Pathogenicity and Teleomorph-Anamorph Connection of *Botryosphaeria dothidea* on *Sequoiadendron giganteum* and *Sequoia sempervirens*

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ABSTRACT

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Pathogenicity of *Botryosphaeria dothidea* (=*B. ribis*) to giant sequoia (*Sequoiadendron giganteum*) and coast redwood (*Sequoia sempervirens*) was demonstrated in greenhouse inoculations of both hosts and in field inoculations of giant sequoia. Both the teleomorph and anamorph were found on giant sequoia, and their identity was confirmed by single-ascospore isolations and inoculations. No evidence for host specificity was found.

Botryosphaeria dothidea (Moug.: Fr.) Ces. & de Not. (= *B. ribis* Grossenb. & Duggar) has been reported to cause dieback or canker on many woody hosts (7,11). Although some authors (3,7) consider *B. ribis* to be separate from *B. dothidea*, the synonymy proposed by von Arx and Müller (14) will be followed in this discussion.

Modern taxonomic interpretation of the Coelomycetes suggests that *Fusicoccum* may be an appropriate genus for the anamorph (13). Typically, only the anamorph is found in the field and in culture, and the fungus is identified on this basis (3,4,8). The anamorph-teleomorph connection has been demonstrated on hosts where the teleomorph does occur (e.g., currant, apple [10], and willow [15]), but in most cases, this has not been possible. Proper identification of a pathogen as *B. dothidea* in the absence of the teleomorph can present a problem. The amount of stromatic tissue and the form of the conidioma, whether *Macrophoma*-like or *Dothiorella*-like, can be influenced by host (10), constituents of synthetic media (8), and lighting regime (12). In addition, such conidiomata can be confused with the immature forms of *Diplodia* or *Botryodiplodia*.

In California, a common twig and branch dieback of giant sequoia (*Sequoiadendron giganteum* (Lindl.) Buchholz) planted outside its natural range is generally considered to be caused by *Botryosphaeria dothidea* (9). However, Koch's postulates apparently have not been demonstrated and there is no record of fruiting bodies associated with the disease. In early spring of 1983, we received a number of samples of coast redwood (*Sequoia sempervirens* (D. Don) Endl.) that had stem cankers associated with dead branches. Similar damage to redwood had been observed previously and attributed to *B. dothidea* by W. Wagener (J. R. Parmeter, *personal communication*).

The occurrence of a saprophytic form of *B. dothidea* (2) indicates the necessity of demonstrating the pathogenicity of the fungus on new hosts. Accordingly, we began research on the problem with the objectives of demonstrating pathogenicity of the fungus on both giant sequoia and coast redwood and testing for host specialization, because cultivar-specific races of *B. corticis* are recognized on blueberry (5). Our discovery of ascocarps of *B. dothidea* in addition to macroconidial fruiting bodies on giant sequoia also enabled us to verify the conspecificity of the two fruiting types and to test pathogenicity of single-ascospore isolates of *B. dothidea*.

MATERIALS AND METHODS

Isolations from diseased tissue were made onto water agar, and cultures were maintained on potato-dextrose agar (PDA). Ascospores were obtained by allowing ascomata to eject spores into sterile water. The ascospore suspension was plated onto water agar and incubated for 24 hr. Single germinating ascospores were then transferred to PDA.

Two-year-old seedlings of coast redwood and giant sequoia were potted in a sand-peat mix and placed in the greenhouse for 2 mo. Seedlings were inoculated after removing a section (5×5 mm) of bark about 8 cm above the soil line. A PDA plug (4 mm in diameter) from an actively growing culture was placed on the wound and the area was wrapped with laboratory Parafilm. Two isolates from diseased tissue of giant sequoia (308 and 314), two single-ascospore isolates (SS1 and SS2), and one isolate from coast redwood (360) were inoculated into giant sequoia (five seedlings each). Coast redwood seedlings (six each) were inoculated with one diseased-tissue isolate from each host (314 and 360). Uninoculated, wounded controls were included, and one combination (giant sequoia × isolate 360) was also inoculated without wounding. Disease was evaluated 1, 3, and 5 wk after inoculation.

