Selective Medium for *Verticicladiella procera*

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ABSTRACT

McCALL, K. A., and W. MERRILL. 1980. Selective medium for *Verticicladiella procera*. Plant Disease 64:277-278.

Acid malt agar containing 500 mg per liter of cycloheximide is a selective medium for *Verticicladiella procera*. This medium can be used to isolate *V. procera* from white pines in various stages of root decline.

Verticicladiella procera Kendrick causes a root decline of *Pinus strobus* L. (eastern white pine). Affected trees have chlorotic, drooping needles, crooking of new shoots, reduced shoot elongation, and retention of needles for a year or more after the tree's death. There may be diffuse stem cankers near the ground line. The basal stem and the roots have resin-soaked, irregular zones of dark brown discoloration (3).

White pine root decline is suspected in scattered areas of Pennsylvania. This has not been confirmed because of difficulty in isolating *V. procera*; the fungus has been recovered in as few as one of 528 isolations (4).

Cycloheximide (Actidione) at a concentration of 100 ppm inhibited the growth of common contaminants such as *Neurospora* sp. and *Penicillium* sp. but did not delay the growth of *Ceratocystis ulmi* (Buisman) C. Moreau (1). Because several species of *Verticicladiella* have *Ceratocystis* perfect stages (2), in vitro and field studies were done to test the selectivity of cycloheximide for *V. procera*.

Contribution No. 1099, Department of Plant Pathology, The Pennsylvania Agricultural Experiment Station; approved for publication as Journal Series Paper No. 5827.

Accepted for publication 15 October 1979.

00191-2917/80/03027702/$03.00/0

MATERIALS AND METHODS

In vitro studies. Water solutions of cycloheximide (3[2(3,5-dimethyl-2-oxocyclohexyl)-2-hydroxyethyl]glutarimide, SIGMA Chemical Company, St. Louis, MO) were added to warm acid malt agar (AMA = 15 g of agar and 20 g of Difco malt extract per liter of distilled water, plus 1 ml of concentrated lactic acid per liter added after autoclaving). Plugs of agar and mycelium 5 mm in diameter were taken from the margins of fungal colonies growing on AMA and placed in the centers of plates of medium containing various concentrations of the antibiotic. Colony diameters of the fungi were measured 7–9 days after the plugs were placed on the cycloheximide media.

In the first study, seven concentrations of cycloheximide (0.0, 0.001, 0.01, 0.1, 1, 10, and 100 mg per liter) were tested against four fungi: *C. ulmi, C. fagacearum* (Bretz) Hunt, *Penicillium* sp., and *V. procera*. In the second study, four concentrations of cycloheximide (0.0, 100, 300, and 500 mg per liter) were tested against six fungi: *Fusarium solani* (Mart.) App. et Wr. emend. Snyd. & Hans., *Trichoderma viride* Pers., *Pythium aphanidermatum* (Edson) Fitz., *Penicillium* sp., *Alternaria* sp., and *V. procera*. There were five replicates of each concentration for the first study and six replicates of each concentration for the second study.

Field studies. Wood samples from the basal stems and roots of six eastern white pines known or suspected to be infected by *V. procera* were collected. Chips 2 × 2 × 2 mm were cut from these samples and plated on AMA containing 500 mg per liter of cycloheximide.

In May 1979, three eastern white pines were sampled at the Hanover Municipal Watershed, Hanover, Pennsylvania. Previous studies had shown the fungus to be present in these trees (B. Towers, *personal communication*). Sixty chips were plated from the stem of tree one, which had a basal stem canker, dark brown and bluish discoloration of the sapwood, and heavy bark beetle infestation and had been dead for 1 yr. Thirty stem and 30 root chips were plated from tree two, which was stunted, had reduced shoot growth in 1979, drooping chlorotic needles, and discoloration of the roots and basal stem. Thirty stem and 30 root chips were plated from tree three, which had discoloration of the roots and basal stem and no shoot elongation in 1979.

In June 1979, 30 stem and 30 root chips were plated from tree four, which was growing in a Christmas tree plantation in Dushore, Pennsylvania. This tree was stunted, had chlorotic needles, dark brown discoloration of the basal sapwood and roots, slight resinosis at the tree base, and no shoot growth in 1979.

In June 1979, samples were collected from trees five and six, which were growing in a Christmas tree plantation in Schuylkill Haven, Pennsylvania. Thirty stem chips were plated from tree five, which had been dead for over 6 mo but still had brown needles attached. Thirty stem and 30 root chips were plated from tree six, which was living but had chlorotic needles, reduced shoot growth, and discoloration in the roots and basal stem.

RESULTS AND DISCUSSION

In vitro studies. In the first study, 100 mg per liter of cycloheximide completely inhibited *C. fagacearum,* almost completely inhibited *Penicillium* sp., and had no effect on the growth of *C. ulmi* and *V. procera.*

In the second study, 500 mg per liter of cycloheximide completely inhibited *F. solani, P. aphanidermatum, Penicillium* sp., and *T. viride* and had no effect on the growth of *Alternaria* sp. and *V. procera.*

The results of these experiments showed that AMA containing 500 mg per liter of cycloheximide acted as a selective medium for *V. procera*; it inhibited the growth of the most commonly encountered fungal contaminants but allowed uninhibited growth of *V. procera.*

Field studies. Recovery of *V. procera* was as follows: tree one—1 of 60 stem chips; tree two—20 of 30 stem chips and 27 of 30 root chips; tree three—7 of 30 stem chips and 5 of 30 root chips; tree four—30 of 30 stem chips and 30 of 30 root chips; tree five—1 of 30 stem chips. The pathogen was not recovered from tree six.

The field studies show that *V. procera* can be isolated from eastern white pines in various stages of root decline by using AMA containing 500 mg per liter of cycloheximide.

The pathogen was recovered in small percentages from dead trees. Tree one, which yielded the fungus in 1.7% of the chips, had been dead for a year. Tree five, which yielded the fungus in 3.3% of the chips, had been dead for longer than 6 mo.

The pathogen was recovered in low percentages from a tree that had just begun to show external symptoms. Tree three, which yielded *V. procera* in 20% of the chips, had no outward sign of infection other than no shoot elongation in 1979.

Although tree six showed symptoms similar to those of tree three, *V. procera* was not recovered. It is uncertain, however, whether this tree's symptoms actually were caused by *V. procera.*

The pathogen was recovered in high percentages from trees in an active state of decline. It was recovered in 82% of the chips from tree two and in 100% of the chips from tree four; both trees were stunted and had discolored roots and basal sapwood, retarded shoot growth, and drooping, chlorotic needles.

ACKNOWLEDGMENTS

We thank Barry Towers, Pennsylvania Department of Environmental Resources, and Larry Zang, Department of Plant Pathology, The Pennsylvania State University, for aid in conducting these studies.

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