12. Mold/Fungi

12.1 Purpose

This chapter is intended to aid conservators with identification, evaluation and treatment in order to respond to three types of micro-biological attack:

- a sudden, localized incident that produces fungal bloom on a small number of collection materials
- the cleaning of dry (and possibly old) fungal growth from damaged surfaces
- the conservation treatment of supports deteriorated and stained by prolonged fungal growth.

The chapter is primarily devoted to paper, however, other materials often encountered in museum, archive and library collections are covered to some extent. (The staining pattern known as foxing has been published in AIC/BPG/PCC 13. Foxing, 1993. For response to major disaster, refer to disaster literature.)

It is not necessary to identify the fungal species in order to respond to an outbreak or to treat mold damage to a substrate. An understanding of the organism and its propagation is, however, essential for prevention and appropriate response. This chapter provides conservators with some general information about fungal growth and biodeterioration.

It is hoped that this chapter will provide the incentive for conservators to begin to record, document and develop a body of information regarding the observed interaction of fungi and their substrates.

12.2 Factors to Consider

12.2.1 The Organism

A. Ubiquitous Nature and Survival Capabilities

Filtration of air to remove particulate matter and good housekeeping practices that prevent accumulation of dust can reduce the incidence of mold growth, but cannot eliminate it completely. Mold spores and the potential for growth are present in all environments. Air-handling systems that exclude mold spores from an environment are not feasible for most institutions housing collections. This is not only because such systems are costly, but because contamination of the air would occur with each staff member, researcher or visitor who enters the environment. In addition, many materials in our care have been contaminated with spores during manufacture and require only the right environmental conditions to germinate and grow. Therefore, it is impossible to approach the problem by attempting to exclude mold spores from a collection environment.

In nature, fungi form a necessary function as part of the cycle through which organic matter is reused. In museum, archive and library collections, we are attempting to arrest the cycle through which organic matter is decomposed to release carbon dioxide. The cycle is temperature and humidity dependent. Thus, environmental control is an essential tool for preventing germination and growth.

B. The Morphology of Mold: Structure and Identification

Many of the fungi which are of concern to conservators are in the classes *Ascomycetes* and *Deuteromycetes*, in the family *Eurotiaceae*. These are sometimes called "higher fungi" as they are more complex in structure. They include both the genus *Aspergillus* and the genus *Penicillium*, two of the most common and widely distributed fungi. Many strains of these two groups have been extensively documented. (See 12.5 Bibliography).

(Note: The name of an organism is binomial, that is, it is composed of two words. The first is a noun designating the genus and the second is often an adjective describing the noun. One example is *Aspergillus niger*).

Fungi have two basic structures: vegetative and reproductive.

1. Vegetative

   The vegetative portion is characterized by a branching of thread-like filaments called *hyphae*, which spread from a single germinating spore. These *hyphae*, collectively referred to as *mycelia*, branch out across and within the paper or other substrate, and may or may not be visible to the unaided eye. They form the equivalent of a root system for the plant, drawing nutrients and moisture from the host. Once the mycelia are mature, the hyphae produce "stalks" known as *conidiophores* which are the first stage in the reproductive system. At this stage of development, the *thallus*, or filamentous *soma* or body of the fungus appears soft, downy, usually green or yellow in color, and can be seen without magnification. (It should be noted that there will be hyphae extending out beyond the more mature portions of the thallus.) In non-laboratory situations, visible vegetative growth generally appears after humidity remains high enough over a period of time to germinate spores; and if still, moist conditions have been maintained for at least 2 - 3 days.

2. Reproductive

   The conidiophore produces *sterigmata* (or *phialides*), the reproductive structures, which produce the spores. The structure of the conidiophore is the most valuable diagnostic characteristic at the genus level. The conidiophore of the genus *Aspergillus*, for example, enlarges at its apex to form a globose, hemispherical, elliptical, or long clavate structure known as the vesicle which furnishes an enlarged surface for the attachment of spore-bearing cells. The genus *Penicillium* is characterized by a branching, broom-like structure. Species differentiation is possible only by detailed microscopic examination using a compound microscope.

3. Color

   The color of a mature colony provides only the most general guide to the identification of the organism, and may vary widely in mycologically identical strains, depending on their stage of development, the nutrients in the substrate, the presence of other organisms, age, pH and other environmental factors. For example, the mature *Aspergillus* may vary in color from yellow to black; *Aspergillus niger* may take on a variety of shades from tan to black. *Penicillium* is characteristically green, but may also be blue or yellow.

The center of the mold will usually appear darker in color because this is the oldest portion of the mold, which is now beginning to produce spores. The color of a particular mold may be caused by the micro-nutrients of the substrate, pigments in the substrate, pigments in the mold itself or the color of its spores. (TP)

4. Identification

The identification of species through the distinctive size, shape and color of the spores is beyond the capability of most conservation facilities. The spores, though unique and very distinctive are so tiny as to be impossible to identify at the magnification levels available in most conservation laboratories. Most text illustrations are either drawings from higher magnification or Scanning Electron Microscope (SEM) micrographs.

C. Environmental and Nutritional Factors in Growth and Survival

1. Moisture

The most important factor for germination and growth of mold mycelia is moisture.

High relative humidities (75% or above) may cause a mold spore to germinate, but moisture content of the substrate is critical to its growth and survival. Hyphae (the name given to individual strands of the mold) are analogous to liquid-filled soda straws which require lots of water to transport nutrients from the substrate to the mold and to remain turgid. With these liquified nutrients, the hyphae exude a slime, called glucan, containing enzymes which further break down the substrate. As this process occurs, the mold mycelial mat grows and, in a few days, will be visible to the unaided eye. (TP)

All molds require moisture to grow, to produce enzymes for obtaining nutrients from the substrate on which they are growing, and to reproduce. Organic materials, such as paper, wood, and textiles are hygroscopic and will take up moisture from their surroundings. Water held within cell walls of the substrate is called "bound water", whereas moisture held between the cells is considered "free water". The percentage of moisture content of a substrate is the relationship between the weight of water present in the material expressed as a percentage of the oven-dry weight. For fungal decomposition to occur the moisture content of the substrate must be 20% or above. (TP)

In a practical setting, for example a below-grade library stack area, the general relative humidity of the open spaces may be 55-60%. Most would consider this level to be a "safe" one for such an environment. Yet mold is discovered growing on bound volumes in a corner, down by the floorline. How can this be? If one were to measure the relative humidity in this corner and sample the moisture content of the bound volumes, it would be found that the moisture wicking through the exterior basement walls and floor was sufficient to provide enough moisture in the micro-climate of that corner to not only allow mold spores to germinate, but to allow the mold mycelia to withdraw enough water from the volume covers to burst into a

"mold bloom". Just because the hygrothermograph in the center of a room says everything's "OK", pockets of moisture may still be present in undisturbed areas of the room which will let their presence be known in the form of a mold bloom. Managing moisture accumulations in "dead air pockets" (micro-environments) of collection storage areas is critical to the control of mold production. Simply placing fans in key areas during times of high humidity or prolonged rainfall may often prevent mold blooms. (TP)

Most mold that grow on library/paper based materials become active only when RH reaches 70 -75 and remains at that level for a few days. Higher RH and temperature increase probability of infestation and rate of growth. (LOP)

For a discussion of how RH may be related to the moisture content of various materials, see Arai, et. al., 1988.

2. Temperature

Three critical temperatures for fungi are the temperature below which no growth occurs, the temperature above which no growth occurs, and the optimum temperature, at which the most rapid growth takes place.

Most microbial forms found in collections will grow in temperatures ranging from 59 to 95°F (15 to 25°C). The optimum temperature for the growth of specific molds is usually around 86°F, but is difficult to determine, in part because of other variables in environmental conditions, and in part because culturing organisms in the laboratory differs from the growth of the same organism in uncontrolled conditions. Optimum temperature may also vary by natural selection over time. (RK)

The temperature below which no growth occurs is not synonymous with the temperature at which the potential for growth is destroyed. Fungi and fungal spores can survive long periods at sub-zero temperatures. (Pure cultures purchased from biological supply houses are freeze dried. One need only add moisture to reactivate them.) This ability to withstand extremely low temperatures in a dormant state is utilized in the long term storage of fungal cultures in liquid nitrogen at a temperature of -196°C. Fungi are less tolerant of alternating below-freezing and above-freezing temperatures.

The temperature above which no growth occurs is not relevant in dealing with collections, since temperatures too high to allow mold growth or high enough to seriously damage existing mold growth, would unquestionably be harmful to artifacts and collections. Most hydrated conidia and living hyphae are killed at temperatures just around 40°C and killed by freezing. (MLF)

3. Air Movement

Moving air allows for rapid evaporation and drying, thus preventing the retention of high moisture content which favors growth. Given the same temperature and RH, air movement will sometimes determine whether or not mold grows even in high moisture conditions. (LOP)

4. Nutrients

   Most naturally occurring compounds can be utilized by fungi as sources of carbon and energy. The elements required include carbon, hydrogen, oxygen, nitrogen, sulfur, potassium, and magnesium. Trace elements such as iron, zinc, copper, manganese, and in some cases, calcium may also be required. Certain vitamins may also be needed.

   The growth and development of fungi are significantly affected by the nature of the nutrient source. A rich medium can compensate in part for other adverse environmental factors.

