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FUNGUS CULTURING EQUIPMENT: AN INOCULATING PUNCH, A PETRI PLATE SHIELD, AND A GROWTH TUBE READER

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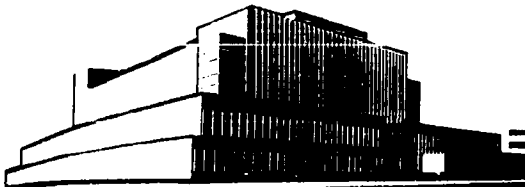
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FOREST PRODUCTS LABORATORY
MADISON 5, WISCONSIN

UNITED STATES DEPARTMENT OF AGRICULTURE
FOREST SERVICE

In Cooperation with the University of Wisconsin

**FUNGUS CULTURING EQUIPMENT: AN INOCULATING PUNCH,
A PETRI PLATE SHIELD, AND A GROWTH TUBE READER¹**

By

JOE W. CLARK, Pathologist

Forest Products Laboratory,² Forest Service
U.S. Department of Agriculture

Summary

↙ Three devices are described that aid in handling cultures of fungi more accurately and conveniently: (1) The inoculating punch permits cutting and transferring uniform-size inocula quickly and easily for tube, plate, or soil bottle cultures; (2) the petri plate shield supports an open plate culture and provides protection against contamination during transfers; and (3) the growth tube reader makes possible accurate quantitative measurements of lineal fungus growth rate or changes of growth rate.

↑
Introduction

While doing research for a doctoral dissertation at Yale University in 1949 and 1950, the author devised three simple pieces of equipment--two for making inoculations with fungi in tubes and bottles, using cultures grown on solid media, and a third for measuring lineal growth of fungi cultured on solid media in large test tubes. During subsequent use at the U.S. Forest Products Laboratory, the equipment was modified somewhat. Recently considerable interest has been shown by other pathologists in this equipment, and several requests have been made for detailed descriptions.

¹This equipment was first developed during the preparation of a dissertation presented for the degree of Doctor of Philosophy at Yale University.

²Maintained at Madison, Wis., in cooperation with the University of Wisconsin.

The three items consist of a punch for cutting and transferring plugs of fungus mycelium and substrate for use as inocula, a petri plate shield to hold the plate of inoculum and protect it from contamination while transferring, and a growth tube reader for measuring lineal growth in tube cultures.

Figure 1 is a general view showing the inoculating punch being used with a growth tube, with the petri plate shield and tubes in the background.

The growth tubes mentioned, and specifically used with the growth tube reader, are 25- by 200-millimeter test tubes that have been modified in the manner suggested by Scheffer.³ Such tubes have the glass drawn in on one side, near the open end of the tube. When the tube is partially filled with media and placed in the horizontal position (fig. 1), a layer of substrate 150 millimeters long and of uniform width and depth is formed.

Inoculating Punch

The inoculating punch, which is a simple adaptation of the old "cork borer" technique, was developed for cutting and transferring small, uniform plugs of fungus mycelium and solid medium substrate for inoculation from petri plate cultures to growth tubes or soil blocks.

Several sizes of these punches have been made at the Laboratory for various uses. Punches used for inoculating growth tubes are 12 to 14 inches long with 0.094-inch outside diameter and 0.070-inch inside diameter. For the inoculation of bottles used in the standard soil-block technique,⁴ a larger inoculum is desirable and the punch is constructed of stainless steel tubing (hypodermic needle stock) approximately 8 inches long and with diameters of 0.203-inch outside diameter and 0.173-inch inside diameter.

Use of the punch offers the advantages of uniform inoculum replicates, ease of transfer with minimum chances of contamination, and rapid and accurate placement of the inoculum in the instance of growth tube or soil bottle inoculation. Transfer of the inoculum plug is made with the material in the punch so that it is relatively unexposed. A 2.5 or 3.0 percent agar medium, malt or other as desired, gives a plated substrate that is firm enough to cut precisely, holds its shape, and is of sufficient rigidity to facilitate pickup from the plate as plugs in the punch.

³-Scheffer, T. C. A Tube for Culturing Fungi. Science 82(2133): 467-468. 1935.

⁴-American Society for Testing and Materials. Accelerated Laboratory Test of Natural Decay Resistance of Woods. ASTM Designation D 2017-62T. 1962.

The punch is shown in figure 2 in detail. The wood handle and plunger knob could be made of metal or plastic. For the stainless steel tubing and rod, hypodermic tubing stock is desirable. The compression spring is preferably wound of a corrosion-resistant, tempered spring wire of approximately 0.016 to 0.020 inches in diameter; tempered steel music wire is adequate although subject to corrosion.

In use the punch is easily and quickly sterilized by flaming. Several punches of a given size are used at the Laboratory, and rotated in use to allow each punch time to cool after it is flamed. Dipping the punch in alcohol for cooling is tedious because liquid remains between the plunger and tube wall and must be removed before use.

