

A LARGE CULTURE PLATE FOR AGAR DIFFUSION ASSAYS, SEED TREATMENT EVALUATION, AND MASS PRODUCTION OF SPORES<sup>1</sup>

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A culture plate providing a large surface of agar medium is essential for a variety of microbiological procedures including agar diffusion assays, determination of fungicide on treated seeds, and the production of fungus spores in quantity for various purposes. Machacek<sup>3</sup> devised a tray assembled from sheets and strips of glass to contain an agar sheet, Lockwood *et al.*<sup>4</sup> used a sheet of glass enclosed in a metal tray fitted with an asbestos board cover. A simplified version developed at this laboratory has been used extensively with highly satisfactory results. Easily assembled from readily available components, it is easy to handle and economical of time and materials.

The components of the plate are simply a Pyrex baking pan approximately 9" x 14" x 2" and a 10" x 15" piece of 1/8" asbestos board (Transite). The asbestos board cover is held in place by means of two spring paper clips of the fold-back type. The assembled plate is wrapped in paper and oven sterilized before use.

Where uniformity of depth of seeded medium is important, as in agar diffusion assays, the procedure is as follows. First, 100 ml. of plain medium (water agar is satisfactory) is poured and allowed to solidify. Then 100 ml. of assay agar containing spores of the test organism is uniformly distributed over this surface. The test material (treated seeds, diffusion cups, paper disks, or paper chromstogram strips) can be applied as soon as this layer is hard. As a guide for the uniform placement of samples an appropriate pattern drawn on paper is placed underneath. The plates can be either stacked or stored in a vertical position during incubation.

Inhibition zones can be measured either directly at the surface of the medium or through the glass bottom of the plate. In the latter case it is not necessary to remove the lid, an advantage when repeated observations are required to detect delayed growth.

One of these culture plates can accommodate as many samples as a dozen Petri dishes. Since several treatments can be tested on the same plate and replicated on similar plates the variability factor is considerably reduced.

For mass production of spores the plates are prepared by pouring 200 ml. of a suitable agar medium. When this has solidified the surface is flooded with a heavy suspension of spores in sterile water. The excess liquid is poured off immediately.

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<sup>1</sup> Contribution No. 187, Pesticide Research Institute, Research Branch, Canada Department of Agriculture, London, Ontario.

<sup>2</sup> Plant Pathologist,

<sup>3</sup> Machacek, J. E., 1950. An agar-sheet method of testing the efficiency of seed treating machines. Can. J. Research, C, 28: 739-744.

<sup>4</sup> Lockwood, J. L., Curt Leben, and G. W. Keitt, 1952. A culture plate for agar diffusion assays. Phytopathology 42: 447.

This method has been particularly successful in the production of spores of Glomerella cingulata. On a single plate, over  $10 \times 10^{10}$  spores (approximately 1 g. dry weight) can be produced on potato dextrose agar in 3 days. Spores of Monilinia fructicola and Alternaria solani have also been produced in quantity in the same way.

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THE SUSCEPTIBILITY OF POTATO VARIETIES TO STORAGE ROTS  
CAUSED BY FUSARIUM SAMBUCINUM FCKL. F6 WR, AND  
FUSARIUM CAERULEUM (LIB.) SACC.

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The susceptibility of thirteen potato varieties to rot caused by Fusarium species was assessed by immersing artificially wounded tubers in spare suspensions of the pathogens. The experiments were conducted in replicates of four with 20 tubers per varietal replicate. Tuber lots were inoculated with F. sambucinum f6 on January 11 and examined for extent of decay on May-5, 1960. Inoculation with F. caeruleum was effected on February 10 and examinations were made on June 10, 1960.

The value of figures presented in the Tables 1 and 2 is mainly in the comparative ratings of the varieties tested.

Under epidemic conditions which prevail in Prince Edward Island in certain years serious storage rot losses have occurred in harvested tubers of the Sebago variety. Screening results obtained in the current and previous experiments have shown that Sebago is very highly susceptible to rot caused by F. sambucinum f6. Certain other varieties listed below appear only slightly less susceptible, while stocks of Irish Gobbler and F5350 proved quite resistant.

The variety Keswick has shown a high degree of susceptibility to F. caeruleum in the field and it would be expected that varieties approaching or exceeding the ratings established for Keswick in this experiment might be severely affected under epidemic conditions. It is apparent, from the results obtained, that several varieties are quite resistant to "caeruleum" decay.

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