DRIED MOLD COLONIES ON CELLOPHANE

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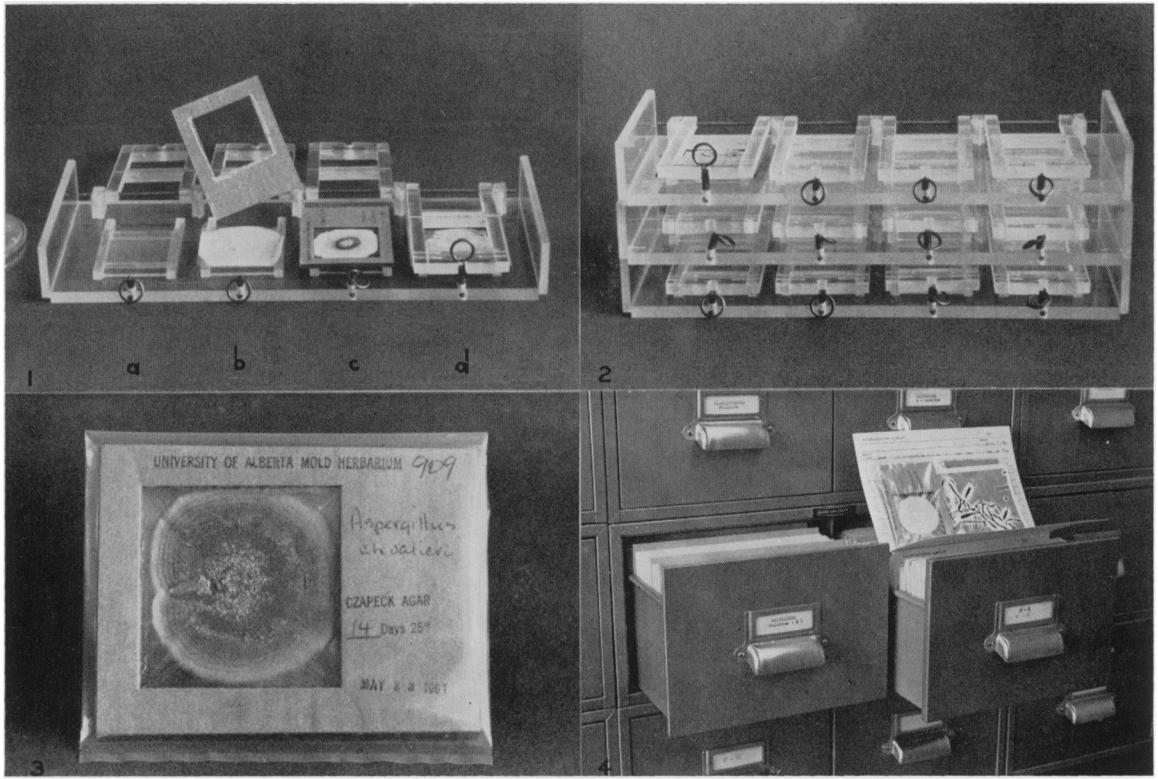
(WITH 5 FIGURES)

Classical herbarium methods are not always suitable for mold fungi. One problem which arises concerns the kind of specimen to be preserved as a permanent record. The usual field collections of molds, on plant parts or bits of this and that, make useful dried specimens when an abundant, fairly pure growth of mold occurs on a suitable substrate. However, when cultures have been isolated from a nonfruiting or thoroughly mixed flora, the logical specimen is something which preserves the colonial and microscopic morphology of the pure culture. To meet this need, some workers save agar slant cultures which have been allowed to dry out in the tube. These are easy to prepare, but the colonial morphology is poorly preserved and early stages of sporulation may be absent. Also the tubes are heavy, bulky and fragile. A better method, which is widely used, is to cut pieces out of a petri dish culture and stick them to the bottom of a thin, flat box such as a single-slide mailing tray. These specimens are easily stored and can preserve both young and mature stages. However, the agar shrinks and darkens and the colonial morphology is again lost.

