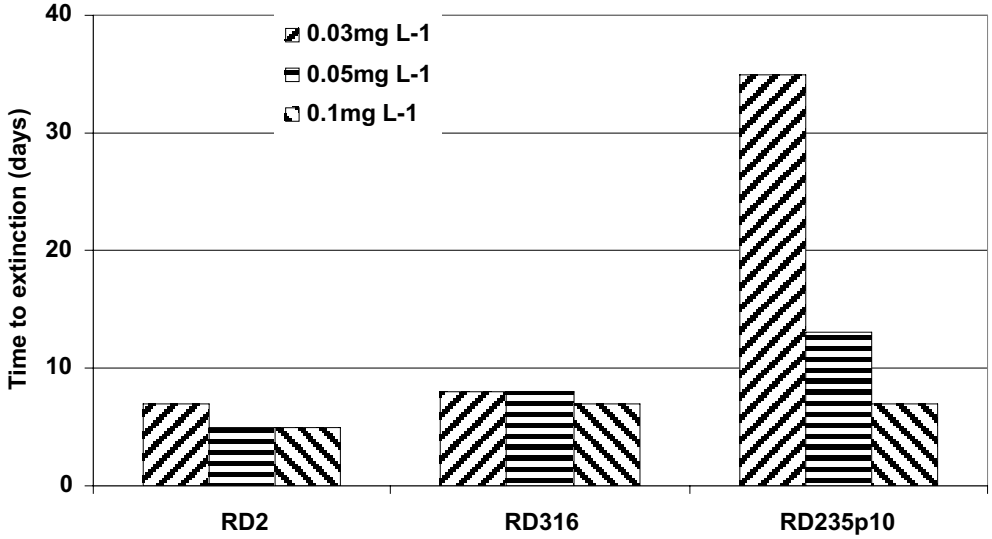

Figure 9. Time to population extinction in cultures of three strains of *Rhyzopertha dominica* (all stages) treated with constant concentrations of phosphine (Hyne and Winks 1997).

Preliminary research into the use of the essential oil of *Melaleuca alternifolia* (tea tree oil) in museum conservation

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Abstract

Melaleuca alternifolia (tea tree oil) has been used for medicinal, antimicrobial, sterilising and sanitation purposes since the 1930's. It's remarkable properties contributed to the Second World War effort and pioneering research was carried out by the Sydney Technological Museum (now the Museum of Applied Arts and Sciences). The museums essential oil research plantation was located at Castle Hill, a site now used by the museum for collection storage.

In this case study, a tea tree oil product called *Bactigas*®(a BOC product) is being assessed for its potential to control recurrent mould in a large object storage area at the Castle Hill site. Alternatives methods of control are also being reviewed.

Factors being considered include spore counts, frequency and effectiveness of treatment, possible surface build up and potential for damage. Toxicity and Occupational Health and Safety issues will be considered. The potential use of this essential oil in museum conservation will also be discussed.

Introduction and history

With growing international interest in environmentally sustainable products and natural therapies, the medicinal properties of essential oils have received renewed attention from researchers, manufacturers and consumers alike.

The oil of *Melaleuca alternifolia*, in particular, has attracted a great deal of attention. This is evidenced by the diverse range of information about tea tree oil that is available on the Internet.[1]

A visit to the local health food store or pharmacy confirms that tea tree oil is marketed as the active ingredient in a wide range of commercial products. Mouthwash, ointment, skin conditioner, head lice treatment, shampoo, pet care; the list of products advocating the effectiveness of tee tree oil as an insecticide, bactericide and fungicide are numerous.

The Museum of Applied Arts and Science has a special interest in these developments. Much of the pioneering research into the properties and use of tea tree oil was carried out by the botany and chemistry laboratories of the museum during a long and distinguished history (1897-1979) of research into essential oils.[2] The work of museum researchers, including H.G.Smith, J.H.Maiden, R.T. Baker, A. R. Penfold, H.H.G. McKern, F. R.Morrison, J. L.Willis and E.V.Lassak, appears regularly in the documented record of the Australian essential oil industry.

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* The Museum of Applied Arts and Sciences incorporates the Powerhouse Museum and Sydney Observatory.

