SPORULATION OF *MYCOLEPTODISCUS TERRESTRIS* ON A SYNTHETIC CULTURE MEDIUM

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Mycoleptodiscus terrestris causes a root and crown rot of red clover (*Trifolium pratense*), alfalfa (*Medicago sativa*) and birdsfoot trefoil (*Lotus corniculatus*) and root and basal stem rot of soybean (*Glycine max*) (Gerdemann, 1954). Screening of forage legume germplasm for resistance to *M. terrestris* has been performed by inoculating plants with a mycelial slurry. This method does not quantify the amount of inoculum delivered with precision. Inoculation with a spore suspension is a more precise method. Sporulation has been reported on natural media sterilized with propylene oxide, ethylene oxide, or γ irradiation, methods non-destructive to plant tissue relative to autoclaving and microwaving, but the number of spores produced is inadequate for large-scale pathogenicity testing (Ostazenski, 1964). Sporulation of *M. terrestris* was obtained on corn meal agar (CMA) and the number of spores was adequate for experimentation.

Spore production on CMA is simple and inexpensive relative to production on sterilized plant tissue. Twenty-five mL CMA (Difco 17 g/L) per plate was poured into 100mm x 15mm petri plates. Plates were then inoculated in the center with a plug 5 mm in diameter cut from a colony of *M. terrestris* grown on potato dextrose agar (Difco 39 g/L). The inoculated plates were sealed with Parafilm® and incubated at 23°C, 50% relative humidity, under fluorescent light (13 μmol $s^{-1}m^{-1}$) on a 12 hour light/12 hour dark cycle. When the colony margin reached the edge of the plate (approx. 7 days) the Parafilm was removed and the plates were allowed to air-dry for 7 days. During this period hyphal aggregations formed giving rise to large, complex conidiophores which produced conidia in a mucilagenous substance (McVey and Gerdemann, 1960). A spore suspension was obtained by washing with 10 mL sterile, distilled water per plate. The water was allowed to sit on the agar surface for 5 minutes to allow spores to disperse. Conidia may be stored indefinitely in sterile, distilled water at room temperature. Spores sometimes clumped making precise quantification using a hemacytometer difficult. Tween 80 (Sigma Chem. Co.) at a concentration of 0.25 mL/L may be added to a spore solution to prevent clumping with no adverse effect on spore percent germination.

Precise quantification of inoculum should result in more accurate and repeatable characterization of resistance to *M. terrestris* among populations of forage legumes. Preliminary tests have shown spores to be capable of causing foliar infection of red clover and soybean. Sporulation on CMA also serves as an accurate method of identifying isolates of *M. terrestris* in culture.

LITERATURE CITED:

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