A SELECTIVE MEDIUM FOR ISOLATION AND IDENTIFICATION OF *BOTRYTIS* SPP. FROM SOIL AND ONION SEED

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The population of *Botrytis allii* in either naturally or artificially infested soils was selectively measured by the soil dilution plate count procedure on a developed synthetic medium supplemented with tannic acid, fungicides, antibiotics and Cu⁺⁺ ions. Conversion of tannic acid into a dark brown pigment was related to the activity of extracellular polyphenoloxidase produced by the fungus. Thus, brown-pigmented colonies were recognized as *Botrytis* spp. The same medium was used for detecting the presence of the fungus on onion seed.

**KEY WORDS:** *Botrytis allii*; tannic acid; selective inhibition.

INTRODUCTION

Synthetic media which combine selective exclusions with pigment production by the desired fungus have been developed for only a few plant pathogenic fungi. Such media were employed for the isolation and quantitative studies of *Fusarium oxysporum* Schlecht. (2) and *Pyrenochaeta terrestris* (Hans.) Gorenz, J.C. Walker and Larson (7).

*Botrytis allii* Munn, a common pathogen of onion (*Allium cepa* L.), affects its host mainly during storage. Loss of 50% of onion bulbs due to the neck rot disease was reported (5). Two main sources of primary inoculum have been described. The organism has been reported to overwinter as sclerotia in the soil and in the bulbs (8) and to be seed-borne (5,6).

A selective medium for the assay of *B. allii* from soil was reported by Lorbeer and Tichelaar (3). Although this medium is quite effective in its selective inhibition, identification of the colonies in question as *B. allii* is corroborated only 8-10 days later, on sporulation. While using their medium for detecting seed-borne *B. allii*, the isolation plates were found to be overrun by various fungi.

In the present study a medium for the isolation of *Botrytis* spp. was developed,

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based on selective inhibition and pigment induction of the organism under study. The latter phenomenon has been treated as a device for rapid identification of the fungus.

**MATERIALS AND METHODS**

Sporulating cultures of *B. allii* isolated from onion bulbs (cv. 'Ori') were maintained by frequent transfers of single conidia to potato dextrose agar (PDA) slants, which were incubated at 24°C. Standard conidial suspensions were prepared by gently washing the surface of 2-week-old cultures with 20 ml of sterile distilled water containing one drop of Triton x-100.

The basal medium (BM) of the following components (g/l of distilled water) was developed: NaNO₃, 1.0; K₂HPO₄, 0.9; MgSO₄·7H₂O, 0.2; KCl, 0.15; glucose, 20.0; and agar, 25.0. The autoclaved medium was cooled to 70°C, and the other ingredients were added: Terraclor (PCNB), pentachloronitrobenzene 75% WP, 7 x 10⁻³; Maneb (manganese ethylene bisdithiocarbamate), 4 x 10⁻⁴; chloramphenicol, 2.5 x 10⁻²; CuSO₄, 1.7; and tannic acid, 5.0 (Tannin, Sigma Co.). The pH of the supplemented basal medium (SBM) was adjusted to 6.0 with 1N NaOH.

**Inoculation technique**

Inoculum was grown in 250-ml flasks containing 15 g of wheat bran and 50 ml of tap water, autoclaved twice for 40 min. Three discs (3-mm diam.) of a single spore culture of *B. allii* grown on BM were transferred to each flask. After 7 days' incubation at 24°C the flasks were shaken for 15 min. to ensure uniform distribution of the inoculum; this was followed by an additional 7-days' incubation. The inoculum, composed of mycelium, sclerotia and conidia, was then mixed with a sieved, air-dried, heat-sterilized sandy loam soil. The infested soil was placed in trays (52 x 31 x 10 cm), each of which was planted with 21 onion sets (cv. 'Ori'). The plants were kept for 21 days at 24°C (14 h, day) and 14°C (10 h, night), after which time the final disease assessment was made.

**Isolation from soil**

Ten g of freshly infested soil was suspended in 100 ml of 0.1% water agar and shaken for 15 minutes. One-ml aliquots of 10⁻³ soil dilution were pipetted into 90-mm diam. petri dishes and spread evenly on the surface of the medium.

**Seed test procedure**

Commercial onion seed (cv. 'Ori') (60 seeds/plate) was incubated on SBM for 72 h at 24°C.

**RESULTS AND DISCUSSION**

Addition of tannic acid to culture media provides an easy means of testing for the presence of phenoloxidase in fungi (4). Clark and Lorbeer (1) have reported that *Botrytis cinerea* developed a brown color on catechol-containing medium.