Sir Alexander Fleming and George Smith (**1944**) suggested covering the troublesome agar with a cellophane disc which would serve as a carrier to lift the mold colony away from the medium. This got rid of the agar, but cellophane itself shrinks and wrinkles when it dries. Fleming and Smith got around this difficulty by sticking the wet cellophane to glass plates. This method yields nice colony preparations, but they are fragile and heavy and the method has not become popular. Walter Jones (**1956**) reported that he could get reasonably flat preparations by drying the cellophane between heavy wooden blocks. This method dispensed with the glass plates, but the cellophane still wrinkled and shrank considerably and the colony was mashed flat by the blocks. Kondo et al. (**1959**) published some additional modifications, but two problems still remained: 1) how to dry the cellophane without shrinkage, and 2) how to protect the dried colony from abrasion without making the specimen heavy or bulky.



FIGS. 1–4. 1. Rack with four drying presses showing stages of procedure. 2. Three racks showing method of stacking. 3. Dried colony preparation in plastic envelope. ("Czapeck" should be Czapek). 4. Filing system. The folder for each isolate serves as a label and data sheet. The protruding colored cards serve as "species covers."

After a great deal of trial and error, I have evolved a quick, simple method which results in very satisfactory specimens. Briefly, it consists of growing the colonies on cellophane squares, drying the squares on a special rack to prevent shrinkage, mounting the squares on corrugated cardboard frames, and enclosing the entire preparation in a transparent plastic envelope.

MATERIALS AND EQUIPMENT

The *cellophane* used is plain, transparent cellophane about 30 μ thick. In North American commerce this cellophane is designated as "300 PT." We purchase large sheets of cellophane from a paper wholesaler and have the sheets cut into $2\frac{1}{2}$ inch squares at a printing shop. The *corrugated cardboard frames* are cut by a printing shop from 275 pound

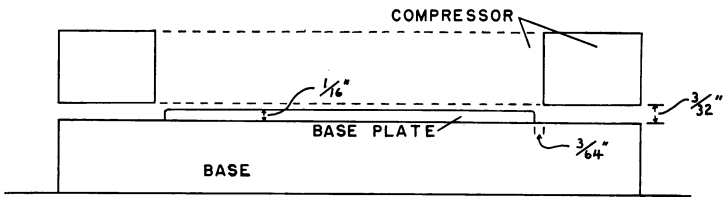


FIG. 5. Cross-section of drying press showing critical dimensions, × 1. Compare with FIG. 1.

test corrugated cardboard stock. This stock is about $\frac{1}{10}$ of an inch thick. The outside dimensions of the frame are $3 \times 3\frac{3}{4}$ inches. A 2 inch square window is die-cut in one end so as to leave a $\frac{1}{2}$ inch margin on three sides of the window (FIG. 3).

The lucite plastic *drying presses* were made up for us by a local equipment maker. FIG. 1 shows a rack with four presses. FIG. 2 shows the manner in which the racks may be stacked to save space. Important dimensions of the press are shown in FIG. 5. The function of the base plate (FIG. 5) is to push the cellophane up into the window in the cardboard frame. This produces a small amount of slackness in the cellophane and prevents the cardboard from buckling when the dried preparation is removed from the press.

The *protective plastic envelopes* are made from $1\frac{1}{2}$ mil (38 μ) thick polyethylene and measure 4 inches wide by 6 inches deep.

PROCEDURE

Place a supply of cellophane squares on edge in a wide-mouthed, screw-capped jar filled with 70% ethyl alcohol and sterilize by heating

in a 55° C water bath for two or more hours. Remove the squares, one at a time, with sterile, angle-tipped forceps, drain briefly on a sterile paper towel, and lower on to the surface of any desired agar medium in standard 10 cm petri dishes. If any air is trapped under the cellophane, smooth it out to the edge with the back of the forceps. On some media, the cellophane may wrinkle slightly after a few minutes, but the sheet flattens out again later. Leave the plates overnight at room temperature before using or storing in the refrigerator. During this period the surface film of water and alcohol either evaporates or is absorbed by the medium. The small amount of alcohol which may remain in the dish has no apparent effect on colonies grown on ordinary media, but for growth tests on particular carbon sources the cellophane should probably be sterilized by autoclaving in water.

The mold to be preserved is inoculated onto the surface of the cellophane square and the culture is incubated at the desired temperature. The growth rate and the colonial morphology of most fungi are not changed very much by the presence of the cellophane between the colony and the nutrient medium.