The Type physiological form of *Melaleuca alternifolia*, recommended as the only type suitable for medicinal and dental purposes, because of its low percentage of cineole and high percentage of 1 Terpinen-4-ol, was recognised by Penfold and Grant in 1925.[3]

Given this background, it is not surprising that we should choose to trial the application of Bactigas®, a product developed with 0·3% tea tree oil as its active ingredient, for the control of a large outbreak of mould at Castle Hill museum stores. [4]

Interestingly, the site of the store, at Castle Hill, in the northwestern suburbs of Sydney, was originally developed as a plantation to grow elite, oil rich Australian plant species. Selection and propagation of these plants enabled detailed research into the properties and oil yield of different physiological and regional variants of the same plant.

It can be assumed that selection of the site was based, in part, on the needs of the plants. Given that many *Melaleuca*, including *Melaleuca alternifolia*, require boggy conditions, we conclude that the site was carefully selected because it has a higher local rainfall than other potential sites in the Sydney basin. This view was clearly expressed, in the late 1970's, by the last manager of the plantation site.

Since the 1970's, the Castle Hill site has mainly been used for collection storage. Development of the site has been managed so as to ensure that many of the elite, oil-bearing trees remain, as evidence of the significant scientific research carried out there.

The first collection storage buildings built on the site were designed to meet only basic requirements and they continue to pose environmental problems. The estimated cost of substantially improving these older stores was estimated, more than a decade ago, to be uneconomically high so that only minor improvements have been made to them, generally to improve insulation and seal the buildings against rodents and insects.

Recently, a large new store was designed and built, to preservation specification, providing opportunities to improve the storage conditions of the stored collection on this site. Relocation of the more vulnerable collection items to this store is currently underway.

In interim, the high humidity of the site and the control of associated mould growth in the older buildings still pose a conservation and health and safety challenge.

Case Study: The Reduction of a Mould Outbreak at an Off Site Museum Store.

Background

A mould outbreak occurred in a large off-site store. This Store is 1030sq metres and 7 metres high, made of a lightweight construction with roller doors at both ends. The store has no environmental control or regular cleaning. Previously a project was carried out in this Store that required daily access for an extended period. During this time no mould was evident on the surfaces of the stored collection. Once this

work was complete the Store went back to being visited once a week. At the beginning of the year 2000 a mould outbreak was found in this store and a mould reduction and disinfestation project began.

Influence of environmental conditions on mould growth.

Environmental conditions were obtained from the Bureau of Metrology, Sydney. Annual minimum and maximum temperature and relative humidity were plotted. Results are shown in Figure 1.

The weather conditions over the last 18 months (January 2000 to June 2001) were reviewed. During this period the recorded climate was humid and there was more rainfall than normally expected for this period. During this period the average relative humidity was over 65% for 10 months and the store was relatively undisturbed for this time. This would provide an ideal opportunity for mould spores to germinate and grow. Fungal filaments do not absorb water vapour from the air. They absorb water from materials through the very tips of the hyphae where the water impermeable cell wall has not been completely formed. Water in materials is influenced by the relative humidity, the materials physical characteristics eg: porosity and surface area and chemicals in the materials (salts, tannins, glycerol, sugar etc).
If the moisture content in the materials is appropriate, the conidia if viable and activated will germinate.[5]

