A Simple Method for Producing Cercospora arachidicola Conidial Inoculum

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ABSTRACT

Abundant quantities of *Cercospora arachidicola* conidia were produced on a peanut leaflet-oatmeal agar medium when cultures were continuously illuminated under cool-white fluorescent lights for 14 days at 28 C. Phytopathology 61:1414.

Additional key words: peanut leaf spot, sporulation.

The Cercosporae have fastidious requirements for in vitro growth and sporulation (2, 6, 7). *Cercospora arachidicola* Hori, one of the principal peanut leaf spot fungi, grows slowly and sporulates poorly in vitro (1, 3, 5, 8). Landers (4) developed a chemically defined medium for *C. arachidicola* which is useful for quantitative measurement of vegetative growth. Abdou (1) found that *C. arachidicola* sporulated on peanut leaflet extract, oatmeal, lima bean, and mycophil agar media.

The purpose of this note is to describe a technique for producing abundant quantities of conidia for use in pathogenicity studies.

Peanut oatmeal agar (POA) used in this experiment is prepared as follows: *Arachis hypogaea* L. 'Argentine' leaflets (50 g) + 500 ml of distilled H_2O are placed in a Waring Blendor for 10-15 sec, and the resultant slurry is filtered through cheesecloth. Oatmeal (15 g in 500 ml distilled H_2O) is boiled for 15 min, then filtered through cheesecloth. Equal volumes of peanut leaflet and oatmeal filtrates are combined with 20 g agar/liter and autoclaved for 15 min at 121 C.

Suspensions of spores and mycelium of *C. arachidicola* are stored in sterile distilled H_2O at 5 to 6 C. To prepare inoculum for greenhouse pathogenicity studies, a plastic syringe (manufactured by Becton, Dickinson & Co., Rutherford, N.J.) with a 23-gauge needle is used to flood each POA plate with 2 ml inoculum. The narrow orifice of the needle results in a more uniform dispersion of the spores and mycelium than does a pipette with a larger orifice.

Plates of POA are incubated for 14 days at 28 C. A single layer of cultures is continuously illuminated with two 15-w cool-white fluorescent lights at a height of 31 cm above the level of the cultures.

To prepare the inoculum, 20 ml sterile distilled H_2O and two drops of Tween 20 (polyoxyethylene sorbitan monolaurate) are added to each 90 mm petri dish culture. Conidia are easily suspended with a sterile camel's-hair brush.

We have used this method to produce inoculum for numerous pathogenicity tests and to screen more than 800 peanut introductions for resistance to *C. arachidicola*. With 10-15 POA plates, it is possible to obtain enough conidia to inoculate all leaves of 400 3-week-old plants. The reproducibility and simplicity of this procedure should encourage additional research on the pathogenicity of *C. arachidicola*.

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