# A Medium for Enumeration and Isolation of Calonectria crotalariae from Soil

S. C. Hwang and W. H. Ko

Graduate Research Assistant and Associate Professor, respectively, Department of Plant Pathology, University of Hawaii, Beaumont Agricultural Research Center, Hilo 96720.

Supported in part by PSW Grant No. 3 from the U.S. Forest Service. Journal Series Paper No. 1855 of the Hawaii Agricultural Experiment Station.

ABSTRACT

*Calonectria crotalariae* produced brownish colonies with visible strands of microsclerotia on acidified medium containing 2% dextrose, 0.02% peptone, and 2% agar. The colony size and growth of aerial mycelia of soil fungi were greatly reduced on this medium due to the high dextrose-peptone ratio. With this medium 83 to 93% of *C. crotalariae* propagules were recovered from artificially inoculated soil. The population of this fungus in the vicinity of diseased papaya seedlings ranged from 350 to 3,050 propagules per gram of dried soil.

Phytopathology 65:1036-1037

*Additional key words: Cylindrocladium theae.*

---

*Calonectria crotalariae* (Loos) Bell & Sobers, with the imperfect stage of *Cylindrocladium crotalariae* (Loos) Bell & Sobers, causes black rot of peanuts, collar rot of koa and papaya, and stem and root rot of blueberry (1, 2, 5, 7). It produced conidia and ascospores on diseased tissue of various crops (1, 2, 5), and microsclerotia in infected papaya stems and peanut roots (3, 9). Azalea leaves and peanut plant have been used for isolation of *C. crotalariae* from soil (6, 8). We report here a medium for determining population of *C. crotalariae* in soil.

MATERIALS AND METHODS.—Conidia of *C. crotalariae* were obtained by growing the fungus on V-8 juice agar for 4 days at 24 C with continuous fluorescent light. Perithecia, which were produced on the same medium after 15 days of incubation, were transferred with a needle to a test tube and were freed of conidia by washing with sterile distilled water five times by sedimentation. Ascospores were separated from crushed perithecia by sedimentation before use. Microsclerotia were obtained by growing the fungus on a piece of cellophane (90 mm in diameter) laid on the surface of dextrose-peptone agar for 2-3 weeks at 24 C (4). The cellophane with microsclerotia, conidia, and mycelia were removed from agar medium and triturated with distilled water in an Omni-Mixer at 15,000 rpm for 1 minute. After removal of cellophane, the suspension was passed through a 38-$\mu$m sieve which retained only microsclerotia and larger mycelial fragments. Microsclerotia were further separated from larger mycelial fragments by sedimentation. Propagule concentrations were determined by the microsyringe method (4).

The medium consisted of 20 g dextrose, 0.2 g peptone, and 20 g agar in 1,000 ml distilled water. After autoclaving, it was acidified to pH 4.0 by mixing 0.14 ml of 20% lactic acid with 100 ml of medium. Soil suspensions were prepared by mixing 10 g of soil inoculated with $10^5$ conidia, $10^5$ ascospores, or $3 \times 10^4$ microsclerotia with 50 ml of water in an Omni-Mixer chamber at 6,500 rpm for 1 minute, and were further diluted to $10^{-2}$. One milliliter of diluted soil suspension was distributed on a plate of the dextrose-peptone medium. The number of *C. crotalariae* colonies was

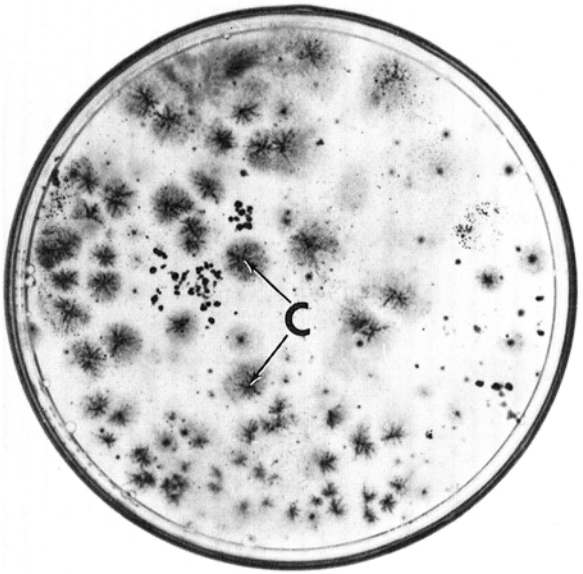


**Fig. 1.** Colonies of fungi from soil on the dextrose-peptone medium. Those colonies (c) with dark strands of microsclerotia are *Calonectria crotalariae*.

recorded after 1 week of incubation at 24 C. Five plates were used for each treatment.