In June 1983, four giant sequoia trees were selected in the field and twigs < 1 cm in diameter were inoculated about 30 cm from the tip with a technique similar to that used in the seedling inoculations. Two trees were near Berkeley, CA, and two trees were selected in Walnut Creek, a warmer, inland area. Both sites are outside the natural range of giant sequoia. Disease was evaluated 2 mo after inoculation. Trees were considered infected when extensive necrosis was present around the inoculation point. Foliar symptoms were also noted.

RESULTS

On giant sequoia, twig and branch dieback was observed, whereas on coast redwood trees, cankers were found on the main boles. Occasionally, top-killing was found on both hosts (restricted to tops < 4 cm in diameter on giant sequoia).

Cultures obtained from ascospores, conidia, and diseased host tissue on giant sequoia were indistinguishable morphologically, and conidiomata were produced in cultures from all sources. Cultures obtained from coast redwood and giant sequoia were also indistinguishable. Conidiomata on infected branches of giant sequoia (Fig. 1) and in cultures from such material were identified by E. Punithalingam (Commonwealth Mycological Institute, Kew, U.K.) as the conidial state of *B. ribis* (= *B. dothidea*). Conidiomata were 0.5–1 mm in diameter and multiloculate (Fig. 1). Occasionally, locules were confluent. Conidia from giant sequoia measured 8–10 × 24–32 μm (mean = 8 × 27 μm). Conidial development appeared similar to that observed on *Rubus* spp. (4) (Fig. 1). An isolate from giant sequoia has been deposited with the

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American Type Culture Collection (ATCC 60344).

Isolates from both hosts, including the single-ascospore cultures, infected all inoculated seedlings of both species in wound inoculations in the greenhouse. Four isolates (308, 314, SS1, and SS2) caused 100% mortality of giant sequoia within 5 wk. The fifth isolate (360) killed four of the five seedlings within this period. Coast redwood seedlings suffered less mortality; isolate 314 (originating from giant sequoia) killed four of six seedlings, and isolate 360 (originating from coast redwood) killed no seedlings. Five of the six seedlings inoculated with isolate 360 showed wilting and some foliar necrosis, however. The fungus was successfully reisolated from diseased seedlings. No infection or symptoms were observed in controls or in giant sequoia inoculated without wounding.

Field inoculations of giant sequoia confirmed the pathogenicity of isolates from both hosts as well as that of the single-ascospore isolates, though differences among isolates were apparent (Table 1). Chi-square analysis showed that isolate 308 infected significantly fewer twigs than did the other isolates ($P = 0.01$). Although both single-ascospore isolates infected almost all inoculated twigs, SS1 caused advanced foliar symptoms in all twigs and SS2 did so in significantly fewer infected twigs ($P = 0.01$). Site differences appeared to affect isolate 360, which infected and caused advanced symptoms in all twigs at Berkeley but infected only one twig at Walnut Creek.

Field inoculations of unwounded branches were generally unsuccessful. The single twig infected in the unwounded inoculation of SS1 on tree 2 (Table 1) was killed proximal to the inoculation point and may have been killed by naturally occurring infections, which had been noted on that tree.