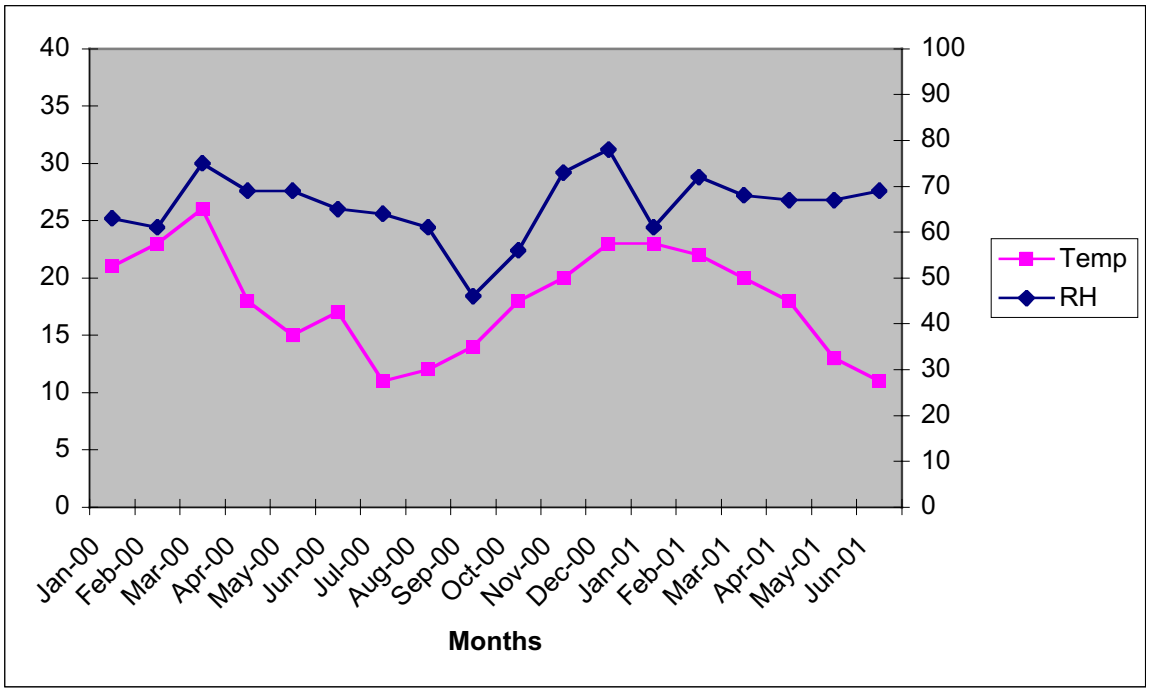

Figure 1: Mean Temperature and relative humidity for January 2000 to June 2001
(Measured from the closest Bureau of Metrology site)

In the Store, it was found that mould grew on organic (eg: leather strapping, wood) and inorganic (glass, vinyl, painted signs) materials. Growth on inorganic materials can best be explained by dust being the most common microenvironment that supports fungal activity on the surface of materials. The dust captures the conidia and other particles, which absorb enough moisture to allow the conidia to germinate and support, limited growth.[6]

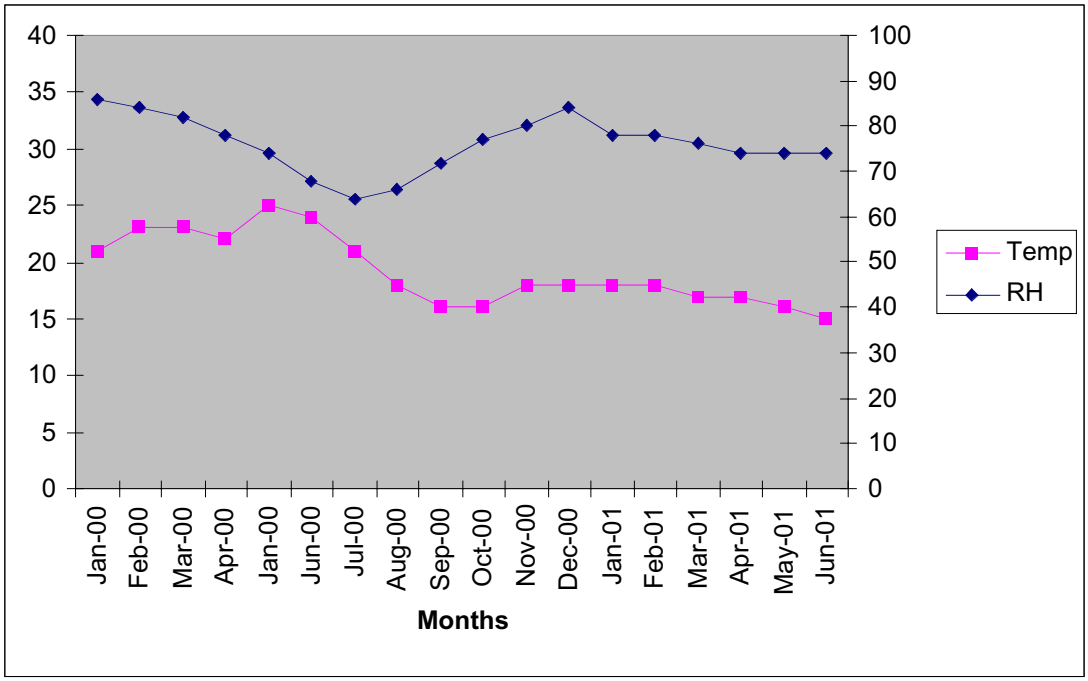

Figure 2: Maximum temperature and relative humidity measurements inside the Store

Comparing the outside conditions to the inside Store conditions showed that inside the Store the maximum and minimum temperatures were not as extreme as the outside. The inside average temperature was lower compared to the outside and the average relative humidity was higher when compared to the outside. Therefore the Store environment and conditions provided an excellent opportunity for mould growth.