5. pH

   Fungi prefer a slightly acid medium for growth, with a pH of 6 being near optimum for most species. (Alexopoulos & Mims, p. 19) There is some anecdotal evidence that pH in either the upper or lower range of the scale inhibit growth, but this may be due to other variables. Research has shown that pH significantly affects stain intensity and color (Szczepanowksa and Lovell, 1992). The pH of the substrate may be altered by fungi metabolic products. (MLF)

6. Light

   The role played by light in fungal growth is not well defined. (MLF) Because fungi lack chlorophyll, light plays a minimal role in the growth (metabolic processes) of fungal species. Some species of fungi are diurnal, that is, light actually inhibits growth during the day and growth is accelerated at night. The mold ends up with a growth pattern of concentric circles. (TP)

   Light may trigger sporulation in fungi that require it. Cochrane speculates that light checks growth, thus initiating a chain of events that lead to sporulation. Belayakova reported exposure to ultraviolet light affected pigment production. However, it does affect the reproductive processes. Light is essential for the formation of *conidiophores* and spore production in many species (Cochrane, 1958).

   Light also plays an important part in spore dispersal since the conidiophores of many fungi are positively phototropic and discharge their spores toward the light. Research has shown that exposure to ultra-violet light is injurious or lethal for some species. (Belayakova, p. 73)

12.2.2 Effect of Organism on Substrate

A. Decomposition

Fungi are *saprophytic*, organisms that live on and derive their energy from dead or decayed matter. The mycelium grows on the surface or within the substrate. The hyphae obtain nutrients by osmosis through the hyphal walls, causing the disintegration of the organic matter they utilize. Fungi secrete enzymes to break down proteins into amino acids, carbohydrates to sugars and fats to fatty acids and glycerins. The organic substrate is broken down by

enzymes into the necessary nutrients which are absorbed through the hyphae walls. (MLF)

Any amount of fungal growth for any period of time will decompose the substrate on which it feeds, however, damage to cellulose is generally observed only after extended period of growth. The shorter the period of exposure, the lesser the damage.

Fungi are opportunists and will digest what is most easily available. For example, they may digest only media or sizing (starch or protein) between paper fibers. (MLF)

B. Staining

The exact cause of the stains produced by mold growth or in dead or dormant colonies is difficult to determine. Stains may be caused by metabolic processes, such as acids produced during the hydrolysis of the cellulose or other nutrient matter; chemicals produced during the digestive process and excreted by-products; or simply by pigments present in the fungal structure itself. Certain molds are known to produce pigments, and may cause extensive color changes in the substrate, even though their growth is limited (Beckwith, 1940, p. 331.) Belyakova identified numerous *genera* which produce stains on paper due to the production of pigments. The color of the stain is not an accurate guide to the specific mold which caused it, since the nature of the substrate effects the morphology of the organism. Belyakova noted that *Penicillium frequentas* produced yellow stains in some instances, pink stains in others.

There is some evidence that staining is most prevalent in mature colonies that have been allowed prolonged growth and development, and is most pronounced in those areas where the older hyphae have deteriorated. Staining seldom occurs when the growth is removed during the vegetative stages, or before the mature organism begins to deteriorate. Staining may also result from attack on the organism, including adverse environmental conditions designed to retard its growth, or even fumigation.

12.2.3 Vulnerability of Materials

A. Paper - Cellulose, Sizes, Coatings

In 1940, Beckwith and his coworkers isolated 55 different mold cultures from old book papers. This included eleven *genera*, of which *Penicillium* and *Aspergillus* were the most commonly found. In the study, spores were removed from the papers, transferred to a culture medium and grown under laboratory conditions. Not all of them may use cellulose as a medium for growth, but certainly some of the strains of *Aspergillus* and *Penicillium* would be likely to attack cellulose or paper additives, sizes or coatings. At least 180 genera or species of mold are known cellulose destroyers; i.e., they use the cellulose fiber as a nutrient. (Belyakova, p. 184) The effects of severe fungal decomposition on paper can easily be seen. The paper loses strength and becomes soft and spongy, often with areas of loss or thinning clearly visible. Less severe cases are often apparent only when viewed through transmitted light, or evidenced by differential wetting characteristics during treatment. Damage to cellulose is generally observed only after extended period of growth.

Other fungi that do not actually consume cellulose may damage paper by weakening the fiber bonding as they feed on other materials in the paper. The sizes and coatings added to the paper during manufacture to improve printability, texture, color or brightness are a potential source of nutrients, and may include starch, gelatin and casein. Very little is known about the various synthetic sizes, as much of the research in this area took place before they were in common use.

Paper in bound volumes is less vulnerable to high ambient relative humidity and spore deposition than unbound paper. Cryptogamic fungi seldom occur in closed volumes under such conditions, but rather on the bindings and on unbound sheets of paper exposed during prolonged periods of dampness. In cases of flood or other severe wetting, book paper may be considered to be more vulnerable, since the bulk of the volume and the compression of the paper at the spine slow the drying process considerably.

B. Media

Fungi may damage media if the media layer is more accessible than the paper and if there are attractive components that it may be utilized as nutrients. Mold can destabilize iron gall ink and increase its solubility in mold damaged areas. Pastels are particularly susceptible to mold damage because of the gum binder. (LOP)

C. Bookcloth

Many bookcloths, including those of cotton and linen, are cellulosic and are vulnerable to the same range of mold species that affect paper. Like paper, the fillers and coatings added during manufacture provide an additional source of nutrients. The unsized cloth frequently used in bindings from India and Southeast Asia is particularly vulnerable. Because it is often thin, the adhesive used in attaching the cloth to the boards often penetrates the weave of the cloth, allowing mold to grow on the surface. Starch-filled buckram, commonly used in more temperate climates, is also an excellent source of nutrients and contamination. Manmade fibers,or natural fibers coated with synthetic resins, i.e., pyroxylin cloth and acrylic-coated buckram, are more resistant to mold, but not entirely immune.

D. Leather

Collagen in tanned leather is more resistant to mold growth than in untanned leather. Chrome-tanned leathers are relatively impervious, vegetable-tanned leathers considerably less so. Book leathers are, unfortunately, vegetable tanned, chrome leathers being used primarily in shoes, luggage and other such items.

Studies indicate that mold growth does not affect leather in the same way that it does cellulose. The mold apparently does not attack the hide-tannin complex itself.

The components of leather which support mold growth are the lubricants, the conditioning materials and the finish. It would seem from the literature cited

above that high ambient relative humidity rather than mold damage is the primary cause of deterioration of leather.

Special book binder's leather is now available tanned with non-hydrolyzable vegetable tannins. (MLF)

E. Adhesives

Pastes (made from vegetable starches), glues (made from animal products) and gums (made from vegetable resins) are all subject to mold growth to varying degrees.

Synthetic adhesives, including cellulose ethers, polyvinyl acetate emulsions (the so-called "white glues" which vary enormously in composition and properties), pressure sensitive adhesives on tapes and labels, heat set adhesives such as those used in dry mount papers, and aerosol spray adhesives such as those used in dry mount papers, are more resistant to mold, but not entirely immune.

F. Photographic Materials

Almost all photographic materials have a common structure consisting of a support layer (substrate) which carries a binder layer (emulsion) containing the microscopic image particles. While most photographic materials since the turn of the century have a gelatin binder layer, the two predominant processes from the nineteenth century have binders made of either albumen or collodion. The support layer may be metal, glass, paper or plastic film such as cellulose nitrate, cellulose acetate or polyester. The image particles may be metal such as silver, pigments, or dyes. The format of the photograph may be a negative, a positive print or transparency, a microfilm roll or microfiche. (SW)

All layers in the photographic structure are susceptible to fungal attack and growth. Although gelatin binders provide a rich nutrient source for mold growth, other binders can support fungi given optimum growth conditions. While plastic films used for contemporary film stock are generally resistant to fungal attack (Bard and Kopperl, 1971, p. 53), both paper and glass supports are vulnerable (glass can be etched as fungi extract minerals from the glass matrix). Image materials can be attacked directly in processes having a pigment image such as hand-colored photographs or various pigment/gum processes. Of course, image material can be lost inn all processes as the binder or support layer is consumed by fungal growth. (SW)

12.2.4 Health Hazards

The ability of fungi to grow on different substrates under a wide range of environmental conditions have enabled some of them to colonize living animal tissue. Their invasion of living tissue is responsible for many forms of disease in both warm and cold blooded animals. Such parasitism, however, is incidental to their normal saprophytic life. The production of metabolites toxic when ingested or allergenic when inhaled or otherwise contacted is in no way essential to the survival of the species.

Many of the diseases result from inhalation and are respiratory in nature, including the most common, histoplasmosis, which is connected to certain ascomycetes. Pathogenic

or toxigenic *Aspergilli* have been recognized in all but seven of the species groups. Three types of disease are caused by *Aspergilli*, two of them affecting man, mycosis (either primary or secondary) resulting from the invasion of the living tissue by the fungus, and allergy, which is associated with the inhalation of conidia or other contact with the fungi. Involvement of almost every body organ has been reported; however, *Aspergilli* are most frequently respiratory pathogens.

In general, persons with allergies or respiratory problems should not handle materials affected by mold. Staff should wear appropriate masks, gloves, and protective clothing and avoid breathing in mold residues. Materials should be handled under a hood and fungal growth should be vacuumed away using vacuums, rather than brushed, to prevent inhalation and dispersal. (Note: If vacuums are used in a fume hood which do not contain specialized filters that prevent contamination, special precautions will be required when disposal.)