Petri Plate Shield

The petri plate shield, figure 3, involves a plastic sleeve supported on a wire frame and hinged to a wood base. On the inside face of the wood piece, which forms the closure at the hinged end of the sleeve, are two bracket hooks that support a petri plate culture of inoculum in a vertical position. Thus supported, the culture is open and accessible while protected from air-borne contaminants. Plugs of inoculum can be removed easily with the inoculating punch or by other means. The sleeve can be disinfected quickly with zephiran chloride or other disinfectant.

Growth Tube Reader

The growth tube reader consists of a light box with a slotted top, a rigid slide housing on a fixed track, and a scale with 0.5-millimeter divisions (fig. 4). Two glass microscope slides cemented in the slide housing have blackened edges that serve as cross hairs for alignment with hyphal tips or the advancing edge of the colony to be measured. A third glass slide is cemented to a lateral extension of the slide housing and provides a reference line immediately over the scale for readings. A fixed wood block at the lower end of the light slot provides a stop against which the closed end of the tube rests and ensures exactly the same positioning for each reading.

Materials used in the construction of the reader consist of 26-gage sheet brass for the slide housing, 1/4-inch square brass rod for the fixed track, 5/16-inch square brass tubing for the slide housing track guide (which is soldered to the slide housing), an accurate 15-centimeter scale, two small light sockets and bulbs, and assorted small bolts, washers, and nuts.

In use the tubes may be inoculated either near the closed or open end and reading taken at intervals of a day or several days, depending on the rate of growth. For slow-growing organisms it may be advantageous to make the

inoculation in the center of the medium strip and take two sets of readings, one from each expanding edge of the colony. By either system numerical growth measurements are available to the nearest 0.5 millimeter, and readings of greater precision are possible by interpolation or the use of a finer scale.

In handling and reading the tubes from day to day there is some chance that the usual malt agar medium substrate may slip or shift in the tube. This chance can be minimized by using a slightly stiffer medium of 2 to 2.5 percent agar in place of the 1.5 percent commonly used.

Conclusions

The equipment has served most satisfactorily for tests of vegetative growth rate, and for obtaining data on optimum and limiting temperatures using growth rate as a measurable criterion of response. Other uses have included the comparison of fungus isolates on variously modified media.



Figure 1.--General view of the punch being used in an inoculate of a growth tube. The petri plate shield, additional plates, tubes, and punches are in the background.

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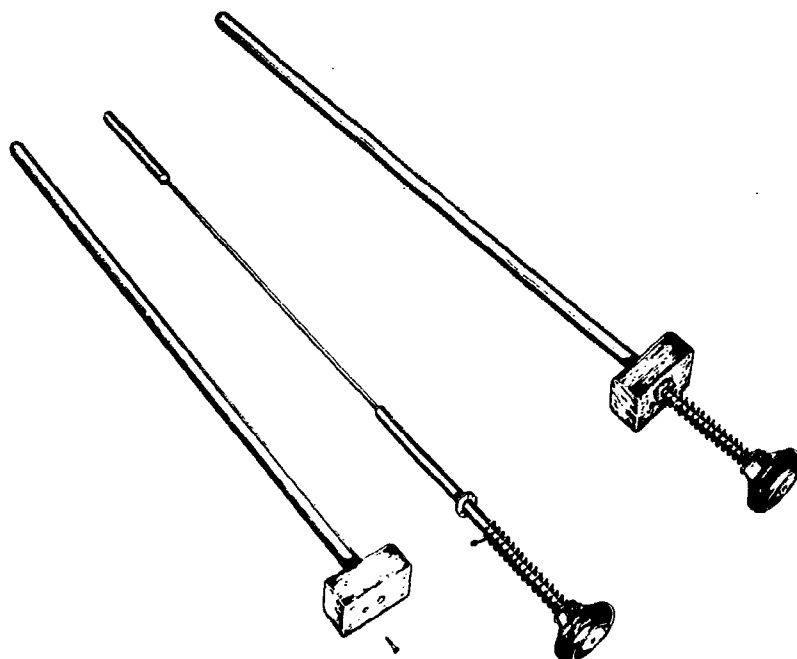


Figure 2.--Details of the inoculating punch, assembled as on the right.

