Characteristics of *Diplodia pinea* Associated with Tip Dieback of Ponderosa Pine Seedlings

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Diplodia pinea (Desm.) Kickx. was recently isolated from tip blighted ponderosa pine (*Pinus ponderosa* Laws.) seedlings at the Fantasy Farms Nursery near Peck, Idaho (James 1984). Blighted seedlings were scattered throughout seedbeds and extensive mortality occurred. Such high levels of seedling disease associated with *D. pinea* have not previously been encountered in this Region. Therefore, investigations were conducted on the isolated fungus to determine if it was similar to previously-described isolates of *D. pinea*.

METHODS

Spore germination, growth characteristics in culture, and *in vitro* pycnidial production of *D. pinea* obtained from blighted pine seedlings were studied. Conidia from pycnidia growing on necrotic needles were tested for germination in sterile distilled water and on 2 percent water agar (WA). Time required for germination and germ tube elongation rate and branching habit were determined. Spores were germinated at 25°C under cool fluorescent light. Cultures of *D. pinea* were grown on potato dextrose agar at 15°C, 25°C, and 35°C under cool fluorescent light. Linear growth rate, hyphal pigmentation, and pycnidial production were determined at periodic time intervals.

RESULTS AND DISCUSSION

Most mature conidia from the isolates of *D. pinea* obtained from necrotic seedling tissue were slightly brown colored and one-celled (figure 1). Immature conidia were hyaline and only a few conidia from mature pycnidia on necrotic needles were septate (two-celled). Although the genus *Diplodia* is defined by its production of two-celled pigmented spores (Barnett and Hunter 1972), many isolates of *D. pinea* rarely produce two-celled spores (James 1979; Peterson 1981a; Peterson 1981b).

A few conidia germinated in sterile distilled water after 6 hours. On WA, very few spores germinated before 24 hours' incubation. Only about 10 percent of the spores from mature pycnidia germinated within 32 hours. Most germinating spores produced a single germ tube which became multibranched after a few hours (figure 2).

Previous work (Chou 1977) indicated that spores of *D. pinea* germinate on sterile water or agar much more frequently and rapidly than on host tissues. Peterson (1981b) found that a high percentage of conidia germinated within 2 hours on WA. Chou (1978) reported that spore germination commenced on host surfaces within 3 hours of inoculation and was nearly complete within 6 hours. My tests indicated that most spores from mature pycnidia were not viable and the few that were took longer than expected to germinate. This may be because blighted seedlings were collected in the late fall when the fungus might not have been active.

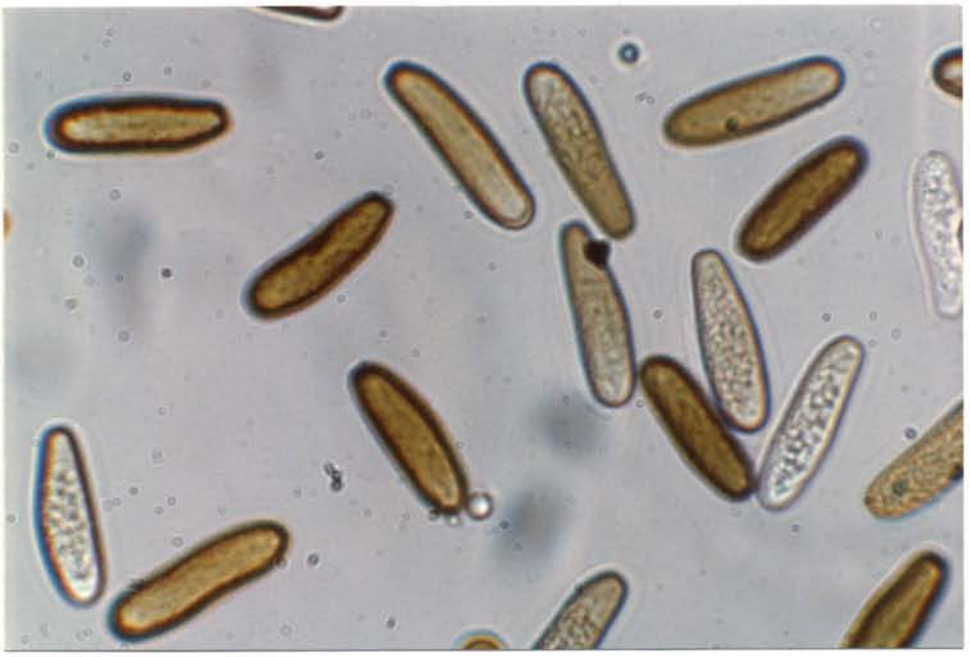

Figure 1.-Conidia of Diplodia pinea from tip blighted ponderosa pine seedlings (X450). Conidia were initially hyaline, but became pigmented as they matured. Most conidia were one-celled.