Persons with diabetes or impaired immunity as well as those taking steroids should also avoid affected areas and materials. (LOP)

12.2.5 Response

A. Inactivation

Proper environmental maintenance is universally agreed to be the most effective means of preventing and controlling fungal growth. Reducing humidity and increasing air flow inactivate and effectively kill fungal growth. Inactivation by changing environment is a fungistatic method. Drying, cleaning and correcting the environmental conditions which encouraged growth are considered sufficient treatment. Fungitoxic methods, the use of toxic chemicals, applied topically or via fumigation to attempt to kill spores, is no longer considered necessary or appropriate, except for very serious outbreaks.

B. Identification

The most important reason to identify the type of fungi is for protection for staff who will be handling an outbreak.

The easiest method of mold identification, at least to genus, is to call the mycologist or microbiologist at the closest hospital or university. Most large hospitals have such a professional on staff who is responsible for the control of mold and mold spores in sterile environments, such as operating rooms. These individuals have the expertise, equipment and usually the willingness to assist you. Often the mycologist will incubate the samples, identify your specific molds and give you some idea of where the contamination may have originated. (TP)

C. When Outside Help is Required

Small to moderate outbreaks of mold involving a limited number of items can often be handled in-house if no highly toxic species are present. The amount and type of outside assistance required will depend on the resources of the institution or owner and the type of material affected. A major bloom, involving a large area of a collection or highly toxic mold species will require

outside professional assistance and advice to stop the mold growth, clean the collection and render the affected area safe for use again. The information in this chapter is applicable to small and moderate outbreaks that do not involve highly toxic species. (LOP)

D. No More Thymol Chambers

Paper conservators have used some form of thymol and/or ortho phenyl phenol diluted in a solvent or volatized by heat to "control mold" for many years. Research has shown that neither kills mold very well. Once the moldy object has been taken out of its moist environment, the actively growing mold is killed. There is no need to "treat" it with some sort of chemical "mold control" material. Some conservators and conservation scientists maintain that the conservator has not "done her job" unless some sort of chemical is used against mold. In fact, thymol and other fungicides or fumigants kill only certain kinds of spores which are hit directly, give no residual protection and may be harmful to the object and/or the applicant. Besides, such treatment certainly doesn't prevent mold spores from landing on the piece once the chemical dissipates. The United States Environmental Protection Agency (EPA) regulates the labeling and use of all pesticides. Thymol and ortho phenyl phenol are pesticides. They are NOT LABELED for use as fungicides, as a heat-generated vapor, a topically-applied spray, or a fog, by diluting crystalline material with a solvent. Ortho phenyl phenol is labeled for use as a disinfectant under the brand name Lysol, and for commercial use in latex paints and other liquids as a mold retardant. It cannot legally be used in a manner inconsistent with this labeling. (TP)

In some studies, thymol has been shown to favorably affect the growth of some species of fungi. (TP, HS)

E. Fumigation

Because all materials toxic to fungi are also harmful to humans and collections and because no fungitoxic compound provides residual protection against future outbreaks, fumigation is no longer recognized as a necessary step in response to fungal growth. In recent years, the conservation field has benefitted from contact with microbiologists and specialists who stress drying and changing environmental conditions as non-toxic, fungistatic measures that effectively kill fungal growth. Also, increased awareness of health risks to staff and more stringent regulation on the uses of toxic chemicals have made the use of in-house fumigation chambers expensive and impracticable.

Fumigation with a toxic gas can only be performed by a licensed professional fumigation firm or by an individual who is properly trained and licensed for such applications. If the facility relies on in-house, licensed staff to perform such fumigations, special insurance is required for this type of work. If an institution has a fumigation chamber and operates it without licensed personnel, they are doing so illegally and leaving themselves wide open to the possibility of not only legal action by EPA and/or State authorities, but also to liability claims of staff and others who might come into contact with the treated materials. Most vacuum chambers in museums and libraries do not

meet current requirements for proper aeration of fumigated materials. The operator of such a chamber is therefore unwittingly exposing personnel to possible harmful effects of a toxic fumigant. (TP)

Materials and methods traditionally or currently used for insect control are not effective against mold or have not been evaluated for their effectiveness. This includes sulfuryl fluoride (Vikane), para-dichlorobenzene, Vapona no-pest (dichlorovos) strips, freezing, inert gas treatment with argon, nitrogen and carbon dioxide and oxygen scavengers. (LOP)

F. Fogging

In some instances of large outbreaks of fungal growth in library or archival stacks, fogging the stack area with a fungicide, such as ortho phenyl phenol, has been part of the recovery procedure. This step preceded drying and vacuuming of collection materials.

OPP is not an effective fungicide in its gaseous state, but as a liquid dissolved in ethanol or water (Lysol), it is effective when it comes in direct contact with the mold. Dissolved in alcohol, it has been used for fogging areas with serious mold blooms. Fogging produces tiny droplets like a mist. (LOP)

Such measures must be undertaken only by professionals. Some specialists, however, would question this added step in the recovery process, which delays the drying process and may produce more extensive staining on surfaces of collection materials than simple drying and vacuuming because the fungi may produce more colored compounds due to metabolic changes and mutations when under attack. Recently, a large outbreak in library stacks was successfully treated by drying and cleaning without fogging.

(See Kovacic, E.S. and L. S. Wolfson, "Moldbusters,"Conservation Administrator's News, No. 50 (July 1992): pp. 6-7, 28.

12.3 Materials and Equipment

12.3.1 Removing Mold Growth on Dry Materials

(Items A through C were prepared by Lois Olcott Price with additional comments from Dr. Thomas Parker.)

A. Fume Hood

Whenever possible, materials should be cleaned in a fume hood to reduce the spread of spores to other areas of a lab or facility. If a fume hood is not available, moldy material should be cleaned outside or in an isolated enclosed area or room that can be thoroughly cleaned after mold removal is complete. If necessary, an isolation booth or make-shift fume hood can be created with plastic sheeting and a window with a strong exhaust fan.

B. Masks and Protective Clothing

Anyone participating in mold removal that involves more than a few minor spots of infestation needs to take serious precautions. A respirator with high efficiency particulate air (HEPA) filter should be used. Dust masks are not adequate because mold spores are so small they pass through them easily.

Protective clothing should also include, at a minimum, disposable gloves and safety glasses. Coveralls and protective hair and shoe covers should be used whenever the mold removal procedure releases a significant quantity of mold spores into the air. These items should be discarded or washed in disinfectant at the end of each mold removal session. Personnel involved should shower as soon as possible.

C. **Vacuum Cleaners**

Vacuuming is the most effective means of removing mold, but the choice or modification of the vacuum is critical to avoid exhausting spores back into the work area. Vacuums fitted with a high efficiency particulate air filter (HEPA) or a water bath filter are acceptable for small outbreaks involving a few items. For larger clean-ups, a wet-dry vacuum can be modified. Place several gallons of a fungicide such as Lysol (which contains ortho phenyl phenol as its active ingredient) diluted with water according to label instructions in the tank. Extend a plastic tube from the hose inlet into the solution so that incoming moldy air passes through the fungicidal solution.

If a vent or rheostat for controlling suction is not part of the vacuum design, some control can be achieved by drilling a hole(s) in an appropriate part of the hose and covering and uncovering the holes with duct tape. The most appropriate attachment for any particular job should be chosen from those available. The crevice tool or the small upholstery attachment are usually the most useful. If the material to be vacuumed is not under a protective screen (see cleaning section), the attachment should be covered with a soft porous cloth, such as cheese cloth, to avoid sucking in any part of the object. (LOP)

Standard household vacuum cleaners should NOT be used for vacuuming mold from artifacts. The exhaust from such a vacuum cleaner will blow mold spores and bits of mycelia into the room. If a vacuum cleaner is to be utilized for conservation of moldy materials, first be sure the materials to be vacuumed are thoroughly dry. Use a wet-dry vacuum cleaner with a soft bristle brush for materials which can withstand this type of treatment. The brush is essential for dislodging matted mold and for getting mold out of cracks and crevices. Although a crevice cleaning tool creates a stronger vacuum, it is usually made of hard plastic and may abrade the materials being vacuumed. Mix a dilution of Lysol and water and place it into the vacuum cleaner reservoir. Use sufficient liquid to ensure that all pieces of mold, mold spores and debris will be trapped in the water and disinfected. To further insure that no particulate matter will be blown around the room, one may modify the inlet side of the wet-dry vacuum cleaner so that the incoming air flow will be directed via plastic piping to enter beneath the Lysol/water level. This bubbling action will trap all debris. Another type of large vacuum cleaner which is sometimes used for vacuuming mold is one equipped with a HEPA filter. HEPA filters are so fine they will trap most mold spores before they have a chance to exit the vacuum cleaner. However, there are mold spores small enough to escape a vacuum cleaner equipped with a HEPA filter. HEPA filters are expensive and are only available for certain types of commercial vacuums. A distributor of

supplies and equipment to the carpet cleaning trade would be a source for such equipment. (TP)

D. Mini-Vacuums

Mini-vacs are used for the removal of mold from the surface of paper. They are most useful where mold is an infrequent occurrence. Most models may be operated by either direct electric power or with batteries. They are available through camera and electronic equipment suppliers. (Note: The use of mini-vacuums, while convenient, do not contain HEPA filters, and require appropriate measures for disposal so that work areas will not be contaminated.)

E. Vacuum Aspirators

Vacuum aspirators, like the mini-vacs, are used for the removal of mold colonies from the surface of both books and paper. They are more effective than the mini-vacs and are a worthwhile investment where mold is a recurring problem.

