Growth of *Cytospora abietis* on Media Made from Dwarf Mistletoe-Infected and Uninfected Branch Tissues of Red Fir

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ABSTRACT

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Cytospora abietis invades dwarf mistletoe-infected branches of red fir in the field more often than uninfected branches. Growth of the fungus on media prepared with dwarf mistletoe-infected branch tissues was compared with that on media prepared with uninfected tissues. Neither linear growth nor dry weight of the fungus differed significantly between the two media. Results indicate that autoclaved dwarf mistletoe-infected tissues of red fir contain no nutrients or other factors that favor growth of *C. abietis* in culture.

The fungus *Cytospora abietis* Sacc. commonly infects true firs (*Abies* spp.) in the western United States (1,4,5). Infection often results in branch flagging and, occasionally, top dieback in young trees.

C. abietis often is found in fir stands infected by dwarf mistletoe (*Arceuthobium abietinum* Engelm. ex Munz.). In these stands, branches infected by dwarf mistletoe often are the infection sites for *Cytospora*, whereas uninfected branches are less often invaded by the fungus. For example, in the southern Cascades and central Sierra Nevada, I found that about 20% of the dwarf mistletoe-infected branches of more than 1,000 white firs (*A. concolor* (Gord. & Glend.) Lindl. ex Hildebr.) and red firs (*A. magnifica* A. Murr.) were invaded by *C. abietis*, while only about 4% of the uninfected branches were invaded (3).

Some of the possible reasons why *C. abietis* invades and kills dwarf mistletoe-infected branches are: 1) dwarf mistletoe reduces the vigor of branches, making them more susceptible to infection by a weak pathogen like *Cytospora*; 2) bark cracks and old dwarf mistletoe shoot bases serve as entrance courts through which the fungus can invade host tissue; and 3) the swollen branch tissue and the endophytic system of dwarf mistletoe are sites of carbohydrate accumulation (2). These tissues may provide particularly favorable substrates in which *C. abietis* can grow.

This paper reports a study of whether extracts from dwarf mistletoe-infected branch tissues provide a more favorable substrate for growth of *C. abietis* in culture than do extracts from uninfected tissues.

MATERIALS AND METHODS

Dwarf mistletoe-infected and uninfected branches of red fir were collected in midsummer 1980 in the central Sierra

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Nevada and stored in plastic bags in a freezer until winter, when they were used to prepare media. The outer bark was carefully removed with a scalpel and discarded; then the living cortical and phloem tissues were removed. All necrotic tissues from the infected branches were discarded. The tissues were dried at 60 C for about 24 hr, then ground in a Wiley mill to pass a 20-mesh screen. The ground tissues were stored in separate containers in the freezer.

Both solid and liquid media were prepared. The solid medium consisted of 2% water agar containing either infected or uninfected tissue at a concentration of 10 g/L. The agar medium was autoclaved for 15 min at 1.4 kg/cm^2 (20 psi) and poured into sterile 100-ml petri dishes, 20 ml per dish. Synthetic liquid growth medium including either infected or uninfected tissue had as basic components 2 g of asparagine, 0.75 g of $MgSO_4 \cdot 7 H_2O$, 1.75 g of KH_2PO_4, and 1 mg of thiamine in 1 L of distilled water. No source of carbon other than asparagine was added except for that provided by the host tissue. Amounts of tissue used were 1, 5, and 10 g/L. The liquid media were autoclaved for 15 min at 1.4 kg/cm^2 (20 psi), filtered to remove solid tissue particles, dispensed into 250-ml flasks, 100 ml per flask, and autoclaved again.

A pure culture of *C. abietis* isolated from dwarf mistletoe-infected white fir in northern California was grown on potato-dextrose agar in petri dishes (20 ml per 100-ml dish) for 1 wk at room temperature (20–22 C). A disk (5.7 mm diameter) of agar containing mycelium was cut from the culture dishes and placed in the center of each test dish and in each flask of liquid medium. The test dishes were placed in plastic bags and incubated at a constant 25 C. The liquid cultures were placed on a rotary shaker at room temperature.

At periodic intervals, colony diameter was measured to the nearest millimeter along two perpendicular lines that met at the center of each dish until the mycelial growth was within about 1 cm of the dish edge. For the liquid cultures, the fungus was grown for 8 days, after which the fungal mats were removed from the flasks, briefly washed in distilled water, and oven-dried at 80 C for 1 hr. The dried mats were weighed to the nearest milligram. Twelve replicates were measured for diameter growth on solid medium for each tissue, and 10 fungal mats were weighed for each tissue and concentration of liquid medium.

RESULTS AND DISCUSSION

Linear growth of *C. abietis* on solid medium with dwarf mistletoe-infected tissue was the same as that on solid medium with uninfected tissue. An F test of growth among the replicates on the two media indicated that growth of *C. abietis* did not differ significantly between them ($P = 0.05$). Average diameter of the fungus colonies on both media was about 35 mm after 1 wk and about 65 mm after 2 wk.

Results in liquid media suggest that growth expressed as dry weight of the fungus also did not differ significantly in media made with either dwarf mistletoe-infected or uninfected tissues. Growth differed among the three concentrations of tissue media, however. Dry weight of fungus (mg) increased with an increase in tissue concentration in the growth media.

No evidence was found to suggest that nutrient conditions or other growth factors present in autoclaved dwarf mistletoe-infected host tissue favored growth of *C. abietis* in culture. In the field, however, growth of *C. abietis* may differ between dwarf mistletoe-infected and uninfected host tissues. Pathogenicity is not always a function of only fungus growth rate. Perhaps other factors in living dwarf mistletoe-infected branches make them more susceptible to infection.

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