An Efficient Method of Inducing Sporulation of Alternaria solani in Pure Culture

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ABSTRACT

A method of inducing sporulation of *Alternaria solani* in pure culture has been developed. This makes it possible to produce abundant quantities of mature spores under aseptic conditions in 16-20 days. Phytopathology 61:239.

Alternaria solani (Ell. & Mart.) Jones & Grout does not produce spores readily under laboratory conditions. Several workers have attempted to increase sporulation by various techniques. These methods have included mutilation of the mycelium (1, 2, 3, 4, 5, 8, 9), use of different culture media (1, 2, 8), exposure to ultraviolet light (2, 8), exposure to fluorescent light (1, 2, 5), exposure to sunlight (8, 9), dehydration of the medium (1, 2, 4, 8, 9), and chemical treatment of the culture (2).

An efficient method, proven consistently to induce abundant sporulation in numerous isolates of *A. solani,* has been developed. This makes it possible to produce abundant quantities of spores under aseptic conditions in 16-20 days. The isolates used were obtained from typical potato leaf lesions on potatoes grown in Idaho and Maine.

The fungus is inoculated onto the center of a 9-cm petri plate containing 15 ml of an adapted potato-dextrose agar (PDA). This medium consists of 15 g of instant mashed potatoes, 15 g of dextrose, and 23 g of flake agar in 1,500 ml of distilled water. The fungus is allowed to grow for 10-14 days at a temp ranging from 20-26 C. The plates may be placed in constant light or under normal diurnal lighting. Little, if any, sporulation will occur with most isolates of the fungus during this growing period.

An entire culture from one petri plate is cut with a sterile wire transfer needle into approx 4-cm strips and placed into a sterile 250-ml flask containing 50 ml of sterile distilled water. The flask is then shaken vigorously for approx 1 min and left to stand for 10 min. One-and-one-half ml of the liquid from the flask are poured onto a fresh plate of the adapted PDA. The plate is rotated so that the added liquid completely covers the surface of the agar. The inoculated plates are placed in an incubator at 20 C with a constant light source. The light source is a 15-w cool-white fluorescent tube that is located 25 cm above the cultures. The petri plates can be stacked two high, with no apparent effect upon sporulation. After 6 days, with most isolates, there is abundant sporulation over the entire surface of the agar. These mature spores can then be harvested by rinsing with a jet of water or by any other desirable means.

Lukens (5, 6) first reported that light stimulated *A. solani* to grow conidiophores and inhibited the conidiophores from bearing spores. He (7) later reported, however, that conidiophores can bear spores in light when the temp is below 23 C. This appears to be in accordance with the results of this research.

LITERATURE CITED

1. Barksdale, T. H. 1968. A method of screening for resistance to early blight on tomato seedlings. Phytopathology 58:883 (Abstr.).
2. Charlton, K. M. 1953. The sporulation of Alternaria solani in culture. Brit. Mycol. Soc. Trans. 36:349-355.
3. Kunkel, L. O. 1918. A method of obtaining abundant sporulation in cultures of Macrosporium solani E. & M. Brooklyn Bot. Garden Mem. 1:306-312.
4. Ludwig, R. A., L. T. Richardson, & O. H. Unwin. 1962. A method for inducing sporulation of Alternaria solani in culture. Can. Plant Dis. Surv. 42:149-150.
5. Lukens, R. J. 1960. Conidial production from filter paper cultures of Helminthosporium vagans and Alternaria solani. Phytopathology 50:867-868.
6. Lukens, R. J. 1963. Photo-inhibition of sporulation in Alternaria solani. Amer. J. Bot. 50:720-724.
7. Lukens, R. J. 1966. Interference of low temperatures with the control of tomato early blight through use of nocturnal illumination. Phytopathology 56:1432-1433.
8. McCallan, S. E. A., & S. Y. Chan. 1944. Inducing sporulation of Alternaria solani in culture. Contrib. Boyce Thompson Inst. 13:323-335.
9. Rands, R. D. 1917. The production of spores of Alternaria solani in pure culture. Phytopathology 7:316-317.