Vacuum aspirators are relatively easy to construct, and require:

1. A small vacuum pump with regulator.
2. A 3' length of clear plastic tubing of appropriate inner diameter to fit the vacuum pumps intake port.
3. Two sections of 1/4" inner diameter glass tubing, one approximately 8" long and the other approximately 4" long.
4. A 1000 ml Erlenmeyer flask.
5. A two-hole rubber stopper for the mouth of the flask.
6. A 5' length of clear tubing, e.g Tygon, of appropriate inner diameter to fit the glass tubing.
7. An eye dropper with the suction bulb removed.

Clear plastic tubing is preferable, as it can be monitored for the build-up of spores on the inner wall of the tubing and changed as necessary. Opaque rubber or plastic tubing may be substituted if clear tubing is not available. If the air intake port and the glass tubing differ in size, tubing of appropriate size may be joined with plastic tube connectors.

The aspirator is assembled by attaching the 3' length of Tygon tubing to the air intake valve on the vacuum pump regulator. The other length of the tube is attached to the 4" glass tube, and the tube is inserted into the other hole in the rubber stopper. The stopper is then placed in the mouth of the flask. The large end of the eyedropper is inserted into the unattached end of a 5' tubing. The eyedropper and the length of tubing form a tiny vacuum cleaner. The mold is collected in the flask. The mouth of the eyedropper should be smooth, and may be sanded with emery paper if there are any irregularities. When the vacuum pump is plugged in, the pull of the vacuum may be regulated by adjusting the intake valve.

In an emergency, when electrical power may be off for days or weeks, a vacuum aspirator can be improvised using a water tap. A special attachment (called a water-jet pump) is necessary for the tap, and can be obtained from chemical suppliers. A vacuum is created by the flow of water through the faucet, and the pull of the vacuum can be regulated by increasing or decreasing the volume of water. The 3' length of flexible tubing should be attached to the side opening of the water-jet pump and connected to an Erlenmeyer flask as described above. Any local university or high school chemistry department can provide assistance in constructing a vacuum aspirator. They are quite simple to set up and use, but rather difficult to describe.

A water vacuum may not have a strong enough suction to bubble the liquid as described with the vacuum pump. (TP)

Another vacuum aspirator is described by the Canadian Conservation Institute, CCI Note 18/2 - *Making a Mini-vacuum Cleaner*.

When using a vacuum aspirator, the flask into which the mold is collected should contain ethanol or a fungicidal solution, such as Lysol. (LOP)

The flask should have a water/Lysol dilution in it and the inlet tube should be below the level of the liquid. In this manner, the inlet/spore/mycelia-laden air will bubble through the liquid and trap the contaminants. (TP)

F. Magnifiers

The use of a magnifier will aid in the removal of superficial mold growth. A dissecting microscope with a long arm adjustable stand is best, but will not be available to most institutions. A headband magnifier provides an acceptable level of magnification, and leaves both hands free. Hand-held magnifying glasses may be used if no other apparatus is available.

G. Brushes

An assortment of brushes will be needed. Fine pointed artist's watercolor brushes should be used for removing mold growth from the surface of pastels and other fragile media. Wide dusting brushes of rabbit hair should be used for routine cleaning and the removal of powdered Art Gum eraser. These dusting brushes should <u>not</u> be used to remove mold growth.

H. Powdered Erasers

Powdered erasers, for example, Art Gum or Skum-ex, for the removal of mold growth from fragile paper are useful. These are available through most art and drafting supply stores. If not available locally in powdered form, Art Gum erasers can be cut into small squares and reduced to powder in a household blender or grated with a hand-held stainless steel grater. Several different grades or sizes can be made, from relatively coarse to very fine. The larger grains should be used first in order to pick up the mycelium from the paper, followed by the finer grain powder to remove remaining spores. (See <u>AIC/BPG/PCC 14. Surface Cleaning</u>, 1992 for a discussion of erasers appropriate for conservation treatment.) Some erasers containing abrasives

and sulfur have been recommended in this context for the purpose of removing superficial mold growth. These erasers are not generally recommended for surface cleaning paper or photographs because of the potential damage caused by abrasives or chemical reactions with residual sulfur. In the case of superficial mold growth, one must weigh the potential for damage caused by these factors against the consideration of the most effective means for mold removal.)

Vinyl erasers tend to smear fuzzy mold growth and cause more staining. Wishab erasers are also very effective. (SB)

I. Tweezers

Very fine pointed dissecting or surgical tweezers may be used for lifting mold from fragile surfaces and pastels.

12.3.2 Culturing Fungi

While culturing and identification are not necessary for treatment of mold damaged collection materials or response to an outbreak of mold growth, in order to protect staff who may be handling mold-damaged materials, a mycologist at a local hospital may provide identification. Some mold species are very dangerous if inhaled and may cause serious illness. This list of equipment and materials for culturing fungi is included because some conservators may be interested in more in-depth study of fungal growth. Growing and cultivating fungi can be a health hazard and appropriate measures must be taken to prevent inhalation and contamination of work areas.

A. Petri Dishes

Glass or disposable plastic may be used. For common fungi pre-sterilized agar plates are often available from Biological Supply House.

B. Transfer Needles

Usually Nichrome or platinum No. 22 or 24 gauge wire, sharpened to a needle point. Nichrome needles can be flame sterilized repeatedly without resharpening.

Commercial needles are also available, including plastic disposable ones.

C. Loops

Like transfer needles, loops may be hand fashioned of Nichrome, platinum-iridium, or pure platinum wire. Platinum-iridium seems to be preferred. Loops of various standard dimensions may be purchased from laboratory supply houses, and like the needles, they can be purchased in disposable plastic.

D. Culture Media

There are innumerable formulae for culture media, and these may be made in house or purchased from laboratory supply houses. Pre-sterilized cultures are available for a number of species.

E. Sterilization Equipment

If pre-sterilized, disposable items are not being used, sterilization will be required for all equipment and media. This includes a bunsen burner and an autoclave (or access to one).

F. Glass Slides and Cover Slips

In mounting, the material is first wetted with alcohol, then floated in a small drop of mounting fluid (see below), and covered with a cover glass. Mounts may be preserved by ringing the cover glass with clear nail polish.

G. Mounting Fluid

Although water may be used to temporarily mount conidia or other elements of the fungi, it is generally more satisfactory to use a mounting fluid which neither swells nor plasmolyses the tissues. Raper recommends a lactophenol solution developed by Amann in 1896 (state of the art!) consisting of:

Phenol crystals—20.0 gm
Lactic acid (sp. gr., 1.2)—20.0 gm
Glycerine (sp. gr., 1.25)—40.0 gm
Distilled water—20.0 ml

The carbolic acid or phenol crystals are liquified first by heating in a water bath. The mycological literature should be consulted. This is a toxic substance.

H. Incubators

While simple culturing and tests may be done at ambient temperatures, any serious work will require more precise control. Access to an incubator is certainly desirable. The temperature range required is 15 to 50°C and control accurate to ± 1°C is satisfactory.

I. Microscopes

A low powered, wide field binocular dissecting microscope, with magnifications from 10X to 40X is sufficient for most basic examinations.

A compound microscope with apochromatic lenses is required for serious study. 16mm to 8mm objectives in conjunction with 10X or 15X oculars is adequate for most purposes. A 2mm oil immersion objective is necessary for detailed examination of conidial structures.

J. Photographic Equipment

Photomicrographic attachments, while not absolutely necessary are very helpful.

12.4 Treatment Variations

(Sections 12.4.1 through 12.4.4 were prepared by Lois Olcott Price with additional comments on the treatment of photographic materials by Sarah Wagner.)

collection materials for a limited time. None have been tested for their long term effects on collection materials, so their use is a last resort.

12.4.3 Inactivation

Inactivation steps are fungistatic procedures undertaken to stop active mold growth and prevent further damage to collection materials.

The first steps are to:

1. Isolate affected materials. This can be done by placing them in a plastic bag and moving them to a dry area or by quarantining the affected area with plastic sheeting and reducing air circulation between the affected area and the rest of the building.
2. Locate the source of humidity. Look particularly for building failures (leaky pipe, blocked gutters, etc.) and HVAC failures (heat exchange coils, drip pans, etc.).
3. Lower the humidity and increase air circulation. Fix or adjust the HVAC if it can dehumidify the air. Thermostatically controlled or fan coil systems often cool the air without removing sufficient moisture and can make the situation worse. Turn them off if necessary and use dehumidifiers. Use fans to circulate the air in the affected area. In the case of a major system failure, immediately contract with a desiccant drying service to install emergency equipment to dry out the affected area. These services should be listed as resources in disaster plans.

If the humidity and moisture content of the objects can be lowered quickly and effectively, this may be adequate to inactivate the mold. If these procedures are inadequate in stopping mold growth, there are several options:

1. Undertake small scale drying of damp materials using standard disaster recovery procedures: fan books, interleave materials with blank newsprint, use fans to circulate air, etc.
2. For moderate to large scale outbreaks, particularly those involving failure of an HVAC system and/or uncontrollable relative humidity, contract with a disaster response company that can provide desiccant drying. With this method, moist air is pumped out of an affected area and dry air is pumped back in.
3. Freeze the material using standard disaster recovery procedures. Freezing will inactivate the mold and prevent further damage. Material can then be freeze dried or thawed and air dried when circumstances permit.