When a suitable colony has formed, a cardboard frame is labelled and a bead of water-resistant, resin glue is run around the edge of the window (FIG. 1 b). The cellophane square is then lifted off the culture medium and placed on the base of the drying press. If the colony has grown beyond the edge of the square, the mycelium should be cut through with a scalpel around the edge of the square before attempting to lift it off the agar. The cardboard frame is then lowered onto the cellophane and clamped firmly in place with the hinged compressor and securing spring (FIG. 1 d).

When the colony has dried the preparation is removed from the press and slipped into a plastic envelope (FIG. 3). I merely fold over the top of the envelope, but the plastic could be heat-sealed if desired. This would provide permanent protection against moisture and insects.

PROBLEMS AND SPECIAL PROCEDURES

The most important problem encountered in the use of this method is the digestion of the cellophane membrane by cellulolytic fungi. However, moderately cellulolytic fungi can be handled if the incubation period is not too long and if the colony is removed carefully from the culture medium. The cellophane may be penetrated or weakened, but useable dried colonies can be obtained. Species of *Fusarium, Alternaria, Botryotrichum* and similar cellulolytic molds yield quite satisfactory colonies

after 7 to 10 days incubation. When the cellophane has been completely digested, we cut out or peel off pieces of the colony and stick them to a fresh sheet of cellophane which has been glued to a frame and placed in the press in the usual way. Pyrenomycetes which fruit after several weeks' growth usually must be handled in this manner. Often, even though the cellophane is digested, its presence restricts the colony mainly to the surface of the medium and allows the removal of the colony with a minimum of adhering agar. The problem can be completely overcome by substituting a different membrane material. In a limited trial, we have found that LB-0053 filter material (Gelman Instrument Co., 106 N. Main Street, Chelsea, Michigan) allows satisfactory growth and is not digested by cellulolytic fungi. Although hyphae penetrate the pores in the filter and grow into the agar, the membrane is tough enough so that the colony can be torn free. The membrane is white, rather than transparent, which hinders microscopic examination of the colonies by transmitted light.

Another problem is the protection of thick colonies or relatively tall fruiting structures. This is easily overcome by adding one or more additional cardboard frames to make up the required thickness.

Since the relative humidity in our laboratory usually ranges from 10 to 60%, the colonies dry in a few hours. In humid climates an oven or desiccator might be necessary to achieve drying in a reasonable time.

DISCUSSION

This simple, inexpensive method yields thin, light, well-protected specimens which provide an adequate record of both the colonial and the microscopic morphology of molds. They can be filed in packets on the large herbarium sheets which are necessary for dealing with higher plants. However, a much more convenient filing system for these small specimens is to use vertical folders in file drawers designed for 8×5 inch cards. We use a separate folder for each isolate and file any notes, drawings or photomicrographs along with the dried colonies (FIG. 4). Bartholomew (1931) has discussed the merits of vertical filing systems.

In addition to their elegance as herbarium specimens, dried colonies can be a valuable aid in the maintenance of a culture collection. Each time a culture is transferred, the resulting colony can be checked against the original dried colony for evidence of contamination or strain degeneration. Colonies are also useful teaching aids. They are particularly helpful for demonstrating to students the range of variation which may occur in different strains of the same species.

SUMMARY

A method is given for preparing dried mold colonies to serve as reference specimens. The mold is grown on a square of cellophane which has been laid on the surface of the agar culture medium. When the colony has grown, the square is lifted off, glued to a protective cardboard frame and dried in a special press. The mounted colony is then placed in a transparent plastic envelope for display or storage.

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LITERATURE CITED

Bartholomew, E. T. 1931. Herbarium arrangement of mycological specimens. Mycologia **23**: 227–244.

Fleming, A., and G. Smith. 1944. Some methods for the study of moulds. Brit. Mycol. Soc. Trans. **27**: 13–19.

Jones, W. 1956. Permanent mounts of fungus cultures on cellophane. Phytopathology **46**: 131–132.

Kondo, W. T., S. O. Graham, and C. G. Shaw. 1959. Modifications of the cellophane culture technique for photographing and preserving reference colonies of microorganisms. Mycologia **51**: 368–374.