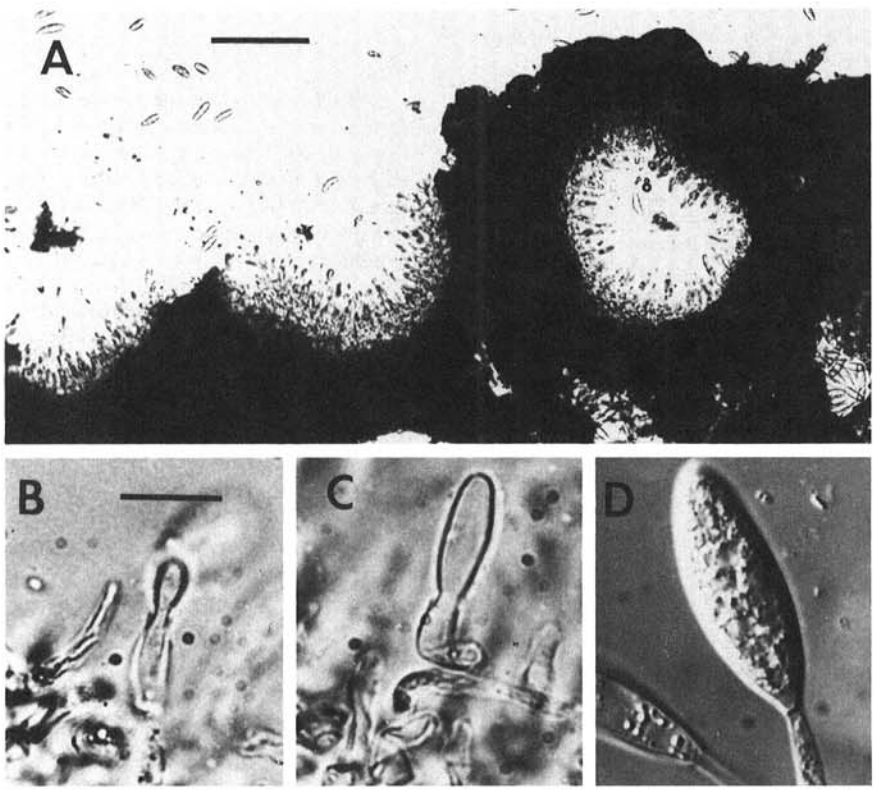

Fig. 1. Conidioma and developing conidia of *Botryosphaeria dothidea* from *Sequoiadendron giganteum*. **(A)** Vertical section of conidioma. Some stromatic tissue was removed from upper lefthand corner during sectioning. Scale bar = 100 μm. **(B)** Early, **(C)** intermediate, and **(D)** late stages of conidial development. Scale bar for B–D = 10 μm; Nomarski differential interference contrast in D.

Table 1. Infection and symptom development in twigs of *Sequoiadendron giganteum* inoculated in the field with isolates of *Botryosphaeria dothidea* from *S. giganteum* and *Sequoia sempervirens*

		Number of twigs infected (number developing foliar symptoms)							
		Twigs wounded						Twigs unwounded	
Site	Tree	308[a]	314	SS1	SS2	360	Control[b]	SS1	360
Berkeley	1	0 (0)[c]	2 (1)	3 (3)	3 (0)	3 (3)	0 (0)	0 (0)	0 (0)
	2	1 (1)	3 (3)	3 (3)	3 (2)	3 (3)	0 (0)	1 (1)	0 (0)
Walnut Creek	3	0 (0)	NT[d]	3 (3)	2 (1)	0 (0)	0 (0)	0 (0)	0 (0)
	4	1 (1)	NT	3 (3)	3 (2)	1 (0)	0 (0)	0 (0)	0 (0)

[a] Isolates 308, 314, SS1, and SS2 are from *Seguoiadendron giganteum* and isolate 360 is from *Sequoia sempervirens*. SS = single-ascospore isolate.
[b] Controls were inoculated with sterile PDA plugs.
[c] First value is the number of twigs infected of three inoculated, value in parentheses is the number developing foliar yellowing and necrosis.
[d] Not tested.

DISCUSSION

The pathogenicity of *B. dothidea* to *Sequoiadendron giganteum* and *Sequoia sempervirens* has been demonstrated. Results indicate that wounding is necessary for infection on these hosts. However, weakened or dead bark may be infected in the absence of wounding, as appears to be the case with apple (1).

Results also confirm that the teleomorph and the associated anamorph occurring on giant sequoia belong to the same fungus. Single-ascospore isolates were no less virulent than isolates from diseased tissue. Symptom development, however, differed in response to inoculation with the two single-ascospore isolates under field conditions (Table 1). This difference was not observed in the seedling inoculations, perhaps because greater susceptibility of the seedlings masked differences in virulence.

There was no evidence of host specialization in isolates from the two hosts. This is consistent with what is known of *B. dothidea* on other hosts (11) but contrasts with what is known of other *Botryosphaeria* species such as *B. corticis* (5).

Size ranges of conidia from giant sequoia were near the upper limits of those given in most descriptions of *B. dothidea*, though descriptions of conidia as well as conidiomata of *B. dothidea* vary considerably (2,4,6,7,13–15). For instance, whereas conidiomata observed on *Rubus* spp. were unilocular (4), those on our hosts were larger and multilocular. Pennycook and Samuels (6) consider *B. dothidea* sensu lato to be a widespread species complex and have described several new species of the complex from New Zealand. A detailed examination of the complex over a wider geographic area would facilitate future work with this group.

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