   The preferred method of recovering photographic materials is to air dry (preceded by soaking in cold water if items have started to stick together). For large quantities requiring mass recovery techniques, photographs may be frozen, then thawed and air dried or if necessary, vacuum freeze dried. Vacuum thermal drying is not recommended for photographic materials. Unlike vacuum freeze drying in which ice sublimes away during the below freezing drying cycle, vacuum thermal drying introduces heat during the drying cycle which causes stacks of photographs to fuse together. Because of their poor recovery rate after immersion in water, or after any type of freezing process, collodion photographs (tintypes, wet plate negatives, ambrotypes) should be immediately air dried. (See Hendricks and Lesser, 1983 and Albright, 1992.) (SW)

4. Expose the affected material, for a period under 30 minutes, to sunlight or artificial source with a high ultraviolet light content. UV light acts as a fungistat, particularly when combined with drying. Active mold usually responds to this treatment and begins to show visible signs of change within ten minutes. Because paper base materials are damaged by UV light, exposure should be minimized.

12.4.4 Cleaning

A. Cleaning of Objects

Cleaning of affected materials, whenever possible, should be undertaken after the mold has become dormant and assumed a dry powdery character. In this state it can be more readily removed without becoming further embedded in the paper and causing additional disfigurement. In some situations, however, it may be necessary to clean active mold. Removing active mold can reduce damage to the material if inactivation of the mold promises to be a slow process due to adverse environmental conditions or the structure or condition of the object.

The preferred method for removing mold is some form of vacuuming because it avoids spreading or further embedding the mold. Specific types of vacuum are described in section 12.3.A.3. The choice of vacuum and method depends upon the types of materials involved, the available resources and the extent of the infestation.

1. Aspirators allow generally safe and meticulous removal of mold even from fragile material. The procedure is very time consuming, but is appropriate in small outbreaks and/or for materials of high value. The pipette opening is placed next to the mold which is sucked into the tube. Small brushes are sometimes useful in dislodging the mold.

2. Mini-vacs are somewhat less controllable, but generally allow the safe removal of mold when occurrences are minor. Unbound material must be carefully weighted or restrained at the edges while the mini-vac is held just above the moldy area. Mold may need to be dislodged with a brush before it is sucked into the vac. Bindings and the pastedowns and gutter areas inside the covers can be vacuumed directly.

3. A vacuum with a HEPA or water filter or a modified wet-dry vac has much stronger suction and is most useful in an outbreak involving library and archival materials. Bindings can be held securely and vacuumed in the manner as described for mini-vacs. Unbound materials can be placed under a fiberglass screen and vacuumed through the screen. The screening should be stretched on a wooden paintings stretcher to facilitate handing and keep it taut. Alternately, the paper can be weighted at the edges or otherwise restrained, the vacuum can be held a few inches from the moldy area, and the mold can be dislodged with a brush and directed toward the vacuum. These techniques are not as thorough as mold removal with an aspirator, but they are much faster when large quantities of material are involved. Follow-up cleaning with grated eraser may be necessary.

4. Grated erasers, either Art Gum or grated vinyl, are very effective in removing mold residue after the major growth has been removed with some form of vacuum or aspirator. In using grated eraser, the same procedures as those used

for standard surface cleaning should be followed. Special care should be taken in mold damaged areas since the mold may have damaged the sizing and/or the paper making the area weaker, more porous and more fibrous. Crumbs should be carefully and thoroughly disposed of since they are now contaminated with a high population of mold spores. (Note: see comments regarding selection of appropriate erasers under 12.3 Materials and Equipment, 8. Powdered Erasers.)

5. After vacuuming, books may be wiped with a dry or very slightly moistened cloth rather than cleaned with grated eraser. This will remove additional mold residue. The cloths should be discarded or washed in disinfectant.

6. Objects with very fragile or friable surfaces, such as pastels, need special consideration. Because of the nature of the media, mold may need to be removed by repeatedly touching it with a very fine pointed watercolor brush. The brush should be wiped clean frequently and disinfected with ethanol when treatment is complete. (See 12.4.H Conservation Treatment: Photographic Materials).

7. After as much mold residue as possible has been removed, it may be desirable to reduce the remaining spore population as much as possible, particularly if the material will return to a somewhat unstable environment. When the media and paper permit, mold damaged areas may be swabbed with a solution of ethanol to which 20% water has been added. The water makes the spores more vulnerable to the fungicidal effect of the ethanol.

B. Cleaning Storage Areas and Materials

As part of a mold clean-up, the storage materials and storage area must be thoroughly cleaned. The goal here is to reduce the spore population to safe levels. Boxes that exhibit mold growth should be vacuumed and wiped with a dry or very slightly dampened cloth. All surfaces (shelves, walls, floors, etc.) should be vacuumed and wiped with a Lysol-type fungicide diluted as recommended on the product container. Rugs and drapes should be thoroughly dried, vacuumed and cleaned if necessary. Collection materials should not be returned until the area is dry and the environment is stable.

The HVAC system should be thoroughly inspected. Filters should be changed. Heat-exchange coils, drip pans and duct work should be cleaned and disinfected as necessary. (LOP)

12.4.5 Conservation Treatment: Removing Fungal Growth from Media

Local application of a water:ethanol (at least 25% water) solution has been recommended as a method of inactivating any residual spores in the substrate. When removing spores and mycelia from a surface with a fine brush, the brush should be dipped in a water:ethanol solution to inactivate and prevent contamination of tools and the work area. (SB)

Patches of white and brown mold were successfully removed from the surface of a color lithograph by using a combination of steam and drying with cotton swabs as described in Dwan, A. "Conservation of Jasper Johns Decoy: Mold Removal Using Steam," A.I.C. Book and Paper Group Annual, 1992, p. 21.

A really fuzzy superficial growth may be removed with the careful use of pressure-sensitive tape, holding the tacky surface just in contact with the growth and lifting it off. The tape may be used off the roll, fashioned into a fine point or attached to a narrow tool. (SB)

12.4.6 Conservation Treatment: Treatment of Structural Damage

It is sometimes difficult to distinguish damage and staining caused by water from that caused by mold growth. Careful examination and the use of examination aids may be helpful for making such distinctions and for gaining as much knowledge as possible about the extent of damage prior to treatment. For example, mold hyphae fluoresce when viewed with ultraviolet. Mold damaged areas, including those that are not noticeable in visible light, can be seen quite clearly in UV. (LOP)

Traditional lining and mending techniques provide structural support. The only treatment particular to mold-damaged paper, particularly severely mold-damaged paper, is the need for thorough resizing followed by pressing to consolidate what is left of the paper and the process adds a significant amount of strength to the paper. (LOP) See 17. Sizing/Resizing.

A. Localized Support

See AIC/BPG/PCC 25. Mending and 26. Filling of Losses.

The successful treatment of extensively mold-damaged Russian scrolls using leafcasting is described in Stanley, T., "The Treatment of Early Russian Manuscript Scrolls," A.I.C. Book and Paper Group Annual, 1992, pp. 186 - 196.

B. Lining

See 29. Lining.

Resizing and encapsulating is preferable to lining since the encapsulation is far more reversible than the lining if the paper is severely damaged. (LOP)

12.4.7 Conservation Treatment: Stain Removal

An excellent source for understanding fungal growth, why it is so damaging to paper and why the stains are so difficult to remove has been published by Szczepanowska, H. in The Paper Conservator, 1986.

Success in minimizing stains is due to many factors. The staining is complex. It may be fresh or old and caused by a mature fungal colony. The staining observed may be caused by pigments or debris produced from metabolic processes but the effects of mold growth may be difficult to distinguish from tidelines caused by dirty water. Empirically, staining appears to be more extensive when active growth is killed while still on the paper. This has been observed with the use of fungicides, fumigants and fungistatic measures which kill active growth by altering environmental conditions such that the fungi undergo severe metabolic change on the support. The extent of treatment to remove stains is often limited by the condition of a severely damaged or fragile paper support.

Recent attempts to diminish stains produced by fungi on paper using laser irradiation are still in experimental stages (See Szczepanowska, H. "A Study of the Removal and Prevention of Fungal Stains on Paper, JAIC 31(1992):147-60.

Most conservators have reported no success with attempts to diminish staining using non-aqueous solvents.

If the paper is in good enough condition to withstand treatment, local application of a dilute solution of ammonium hydroxide has been used successfully to diminish some staining caused by fungi. This may be due to the general lightening effect of dilute ammonium hydroxide solution on many types of discoloration. (TS)

According to Beckwith, waste products produced by fungi are soluble in alkaline solutions. It may be possible that some portion of the staining on paper caused by fungi is due to waste products and this may be the reason that some conservators have had success diminishing stains using a dilute solution of ammonium hydroxide or calcium hydroxide (pH 7.5 - 8.5) locally, followed by local rinsing or immersion. (SB)

A. Enzymes

Because fungi secrete enzymes to digest nutrients and because some of the staining produced by fungi is due to their metabolic activity, the use of enzymes has been suggested as a possible method for treating staining caused by mold activity. This was briefly touched upon by Mary-Lou Florian as an area that might bear investigation in her lecture, "Conidial Fungi (Mold) Activity on Artifact Materials - a New Look at Prevention, Control, and Eradication," ICOM 10th Triennial Meeting. (SB)

B. Bleaching

Many conservators have found that the only means to diminish dark yellow and purple staining caused by fungi is the use of bleaching solutions. However, as for all treatments of paper damaged by fungi, extreme caution is required and the extent of treatment may be limited by the fragile condition of the paper.

Dilute solutions of sodium borohydride have been used with some success to diminish stains produced by fungal growth, however, one must evaluate the paper condition in each case very carefully to judge whether it will survive the extensive treatment required to diminish the stains. (HS, TS)

To the extent that the support allows it, some conservators have had success diminishing staining connected with mold damage using sun bleaching.