Initial Bactigas® treatment

The Mechanical Services Section, responsible for the building and environment maintenance, undertook an initial treatment using an aerial spray of Bactigas® (composed of 0·3% tea tree oil: 97% liquid carbon dioxide: 2·7% ethanol). Bactigas® has been shown to have antimicrobial and sanitizing properties. It is particularly used for air conditioning duct cleaning and to improve indoor air quality. [7]

Bactigas® use will only be effective with surface contact for reduction of mould spores and growth and requires a time period to reduce air spora. Bactigas® trials were carried out by Bioassay [12] which showed that a Bactigas® concentrate (10% tea tree oil in ethanol) can achieve an immediate microbial reduction of 10^{3} for mould over 7 days. Further testing showed that Bactigas® (3% concentrate and 97% liquid carbon dioxide) has a greater microbial reduction than the Bactigas® concentrate. This can be explained that a dilute concentration is more miscible and has better absorption. At this time there is no standard test for aerosols so the above trials can provide only an indication of Bactigas® effectiveness. Bactigas® is suspended in air as droplets for 2 hours and will require multiple or regular application to kill all the mould.

Current work is being carried out in the USA to develop a range of standard testing to evaluate essential oil anti-microbial activity.

To determine the reduction time for the affected Store it was undertaken to measure the microbial count reduction over a 7 day period by treating the Store with Bactigas®, at a concentration yet to be determined, and measuring viable colony forming units (CFU/vol air) before treatment, after 6 hours, then after 7 days. This test will begin when the budget has been approved. Meanwhile the clean-up work is underway

Clean up of mould affected material.

The clean up work needs to achieve 2 outcomes.

1. kill the active mould and clean affected material by removing the visible surface growth
2. remove dead mould components from Store environment using appropriate filtering systems. Even if the mould is killed, the fungal structures such as conidia and beta-glucanns in hyphal fragments, remain antigenic and mycotoxic. These characteristics are not altered by any treatment. Therefore a health hazard exists even when the mould is dead. [5,8,9]

Clean up procedure

Procedures have been modelled on the Florian Aseptic Technique steps [5]

- Prioritise material to be treated
- Cover and remove objects from the affected Store and move to conservation treatment area which has good ventilation
- Random testing of internal surfaces will be carried out using Aniline blue stain (a stain which penetrates protoplasm of fungi and fungal hyphae are stained blue, making them more visible) to determine if mould is present [9,10]
- Dry each object by placing it outside with forced air circulation
- Clean each object using a variety of dry and wet treatments. Such as brush vacuuming using vacuum cleaners fitted with a HEPA (high-efficiency particulate air) filtering system, electrostatic dusting, localized Bactigas® treatment and swabbing using 70% ethanol. An exhaust hood is used to remove loose debris away from the cleaning area. Safety equipment such as masks and gloves will be worn.
- Keep work area clean by regularly wiping work surfaces and tools with a commercial disinfectant.
- Immediately after cleaning, place object in a Cryovac bag and flush with 45% relative humidity and then seal the bag.
- Return the objects to Store.

While the object cleaning is underway measures to improve to the conditions in the Store such as increased air ventilation to create a weak airflow in the storage area[11] and the installation of a dehumidifier fitted with a HEPA filter have been implemented. Depending on the results from the microbial count reduction a regular Bactigas® treatment and monitoring of airspora may be carried out.

Summary

This work will continue for some time and progress reports will be made. Other issues about Bactigas® use such as how residual it is, reaction with materials and possible future handling problems will be

assessed. The use of Bactigas® to reduce the mould in a large store is considered appropriate, due to the scale of the problem, lack of immediate environmental control and need to reduce further contamination.

Acknowledgments

We are grateful for the assistance provided by Mary-Lou Florian and Robert Ryan in the study.

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