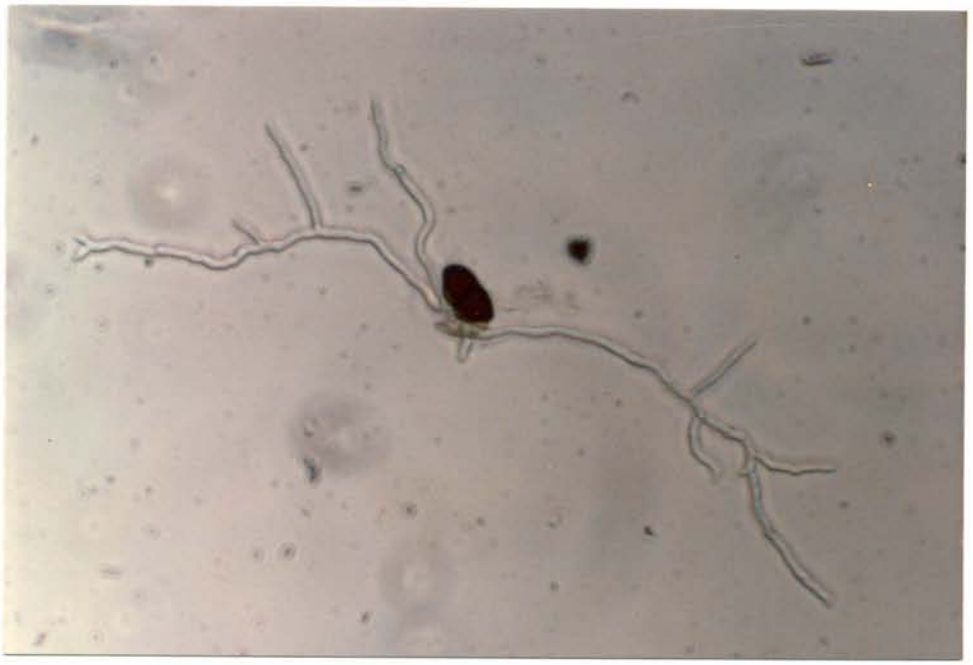

Figure 2.-Germinated conidium of Diplodia pinea on WA after 32 hours' incubation under cool fluorescent light (X200). Most germinated spores produced a single germ tube that became multibranched after a few hours.

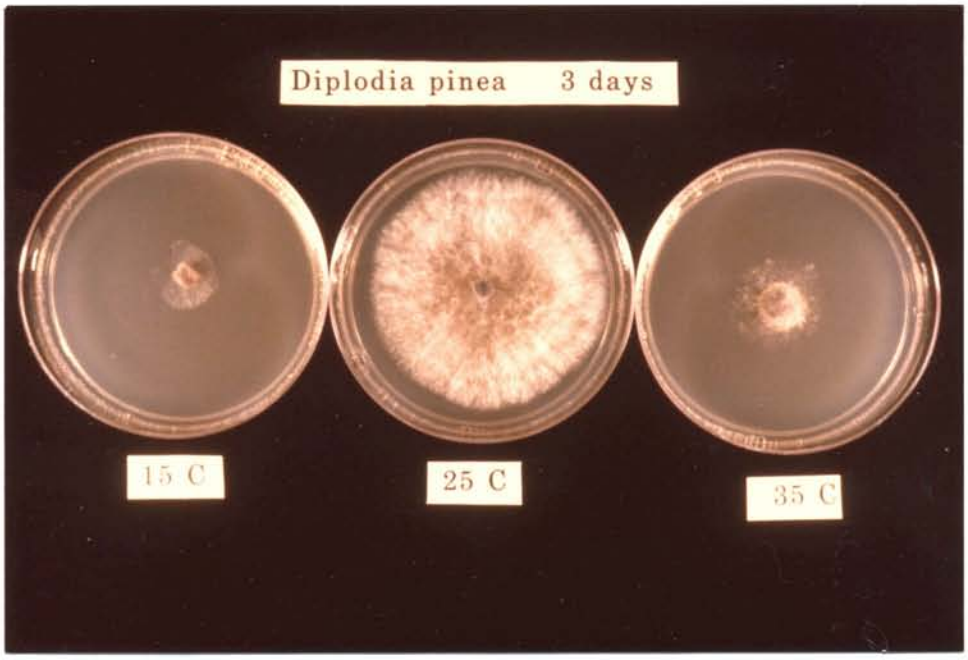

Figure 3.-Growth of Diplodia pinea from tip blighted ponderosa pine seedlings on PDA at 15°C, 25°C, and 35°C after 3 days. Hyphae were initially hyaline to white but became darkly pigmented with age.