C. Learning to Live with It

Conservators have often found that a method which may have been partially successful in one case has no effect on a similar stain in another case. Often, the poor condition of the paper prohibits extensive treatment of stains and one comes to accept the stained condition.

12.4.8 Conservation Treatment: Photographic Materials

(This section is contributed by Sarah Wagner.)

Because of the diversity of photographic materials and possible outcomes of mold damage, this section will be devoted to paper print materials. While there are several Kodak references on mold removal from film materials using Kodak Film Cleaner (Trichloroethane), it is no longer manufactured by Kodak or commercially available. [Kodak Film Cleaner was recommended because it is a non-aqueous solvent which

does not swell gelatin emulsions and because it is not a solvent which dissolves cellulose acetate, cellulose nitrate or polyester film.

In general, mold can be removed or reduced from photographic print materials using the methods described in section 12.4 C. 3. Care should be taken using suction, mini-vacuums, or erasers as binder layers can be as friable as pastels after a fungal infestation.

For a discussion of surface cleaning photographs using erasers, see 14. Surface Cleaning. In general, non-sulfur containing erasers are preferred for use on photographs having a silver based image. While Art Gum and Skum-X both contain sulfur (vulcanized rubber) and are not recommended for routine use with photographs, their use for removal of mold should be weighed in terms of relative dangers, i.e., is the mold more of a danger to the photograph or its image material than any hypothetical fading which sulfur residues may cause? In situations where use of erasers is the preferred choice, Art Gum should be used on the recto rather than Skum-X. Skum-X contains abrasive particles which can scratch delicate binder layers whether or not they are mold damaged.

Careful identification is required before any ethanol or acetone containing solution is used on photographs as a sterilization method. Collodion binders are dissolved by these solvents as are many of the historic varnishes used on photographs. Even undeteriorated gelatin binders can swell when cleaned with reagent alcohol, which always contains a percentage of water dissolved from the atmosphere or from the distillation process. Gelatin which has been deteriorated by mold is partially solubilized and is even more soluble in water. Therefore, it is more likely to swell from the small quantity of dissolved water in ethanol than unaffected gelatin. Likewise, albumen which has undergone fungal attack can react like gelatin in these circumstances and undergo swelling. Of course, water should be avoided in these situations. Although acetone is a likely substitute for ethanol, accurate photograph process identification is necessary in order to eliminate its inadvertent use on photographs with a collodion binder, acetate or nitrate base, or varnish coating.

When in doubt or to reaffirm a treatment approach, consult a photograph conservator who has experience in treating mold damaged photographs. (SW)

12.4.9 Culturing Fungi

Culturing is not necessary for treatment of mold damaged collection materials or response to an outbreak of mold growth. (Identification may be required in order to protect the health of staff who may be handling mold-damaged materials.) Growing and cultivating fungi is not recommended as a general practice or necessity for conservators. It can create a serious health hazard and should not be undertaken lightly. These methods for culturing fungi are included for conservators who are interested in more in-depth study of fungal growth. Appropriate measures must be taken to prevent inhalation and contamination of work areas.

When considering culturing fungi, the conservator should be alerted that some fungi are pathogenic to humans and may cause mycosis, a condition which is difficult to cure. (HS)

One may culture mold for various reasons - academic interest or practical necessity. A mycologist, usually associated with any hospital, can be consulted to provide identification. (LOP)

To culture, just leave an agar plate open in the area. (RK)

A. Horseback Testing (or, fun & games with fungi)

Since mold spores are everywhere, one can easily begin one's mycological studies by sampling the environment in the conservation lab and the storage areas of one's own facility. The materials are readily available.

1. Coffee with sugar
   If you are really not sure you want to get involved, but have a casual curiosity as to what might be lurking in your lab, leave a full cup of coffee with sugar stirred in on the counter in the lab over the weekend. See what you have on Monday morning.

2. Starch paste
   Either rice starch or wheat starch paste will provide a useful, if not scientifically acceptable, culture media. Scoop a good size blob of unthinned paste into petri dishes or other shallow container which can be covered. Expose the paste in an open location in the lab or storage areas. You may wish to vary the length of exposure. Cover the dish, noting the date, length of exposure and location on the lid, and put the dishes away in a location where they will not be disturbed. No incubation should be necessary, as most of what you want to cultivate will grow nicely at ambient temperatures. Check after 72 hours and again every 24 hours to see what you've got and how fast it's growing.
   Examine the growth using a standard dissecting microscope. Note variations in color, growth patterns, note what happens when two apparently different genera come into contact. Colony size may be measured roughly and recorded or photographed. Exposure to hot photographic lights may produce some interesting changes in your fungi's development.

B. Semi-serious Cultivation - The Millipore Test Kit

If your appetite to know more has been whetted, or you have been lulled into a false sense of security by your inability to cultivate a fungal garden, move up to the Millipore Swab Test Kit MTSK-000-25 (see Materials and Equipment section). These are two part pre-packaged test kits with a swab for use in collecting samples, a sterile phosphate buffer for dispersal, and culture paddle with nutrient medium and grid surface for measuring colony size. The swabs allow one to sample flat or irregular surfaces and crevices, rather than simply collecting airborne samples.

A minimum of 25 sample kits must be ordered. Likely locations for testing are the tops of shelves and cabinets, the back of shelves, ventilation ducts, filtration screens or air conditioners, basement storage areas, exhibit cases, or any other location you may have reason to be concerned about. Generally speaking, one should not sample the surface of objects in the collection.

Follow the instructions that come with the test kit with two exceptions. One need not sample 40 linear inches for each test site. These kits are sold to commercial

food processing plants where it is assumed that every surface sampled will have been sanitized. Two to four linear inches are more than adequate in a museum situation. Second, disregard the instructions regarding the use of an incubator. Everything of interest to you will grow nicely at room temperature. Note date and location of sampling site, and follow the examination procedures outlined above. Feel free to improvise. There is always more where that came from.

C. Really Serious Culturing and Cultivation

In order to do serious culturing you may either work from an existing fungal colony on an object exhibiting no visible growth, or from pure cultures obtained from a biological supply house, depending on your interests and needs. In all cases, the mycological literature should be consulted. The information below is only an abbreviated guide, to familiarize conservators with procedures.

1. Culture Media

   Fungi grown on natural substratum may be identified to group from original materials, but differences in substrate may produce marked contrasts in growth characteristics and coloration, and in the morphology of conidial heads. Accurate identification requires isolation in pure culture and examination on culture media of known composition. The organism may then be reintroduced to natural substrates, or the composition of the culture media altered to ascertain the effects of the presence or absence of certain components, and the results observed. It is this latter step that will produce the sort of information useful to conservators.

   There are many formulations for culture media, and the mycological literature must be consulted. Agar is almost universally used as a gelling agent, but the composition of the rest of the formula may vary. Supplementary and often very important information may be obtained by varying the proportions of nutrients, by introducing supplements and by replacing components with widely different substances.

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12.6 Special Considerations

12.6.1 Micro-environments

(This text has been contributed by Dr. Thomas Parker.)

In the workplace, we often encounter moldy materials presented for conservation or we may be asked to participate in cleaning up a "mold bloom" at a site where something has created conditions conducive to a rapid build-up of mold on a variety of materials in situ. Experience has shown that the conservator, with proper training and personal protective measures, can usually handle the restoration of an occasionally moldy item. It is the large scale mold bloom that seems to appear overnight which presents a fairly difficult challenge to the conservator for safely restoring the materials. However, if the reasons and conditions which caused the mold bloom to occur have not been addressed, the stage will be set for a reoccurrence. It is imperative that the source of the moisture problems be found and corrected prior to returning conserved materials to the location from which they came. What are some of the causes for a mold bloom to appear?

Often a malfunction in the air handling equipment results in prolonged periods of high humidity and a resultant mold bloom. In order to increase humidity in a space, some systems are equipped to put steam or water vapor into the airflow during periods of low humidity. Sometimes, because of calcification, these valves and steam jets get stuck in the open position and continuously inject steam into the air flow, elevating humidities for long periods of time. Until the mold bloom appears, often staff members are unaware of this type of malfunction.

Another situation which may place huge amounts of moisture into the air of a space may be caused by an air handling condensing unit filling its drip pan with water. The drain line may be clogged or improperly designed so that the drip pan becomes a "swimming pool." The air flow then directly and constantly adds humidity to the space housing the collections. Again the mold bloom lets everyone know that "something's wrong" somewhere in the air handling system.

Another type of event which leads directly to a mold bloom is a pipe leak or a burst pipe. If materials in this area are wetted, the mold will first appear on the outside of the materials and will soon grow into the interior. Even if materials are not directly wetted, the elevated humidity in this micro-environment may produce a mold bloom. The burst pipe or leak situation requires quick action to prevent the mold from growing through bound volumes and "sticking" the pages together. If the leak is not discovered in time, and materials are wetted, mold will grow into and through the substrate, making conservation problems much more difficult to resolve. Wet materials should immediately be frozen to hold the mold in check until decisions can be made about reclamation. Vacuum freeze-drying of materials may be required prior to conservation work on the materials.

