Method for Production of Sclerotia of Rhizoctonia solani

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Isolates of *Rhizoctonia solani* Kühn differ in their ability to form sclerotia on culture media (5, 6). Isolates that do form sclerotia in culture may produce only a few per plate, presenting a problem when large numbers of sclerotia are required for experimental work.

Other investigators have shown that carbon and nitrogen sources, nutrient levels, temperature, pH, free moisture, and physical limitation of mycelial growth affect the formation of sclerotia in cultures of sclerotium-forming isolates of *R. solani* (1, 4, 6). Henis et al. (2) also showed that nutrition and mechanical damage of mycelium affect the formation of sclerotia in cultures of *Sclerotium rolfsii*. We considered several of these factors.

Seventeen isolates of *R. solani,* from various hosts and geographic locations, were plated on reconstituted dehydrated potato-dextrose agar (PDA). All plates were maintained in diffuse, normal daylight at approximately 27 C on a laboratory bench. Observations on sclerotium production were made over an 18-day period. Several of the isolates produced no sclerotia, the rest only a scattered few. An isolate of *R. solani,* obtained from a bean (*Phaseolus vulgaris* L.) hypocotyl in Delaware, produced the largest number of sclerotia of any isolate examined. This isolate was used for all further experimentation, since the object of these experiments was to develop a method for the rapid induction of large quantities of sclerotia of *R. solani* for use in ecological studies in soil.

This isolate of *R. solani* was plated on PDA at pH 5.6; PDA plus 4 g yeast extract and 4 g peptone/liter (PDA-YEP) at pH 5.5; PDA plus 4 ml 50% lactic acid/liter (PDA-LA) at pH 3.6; Czapek's Solution agar (CZ) at pH 7.2; and PDA made with an extract from 200 g fresh potatoes (FPDA) at pH 5.8. Mycelia of 6-day-old PDA and FPDA cultures of the isolate were heavily scored with a scalpel. Three-day-old PDA and FPDA cultures of the isolate of *R. solani* were placed in 100 ml sterile distilled water in a sterile microblender and were blended for 3 min to form a heavy suspension. A thin layer of this suspension was then poured over the surface of PDA and FPDA plates, respectively. Ten plates were used for each medium and treatment. All plates were incubated as previously described. Plates were observed after 2, 5, and 10 days' incubation for sclerotium production. Data taken represented average values for 10 plates.

Sclerotia were not produced on PDA-YEP, PDA-LA, or CZ plates (Table 1). An average of 10 sclerotia/plate was found on PDA plates after 5 days, and 15 after 10 days. An average of 17 sclerotia/plate was found on FPDA plates after 5 days, and 36 after 10 days. PDA and FPDA cultures that had been scored with a scalpel produced an average of 8 and 10 sclerotia/plate, respectively, only along the cut edges after 10 days. PDA plates overpoured with a blended 3-day-old PDA culture of *R. solani* produced rapid mycelial growth that covered the entire plate surfaces in 2 days. An average of 15 sclerotia/plate was produced after 5 days, and 20 after 10 days. A similar mycelial growth pattern was observed on blended, culture-overpoured FPDA plates. An average of 40 sclerotia/plate was produced after 5 days, and 50 after 10 days.

The blended, FPDA culture-overpour method of FPDA plates was found to be an effective method for the rapid production of large quantities of sclerotia by a sclerotium-forming isolate of *R. solani.* Mechanical damage of mycelium alone did not appear to be a factor in the induction of extensive sclerotium production by this isolate of *R. solani.* A combination of physical limitation of mycelial growth and nutritional

TABLE 1. Effects of culture media and mycelial damage on sclerotium production by an isolate of *Rhizoctonia solani* maintained in diffuse, normal daylight at approximately 27 C

Culture media[a]	pH	Treatment	Sclerotium production[b]		
			2 days	5 days	10 days
PDA	5.5	None	0	10	15
PDA-YEP	5.5	None	0	0	0
PDA-LA	3.6	None	0	0	0
CZ	7.2	None	0	0	0
FPDA	5.8	None	0	17	36
PDA	5.5	Culture scored	0[c]	0[c]	8[c]
FPDA	5.8	Culture scored	0[c]	0[c]	10[c]
PDA	5.5	Blended mycelial inoculum	0	15	20
FPDA	5.8	Blended mycelial inoculum	0	40	50

[a] PDA = potato dextrose agar; PDA-YEP = PDA + yeast extract and peptone; PDA-LA = PDA + lactic acid; CZ = Czapek's Solution agar; FPDA = PDA made with extract from fresh potatoes.
[b] Avg values from 10 plates/medium.
[c] Observations made along cut edges only.

factors in an extract from fresh potatoes appeared to be responsible for the rapid induction of extensive sclerotium production by this isolate of *R. solani*. These results appear to be similar to those reported by Timnick et al. (7) on sporulation of *Melanconium fuligeneum* in response to flooding the agar surface of plates with spores or cut mycelium of this fungus. Rapid exhaustion of nutrients on flooded-plates appeared to be the cause of rapid, extensive sporulation. Lilly (3) also points out that the flooded-plate technique can induce rapid, extensive sporulation in many other sporulating fungi.

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