Production of Fungal Inoculum Using a Substrate of Perlite, Cornmeal, and Potato-Dextrose Agar

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ABSTRACT

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A substrate of perlite, cornmeal, and potato-dextrose agar was used to rapidly produce large numbers of conidia of *Bipolaris sorokiniana* without contamination for field inoculations. The medium was also used for producing large quantities of conidia of *Drechslera oryzae* and *Alternaria alternata*. It should be useful for many other fungi.

Field experiments with plant diseases may be limited by many factors, including the availability of inoculum. Schroeder et al (4) cited by Tuite (5) improved large-scale inoculum production by growing *Helminthosporium* and *Fusarium* species in shallow wooden flats containing a mixture of oats and wheat or a mixture of oats, wheat, and vermiculite. However, maintaining pure cultures in this medium is difficult. Musick (3) improved the possibility of maintaining pure cultures by using a medium of sand, cornmeal, and Czapek-Dox broth to produce conidia of *Bipolaris sorokiniana* (Sacc. ex Sorok) Shoem. for field inoculations, but small quantities of sand in the inoculum caused pumps to wear excessively. The method reported in this paper evolved from these two methods.

MATERIALS AND METHODS

Inoculum was produced in galvanized metal pans (7 × 15 × 21 cm) lined with a double layer of aluminum foil. Perlite, an inert, inorganic, siliceous rock of volcanic origin (1,2) commonly used in potting mixtures, was the matrix for fungal growth. In each pan, 150 g of yellow cornmeal was moistened with 300 ml warm 1% Difco potato-dextrose agar (PDA) and left standing for 10–15 min, then 75 g of perlite was thoroughly mixed with the cornmeal. The flats were covered with two layers of aluminum foil and autoclaved for 1 hr at 1 kg/cm^2 (15 psi). After cooling, the perlite-cornmeal cake was inoculated with 1-cm-square pieces of agar and mycelium from 10- to 14-day-old fungal cultures grown on PDA in petri plates. The pieces from two or three cultures were scraped onto the perlite cake and mixed in with a sterile knife. Fifty milliliters of sterile distilled water was added and the flat was closed. After 2–3 wk at laboratory temperatures (about 25 C), the fungal culture was spread on a tabletop to air-dry. Dried inoculum was bulked and stored in plastic bags at 4 C until needed.

A conidial suspension for field use was prepared by placing 1–2 L of the dried cultures in a fine-mesh nylon bag, agitating in water, and diluting the suspension to a volume of 400 L. Tween 80 was added at 0.25 ml/L of water.

RESULTS AND DISCUSSION

Conidia of *B. sorokiniana*, *Drechslera oryzae* (Breda de Han) Subram. & Jain, and *Alternaria alternata* (Fr.) Keissler were produced by this method. Only one pan in 110 of *B. sorokiniana* became contaminated.

Conidia of *B. sorokiniana* produced by this method were used to inoculate barley (*Hordeum vulgare* L.). Suspensions containing 5 × 10^4 to 5 × 10^5 spores per milliliter (estimated from spores in 10 μl) were sprayed at 2.8 kg/cm^2 (40 psi) from a 60-L tank mounted on a garden tractor every 2 days for 10 or 22 days, beginning when plants were in anthesis. Typical symptoms of spot blotch and kernel discoloration developed.

The perlite-cornmeal substrate has advantages over those used in the past; large quantities of inoculum can be produced with negligible contamination and little expenditure of time. The large numbers of petri plates used previously to produce field inoculum can now be reduced to the few needed to inoculate the pans. The several days required for soaking and autoclaving grain to prepare it for use is reduced to a few hours. Commercial perlite, produced at 1,600 C (2), is free of weeds and most microorganisms (1) and can be sterilized by a single exposure in the autoclave. Substituting perlite for sand in the substrate is desirable because perlite breaks up into fine particles that do not plug spray equipment or damage the pump.

A potentially wide range of adaptions of the perlite-cornmeal substrate could be made by incorporating nutrients appropriate to ensure conidial formation of most fungal species. Large glass jars or clear plastic trays could replace the metal pans when fungal species are grown that require a light regime for conidial development. Because the perlite cake may be easily broken into small fragments, it can be mixed thoroughly into soil to ensure uniform infestation of soil pathogens.

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