The last event which results in catastrophic mold growth is a fire. Wet materials soon grow so much mold that conservation may become impossible. Immediate freeing of wet materials is mandatory in such a case. Decisions can then be made about the value of the damaged collections, which ones can be saved, the amount of conservation required, the costs involved for salvage operation, and which materials should be vacuumed freeze-dried. (TP)

12.6.2 Fungicides and Fumigation: History, Toxicity and Effects on Organic Materials

(The text is adapted from a 1988 UNESCO Ramp study publication by Mary Wood Lee, with additions, updates, and comments from Mary-Lou Florian, Dr. Robert Koestler, Catherine Nicholson, Lois Olcott Price, Dr. Thomas Parker and Sarah Bertalan. It is intended as a review of chemicals which have been used in the past.)

Most librarians, archivists, and museum personnel share a conviction that mold must be killed. It is perhaps more appropriate and effective to concentrate on prevention, inhibition and removal. As noted earlier, molds are admirably equipped for survival. Even a kill ratio of 99 percent "is an almost insignificant loss to a fungus which can produce hundreds of thousands of spores in a small colony started from a single spore." (Haines and Kohler, 1986, p. 54). Fungicides and fumigants broad ranging enough and powerful enough to achieve a 99 percent mortality for fungi are now known to be toxic to man as well. In considering the use of fungicides and fumigants for the prevention or treatment of mold growth, two basic facts should be kept in mind:

- All biocides are chemically reactive, i.e., they are capable of reacting with and altering materials to which they are applied.
- All biocides have some level of mammalian toxicity (Baynes-Cope, 1971, p. 392).

The traditional chemical approach to biodeterioration involves two strategies. One strategy, fumigation, interferes with the vital activities of the organism. The other strategy, topical application of fungicides to an object, interferes with their consequences, that is, with the chemical reactions of the organism and its substrate. The number of compounds in use today is fairly limited. They include certain metal derivatives, organic chemicals (of which the phenols are the most common) and certain organometal compounds. (Van der Kerk, 1971, pp. 3-4). While there is a certain amount of interest in, and testing of more exotic techniques, including irradiation and the use of ozone, "we must not place too much reliance on the hope for brand-new biocidal agents as the solution to the problem (Van de Kerk, pp. 3-4). Both irradiation and ozone have been found to be damaging to certain materials.

It should be noted that the first strategy, interfering with the vital activities of the organism, can be accomplished without recourse to chemical treatment. Modification of the environmental factors required for mold growth is at least as effective as chemical treatments, and certainly far safer for both personnel and materials.

Plant derivatives are becoming more common. Most organo-metals will be severely restricted or banned soon. (RK)

Fungicides

The term fungicide, as used in this study, is limited to those biocides in a liquid medium applied directly to the surface of an affected item. The application may be intended to affect the growth of mold, or to kill the mold once growth has begun. Of the fungicides recommended in the literature, most have proved ineffective in terms of long term protection and are deleterious to the materials themselves. Those who do seem to have some level of residual toxicity are now known to be hazardous to staff and users who may handle the materials later. Exposure may be by inhalation, ingestion, or absorption through the skin. Warnings concerning the use of biocides

should be rigorously adhered to, both with regard to the actual application and possible residual effects.

Beckwith, Swanson and Iliams conducted a comprehensive series of tests on biocides used as paper protectants and found that 28 commonly recommended fungicides were either ineffective in killing mold or damaging to paper. These included mercuric chloride, chloroform and formaldehyde (Greathouse and Wessel, p. 375). As recently as 1971, a British Museum pamphlet on biocides for archival and library materials recommended both chloroform and formaldehyde (Baynes-Cope, p. 383).

Fogging of entire areas with fungicides is most often carried out by professional fumigation companies, and should never be attempted by untrained, unlicensed staff. If fogging is necessary, in-house staff should know precisely what fungicide will be used, and scrupulously observe all restrictions regarding access to the area and exhausting the gas after fogging.

Thymol and ortho phenyl phenol crystals dissolved in alcohol have been recommended as topical fungicides. Indeed, both have been widely used in the conservation field. Their use has been radically curtailed by recent studies showing that both can damage the eyes and upper respiratory system. Thymol is believed to be the more toxic of the two, affecting the liver, kidneys, central nervous system and the circulatory system as well

(Barton and Wellheiser, 1985, p. 63).Thymol was known to cause staining of adjacent papers when used to impregnate interleaving sheets (see Daniels and Boyd, 1986). Ortho phenyl phenol has been shown to cause damage to museum materials by Koestler, et. al., 1993. The solvent power of thymol and the staining it causes as an additive to starch pastes have been well known to paper conservators for some time. (SB)

Any recommendations in the literature that are more than a few years old should be viewed with skepticism, since it is only in the last few years that the toxicity of a wide range of biocides has become a matter of concern. Research is still underway to establish precisely what levels of exposure may be acceptable.

It is a long standing medical principle that one should treat the disease, not the symptom. The application of topical fungicides to items exhibiting mold growth is a classic example of treating the symptom, and fails to address the broader cause of the affliction. Items treated in this manner and returned to the same environment that produced the outbreak are very likely to develop recurring symptoms.

Fumigation

The term fumigation is used in this study to include any treatment which relies on exposure to the fumes or vapor of a biocidal compound to kill mold. The idea of fumigation is appealing because it does not involve the treatment of individual items and is therefore not costly in terms of staff time. Large numbers of items have been treated at one time, in either fumigation chambers or by sealing areas of the building and fumigating entire collections. The reality of fumigation is far less appealing when considered in terms of its uncertain effectiveness, lack of residual protection, possible alteration or damage of materials, and toxicity to staff and users.

Methods of Fumigation

Fumigation has been carried out in various ways, using a variety of fumigant. It must be carried out by licensed professionals.

Of the fumigation chambers commonly in use, those which incorporate a vacuum are most effective in eliminating mold. The vacuum allows greater penetration of the fumigant, and there is a possibility that it may also have adverse effects on the mold structure, removing oxygen required for growth and possibly rupturing the spores themselves. Vacuum chambers are however extremely expensive to purchase and install.

Non-vacuum fumigation chambers are most often used with thymol and ortho phenyl phenol vapors as the fumigant. Many institutions maintain small cabinets for fumigation of a limited number of items. Often these fumigation cabinets are improvised from old refrigerators or metal cabinets which were never intended for use as fumigation chambers.

These improvised cabinets are particularly dangerous for staff exposed to them on a regular basis. Occasionally there are recommendations in the literature that fumigation may be carried out in plastic bags. The standard plastic bag available for the disposal of household trash is not a vapor barrier, and cannot contain fumigation vapors effectively.

Toxicity of Fumigants

Ethylene Oxide

Ethylene oxide was developed in 1859. By the late 1930s it was in common use as a fumigant for grain, and by the 1970s was widely used in museums, libraries and archives. Ballard and Baer provide an excellent study of the history, use, effectiveness, and hazards of ethylene oxide (Ballard and Baer, 1986).

In 1984 the Occupational Safety and Health Administration (OSHA) released a new standard for exposure to ethylene oxide of 1 ppm. Based on animal and human data, OSHA has determined that exposure to EtO "presents a carcinogenic, mutagenic, genotoxic, reproductive, neurologic, and sensitization hazard. Safety requirements for use of the gas include methods of exposure control, personnel protective equipment, measurement of employee exposure, training in use of the gas (a license is required), medical surveillance, signs and labels, regulated areas, emergency procedures and record keeping requirements. The presence of EtO cannot be detected by humans without the aid of monitoring devices until it reaches a concentration of 300 ppm, far in excess of the OSHA standard." (McGiffin, 1985).

By the 1980s, ethylene oxide use was so heavily regulated for health reasons that most museums and libraries could not afford the expense of renovating existing fumigation chambers to comply with OSHA codes. Around the same time, alarming evidence about its interaction with museum materials came to light.

It reacts with cellulose and causes a loss in paper strength of 3% per exposure. It also reacts with chlorine in paper to form ethylene chlorohydrine which is highly toxic, remains in the paper and is absorbed through the skin. Proteinaceous materials, such as leather, retain EtO for several months after treatment. (LOP)

Some studies suggest EtO fumigation alters cellulose and makes it more prone to mold growth. It's use was greatly reduced with stricter EPA rules on toxic gas release. In addition, many studies have documented that EtO lingers in a variety of protein and plastic materials for months. (KN)

EtO is retained by fatty tissues is very toxic to humans. Its use has been virtually abandoned by museums in the USA. (RK)

Methyl Bromide

Methyl bromide was most commonly used in the fumigation of insect infestations.

It is not particularly effective as a fumigant for mold growth. It is a colorless, transparent, easily liquified gas. It is easily detected, having a strong, chloroform-like smell. It is highly toxic by ingestion, inhalation or absorption through the skin. The tolerance level established by OSHA is 5 ppm. Methyl bromide affects the central nervous system, respiratory system, skin and eyes. Acute effects usually occur 30 minutes to 6 hours after exposure and may include convulsions followed by death due to pulmonary and/or circulatory failures. Chronic effects are usually limited to the central nervous system and include muscular pains, visual, speech and sensory disturbances and mental confusion.

Methyl bromide should not be used for the fumigation of any protein based material, as it seriously damages the protein structure. Leather, for example, becomes black and brittle when exposed to methyl bromide fumes.

Methyl bromide is also known by the proprietary names Brom-O-Gas, Brozone, MeBr, Meth-O-Gas and Terr-O-Gas.