RESULTS AND DISCUSSION.—Conidia, ascospores, and microsclerotia germinated 95, 92, and 90%, respectively, on the dextrose-peptone medium. When a soil suspension was plated on this medium, *C. crotalariae* produced brown colonies with visible strands of microsclerotia (Fig. 1). Bundles of cylindrical conidia and stipes with globose vesicles at their tips, typical of *C. crotalariae,* emerged right above each colony. They were, therefore, easily distinguishable from colonies of other fungi. The size of fungal colonies was greatly reduced and very few aerial mycelia were produced on the dextrose-peptone medium. Similar results were obtained when hydrochloric acid and phosphoric acid were used to acidify the medium. However, when potato-dextrose agar and V-8 juice agar acidified to pH 4.0 with lactic acid were tested, plates were covered by aerial mycelia in 4 days and *C. crotalariae* could not be counted and isolated easily. The effectiveness of the dextrose-peptone medium in restricting colony size and aerial mycelium production of soil fungi was apparently due to the high dextrose-peptone ratio because acidified media containing 2% dextrose plus 0.2% peptone, and 2% dextrose plus 2% peptone were not effective.

Using the acidified dextrose-peptone medium for isolation, the percentage recovery of conidia, ascospores, and microsclerotia of *C. crotalariae* from artificially inoculated soil was 83, 84, and 93%, respectively. When this medium was used for determining *C. crotalariae* in naturally infested soil, the population of this fungus in five soil samples collected from the vicinities of diseased papaya seedlings was 350 to 3,050 propagules per gram of air-dried soil.

*Cylindrocladium theae* (Petch) Alfieri et al. and an unidentified *Cylindrocladium* sp. supplied by M. Aragaki also produced brown colonies similar to *C. crotalariae* on the dextrose-peptone medium. Therefore, the same medium may also be useful for isolation of other *Cylindrocladium* spp. from soil.

## LITERATURE CITED

1. ARAGAKI, M., F. F. LAEMMLEN, and W. T. NISHIJIMA. 1972. Collar rot of koa caused by Calonectria crotalariae. Plant Dis. Rep. 56:73-74.
2. BELL, D. K., and E. K. SOBERS. 1966. A peg, pod, and root necrosis of peanuts caused by species of Calonectria. Phytopathology 56:1361-1364.
3. HWANG, S. C. 1974. The biology of Calonectria crotalariae in soil. M.S. Thesis. University of Hawaii, Honolulu. 36 p.
4. KO, W. H., L. L. CHASE, and R. K. KUNIMOTO. 1973. A microsyringe method for determining concentration of fungal propagules. Phytopathology 63:1206-1207.
5. LAEMMLEN, F. F., and M. ARAGAKI. 1971. Collar rot of papaya caused by Calonectria sp. Plant Dis. Rep. 55:743-745.
6. LINDERMAN, R. G. 1972. Isolation of Cylindrocladium from soil or infected azalea stems with azalea leaf traps. Phytopathology 62:736-739.
7. MILHOLLAND, R. D. 1974. Stem and root rot of blueberry caused by Calonectria crotalariae. Phytopathology 64:831-834.
8. ROWE, R. C., M. K. BEUTE, J. C. WELLS, and J. C. WYNNE. 1974. Incidence and control of Cylindrocladium black rot of peanuts in North Carolina during 1973. Plant Dis. Rep. 58:348-352.
9. ROWE, R. C., S. A. JOHNSTON, and M. K. BEUTE. 1974. Formation and dispersal of Cylindrocladium crotalariae microsclerotia in infected peanut roots. Phytopathology 64:1294-1296.