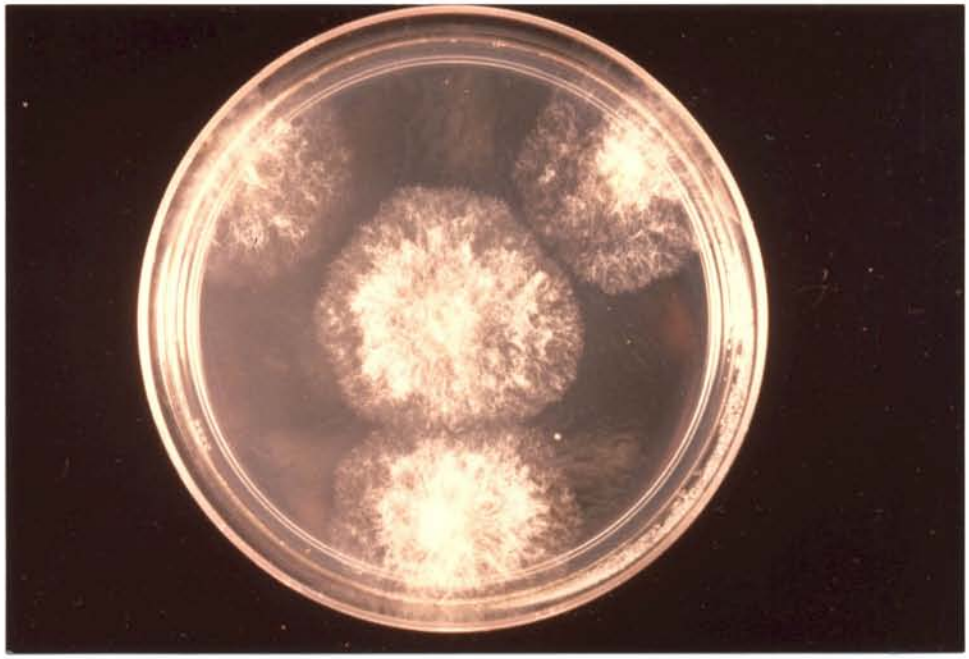

Figure 4.-Early growth of Diplodia pinea from infected needles incubated on PDA. Colonies were white and fluffy, but became dark brown to black after a few days.

Diplodia grew faster at 25°C than either 15° or 35° (figure 3). Daily rates of linear growth were 11.4 mm, 2.9 mm, and 3.7 mm, respectively. This corresponds closely to reported optimum growth rates of between 25°C and 30° C for the fungus (Peterson 1981b; Wingfield and Knox-Davies 1980; Young 1936). Hyphae were initially hyaline to white (figure 4), but after a few days developed light brown pigmentation that darkened with age (figure 3). After 10 days on PDA, cultures began forming stromatic aggregations which eventually produced pycnidia. Mature pigmented conidia were not abundantly produced on PDA even after 30 days, although pycnidial stroma were common. Pigmented hyphae of 1-month-old cultures were multibranched and septate, with constrictions at each septum. Hyphal cells were irregular in size with a granular rather than vacuolate cytoplasm.

Peterson (1981b) reported that isolates of *D. pinea* from tip blighted Austrian pine (*Pinus nigra* Arnold) produced abundant pycnidia on PDA after 10 days. However, viable spores were present in only about one-third of the pycnidia. The isolates from blighted ponderosa pine seedlings in Idaho took longer to form pycnidia and very few spores were produced in PDA culture.

From these studies, it appears that *D. pinea* isolated from ponderosa pine seedlings in Idaho is similar to isolates of the fungus described on the same and other hosts throughout the world. Procedures for reducing losses in nurseries are discussed elsewhere (James 1984). This disease should be closely monitored to elucidate environmental effects of buildup and spread.

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