Methyl bromide reacts with materials containing sulfur which includes leather, most photographic materials, and sulfate and sulfite process papers (i.e. most contemporary papers). (LOP)

Sulfuryl Fluoride

Sulfuryl fluoride, available under the trade name Vikane, is most often used for the fumigation of termites in building structures. It has very high penetration even without a vacuum. It is not known to be effective against mold. It is an odorless, colorless, tasteless gas, and is usually available only to licensed fumigators. The OSHA standard is 5 ppm. It has not been tested extensively, and its carcinogenic and reproductive effects are unknown. It may be ingested by inhalation or absorption through the skin. Acute effects include nausea, vomiting and abdominal pain. Chronic effects include defects in bone and teeth, and in animals, lung and kidney damage have been found.

Research has shown that sulfuryl fluoride is very reactive with many materials in museum collections. It is also probably not very effective against fungi and it's use is being curtailed for museums. (RK)

Thymol

Thymol is a white crystal with a distinctive aromatic odor and taste. It is derived from thyme oil and may be mixed with camphor in its crystalline form. It is moderately toxic by ingestion and inhalation. Studies indicate that exposure to thymol vapors can affect

the central nervous system and circulatory system. No precise level for minimum exposure has been established.

Ortho Phenyl Phenol

Ortho phenyl phenol is considered slightly less toxic than thymol. The Merck Index lists it as a "slightly toxic irritant" when inhaled. It is however moderately toxic by ingestion. In its crystalline form it is a white or cream color and is soluble in alcohol. Several sources recommend the substitution of OPP for thymol whenever the latter is recommended. Relatively little testing has been done regarding the toxicity of OPP, and no exposure level is available.

In tests conducted by Haines and Kohler, ortho phenyl phenol was found to be a not very effective fumigant in its gaseous phase. Of the seven fungi tested, fumigation with ortho phenyl phenol failed to completely halt mold growth even after 10 days of continuous exposure to vapors under controlled conditions (Haines and Kohler, pp. 49-55). In it's liquid phase, dissolved in water (Lysol) or alcohol, OPP is an effective fungicide.

12.6.3 Assessing the Activity of Fungal Growth on Art Objects and Instructions for Taking Fungi Samples from Objects

(See the following two pages for a copy of a handout prepared by Hanna Szczepanowska.)

ASSESSING THE ACTIVITY OF FUNGAL GROWTH ON ART OBJECTS WITH A VIEW TO POSSIBLE FUMIGATION

- Degradation of art objects by microorganisms depends on temperature, humidity and time of growth. Visible manifestation of fungal growth on paper is heavy staining usually exceeding the fungi colony diameter. With time paper becomes weak, spongy and perforated.
  Fungi most frequently encountered on paper are lower fungi, including representatives of the genera: Aspergillus, Verticillium, Chaetomium, Trichoderma, Cladosporium, Mucor.

- In order to assess fungi activity samples of fungi are taken from the art objects using an inoculating loop or needle to place them on the nutrient pads. The following nutrient pads can be used:
  wort
  Sabouraud
  Schaufus-Pottinger
  Incubation conditions: 2-5 days at 28-30°C
  Fungi develop velvety or fluffy whitish or greenish colonies, which can take various colors after conidiospore production.
  Source of ready-to-use nutrient pads: Sartorius Filters Inc.26575 Corporate Ave California 94545
  Procedure of taking fungi samples, see page 2.

- Microorganism cultures must always be handled as carefully as if they contained pathogens. Working with fungi is not dangerous if the safety rules are followed:
  -wash your hands thoroughly before and after working in a laboratory
  -do not touch fungal matter with your hands, use gloves; wear mask
  source of gloves: Fisher brand, PVC Gloves, disposable, ambidextrous
  source of masks: Surgical Products Division/3M, Paul MN 55144
  1800 Molded Surgical Mask
  -prior to and after use, inoculating loops or needles must be sterilized by flaming until they glow red-hot.
  -to protect people and collection from contagious infections live cultures have to be destroyed by disposing them in a suitable container.

- Fungi may cause disease , superficial and systematic mycoses, in various parts of the human body.
  Superficial mycoses involve the skin, the hair or the nails.
  Systematic mycoses occur in internal organs as lung, central nervous system or other organs, and usually result from inhalation of spores.

INSTRUCTIONS FOR TAKING FUNGI SAMPLES FROM OBJECTS

I. EQUIPMENT

1.nutrient pad - wort (pre-sterilized nutrient pad, Thomas Scientific Cat.# 3495-B80 or Sartorius Filters Inc.)
Sabouraud (Sartorius Filters Inc. 26575 Corporate Ave California 94545)
Schaufus-Pottinger, the same source as for wort

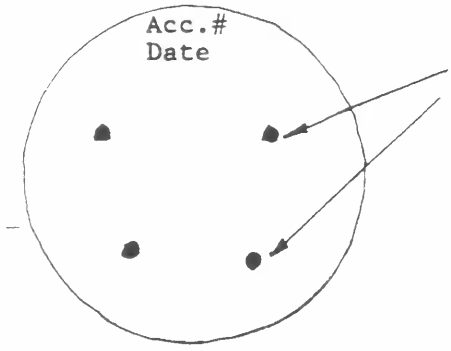
2.tools for picking up fungi samples:
tweezers
sterile inoculating loop or needle

3.alcohol burner - to sterilize inoculating tool

4.graduate (burette) to measure 3.0ml water for each nutrient pad

II. PROCEDURE

1. Sterilize all pieces of equipment by boiling for a few minutes in water. Let the needle cool before using.
2.Prepare the water to wet the nutrient pad:
-boil de-ionized water
-measure the necessary volume with graduate (3.0ml per nutrient pad)
-allow the water to cool to room temperature
-pour the water into the nutrient pad
-cover the Petri dish immediately after adding the water
3. Pick up fragments of the fungus colony with an inoculating tool and place on the wet nutrient pad in the Petri dish, opening the dish as little as possible to prevent inoculation from the environment.
4. Take up to 4 samples of one species and inoculate the dish as shown below:



Fungi samples

Note:After inoculating one species, sterilize the tool by flaming , using an alcohol burner. Cool the tool before taking the next sample. (The safest place to use the alcohol burner is in the fume hood)

5.Inocubation conditions: 2-5 days, temperature 25-30°C, 70-75F.

Hanna Szczepanowska

12.7 Glossary

Archicarp: the initial stage of fructification.

Asexual reproduction: reproduction not involving karyogamy and meiosis. In general, asexual reproduction is most important for the propagation of the species, because it results in the production of many more individuals, and is repeated several times during a season, whereas the sexual stage of many fungi is produced only once a year.

Budding: a form of asexual reproduction in which the somatic cells each bud, producing a new individual.

Colony: a group of individuals of the same species, living in close association; in fungi, refers to the many hyphae growing out of a single spore and usually forming a round or globose thallus.

Conidiophore: a simple or branched hypha arising from a somatic hypha and bearing at its tip or side one or more conidiogenous cells.

Conidium (pl. *conidia):* a non-motile air-borne asexual spore usually formed at the tip or side of a sporogenous (spore producing) cell.

Cryptogamic: a plant that bears no flowers or seeds but propagates by means of spores.

Eukaryotic: any organism or cell with a structurally discrete nucleus.

Fission: a form of asexual reproduction involving the fission of somatic cells into daughter cells, each growing into a new individual.

Fragmentation: a form of asexual reproduction that involves the fragmentation of the soma, each fragment growing into a new individual.

Fructification: any complex fungal structure that contains or bears spores.

Gamete (pl. *gametes):* a differentiated (male or female) reproductive cell, capable of uniting with another gamete to form a zygote that develops into a new individual.

Hyaline: colorless, transparent, as hyphae.

Hypha (pl. *hyphae):* the unit of structure of most fungi; a tubular filament.

Imperfect stage: the asexual (usually conidial) stage of a fungus.

Karyogamy: sexual reproduction through the fusion of two nuclei.

Meiosis: sexual reproduction through a series of two nuclear divisions in which the number of chromosomes is reduced by half.

Morphology: the branch of biology that deals with the form and structure of plants and animals.

Motile: capable of or exhibiting spontaneous motion.

Mycelium (pl. *mycelia):* mass of hyphae which make up the fungal thallus.

Parasite: a plant or animal that lives on or in an organism of another species.

Plasmogamy: a union of two protoplasts bringing the nuclei close together within the same cell. The first stage in sexual reproduction in fungi.

Perfect stage: the sexual stage of a fungus.

Saprophyte: any organism that lives on dead or decaying organic matter.

Septum (pl. *septa):* partitions or cross-walls that divide each hypha into compartments. When the hyphae age, septa are formed in increasing numbers. As portions of the hypha die, the protoplasm is withdrawn toward the growing tip, and a septum that separates the dead portion from the living is generally formed. Those septa that are associated with changes in the concentration of the protoplasm as it moves from one part of the hypha to another are known as "adventitious septa."

Sexual reproduction: in fungi as in other living organisms involves the union of two compatible nuclei. In the more complex fungi, the processes of plasmogamy and karyogamy is sooner or later followed by meiosis. The spores produced can often survive long periods of dormancy, and are often referred to as "resting spores."

Soma: the body of an organism as distinguished from its reproductive organs or reproductive phase.

Somatic: in plants, the vegetative phase, structure or function as distinguished from the reproductive.

Spore: a minute propagative unit (either sexual or asexual) capable of giving rise to a new individual either immediately or after an interval of dormancy. The spore functions as a seed, but differs from it in that a spore does not contain a preformed embryo.

Sporogenesis: reproduction by means of spores, the formation of spores.

Sterigma (pl. *sterigmata):* a small hyphal branch or structure, which supports a sporangium, a conidium or a basidiospore.

Thallus: a relatively simple plant body devoid of stems, roots and leaves; in fungi, the somatic phase.

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