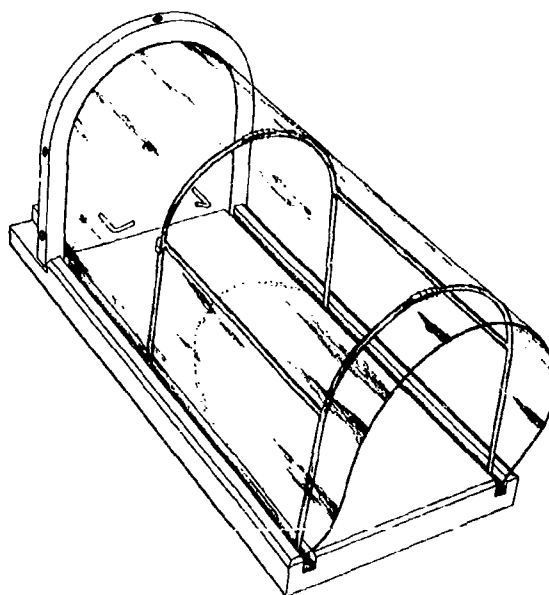


Figure 3.--Petri plate shield, showing the transparent plastic hood supported on a wire frame with a solid wood end and base. This entire hood assembly is hinged to the base at the closed end. The wood base is 6 by 13 inches and the shield is 6 inches high above the base in the closed position. Bracket hooks in the end piece support a petri plate culture of inoculum.

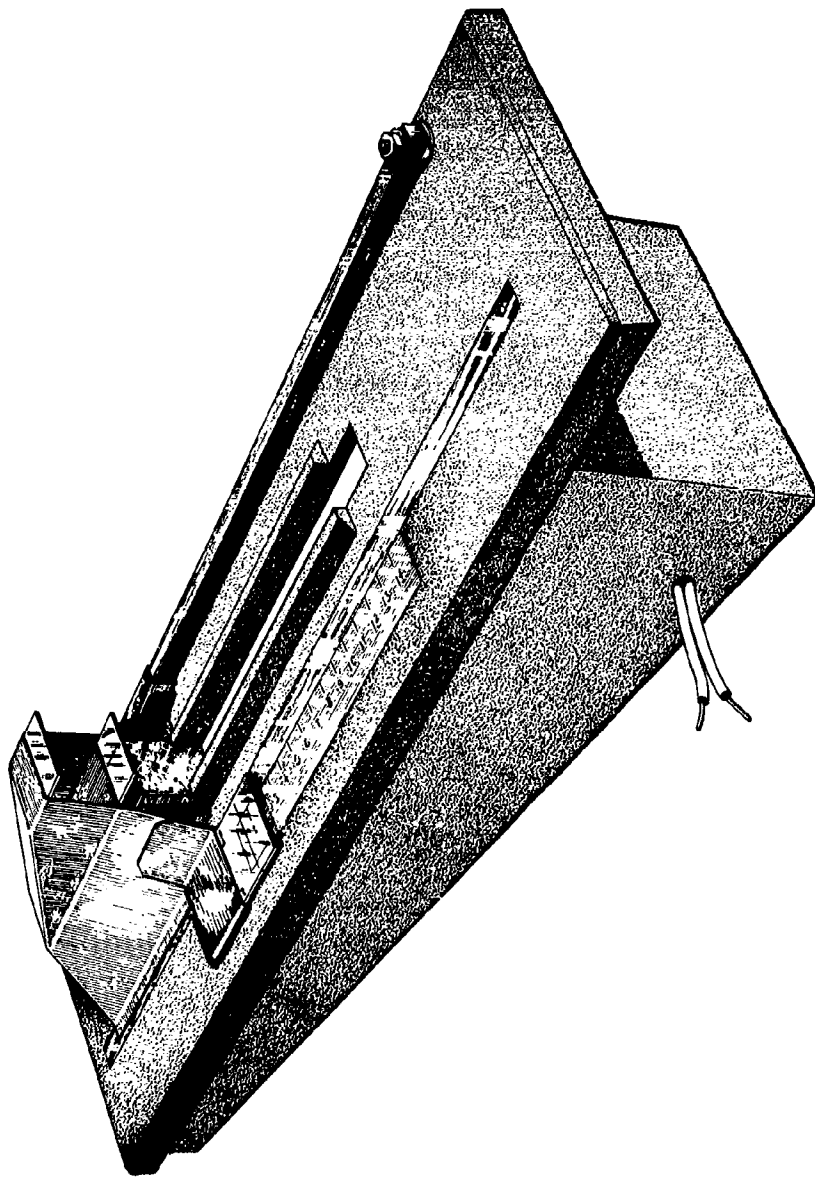


Figure 4.--Growth tube reader. In use, the growth tube is placed with the closed end against the fixed block under the slide housing and resting in the open slot over the light box. Readings are taken by adjusting the slide housing to align the blackened ends of the glass slides with the advancing margin of the growing mycelium. The numerical reading is then taken from the index line over the 0.5-millimeter scale. Base board of the reader is 17 by 4.5 inches.

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List of publications on
Fungus Defects in Forest
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List of publications on
Glue, Glued Products,
and Veneer

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Clark, Joe Waddie

Fungus culturing equipment: an inoculating punch, a petri plate shield, and a growth tube reader. Madison, Wis., U.S. Forest Products Laboratory, 1962.

4 p., figs. (F.P.L. rpt. no. 2262).

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