Quantitative Assay of Macrophomina phaseoli from Soil

Arthur H. McCain and Richard S. Smith, Jr.

Extension Plant Pathologist, University of California, Berkeley 94720. Plant Pathologist, Pacific Southwest Forest and Range Experiment Station, Forest Service, USDA, Berkeley 94701.

ABSTRACT

A new technique for a quantitative assay of *Macrophomina phaseoli* directly from soil has been developed. It uses both wet sieving and a selective medium.

Phytopathology 62:1098

Additional key words: charcoal root disease.

Macrophomina phaseoli (Maubl.) Ashby is responsible for the charcoal root disease which affects many plants in the warm areas throughout the world. The pathogen survives in the soil as sclerotia. Since these sclerotia are both the means of carry-over and the inoculum awaiting the next crop, a method of determining their present population is needed for studies concerned with the biology and control of this pathogen.

Watanabe et al. (2) reported an assay method employing the differential flotation of the sclerotia. Besides being time-consuming, the technique has the disadvantage of requiring a culture of each sclerotium for the determination of viability and identity. Watanabe (1) also describes a plating experiment in which he used sodium hypochlorite in potato-dextrose agar (PDA) as a selective medium. But, as he points out, further research was needed to eliminate contaminants and to develop an efficient, standardized technique.

This note describes a new technique in which quantitative assays of *M. phaseoli* can be made directly from soils by the combining of wet sieving and modifications of the sodium hypochlorite selective medium.

The soil to be assayed is dry-sieved through a 2-mm sieve to remove the larger soil particles. A 1- to 5-g sample of soil is placed in a blender with 250 ml of 0.525% sodium hypochlorite. The sample is stirred at slow speed for 30 sec once every 5 min for a total treatment time of 10 min. The sample is washed through sieves of 0.088 mm and 0.044 mm. The material on the 0.044-mm sieve is then collected by backwashing.

The sample from the 0.088-mm sieve is discarded, and the sample from the 0.044-mm sieve washed with 3 to 5 ml of water into a 200-ml bottle. To that mixture is added 100 ml of melted PDA at 55 C containing 0.5 ml of 5.0% streptomycin sulfate and 0.13 ml of 5.25% sodium hypochlorite. The bottles are shaken by hand, and the agar-soil suspension is poured into six to seven sterile petri dishes. The dishes are incubated at 31 C for 2 to 4 days, then examined. The colonies of *M. phaseoli* are readily identified by the numerous black sclerotia that develop.

In a more detailed sieving of a 20-g moist soil sample (17.6 g air-dry), sclerotia on the various size screens were distributed as follows: 0.037 mm–8; 0.044 mm–21; 0.053 mm–33; 0.062 mm–50; 0.088 mm–7; 0.104 mm–3; 0.177 mm–0; 0.250 mm–1. This distribution indicates that 84% of the sclerotia are obtained when only two sizes of sieves are used: 0.088 mm and 0.044 mm.

This technique has been used in practice on forestry tree nursery soils. Repeated assays of these soils have yielded results with a minimum of variation. This new method has also been used effectively to assay fumigated soils with low populations of pathogens; e.g., 0.1-1 propagule/g of soil.

LITERATURE CITED

1. WATANABE, T. 1967. Populations of microsclerotia of the soil-borne pathogen, Macrophomina phaseoli, in relation to stem blight of bean. Ph.D. Thesis, Univ. California, Berkeley. 97 p.
2. WATANABE, T., R. S. SMITH, JR., & W. C. SNYDER. 1970. Populations of Macrophomina phaseoli in soil as affected by fumigation and cropping. Phytopathology 60:1717-1719.