Twig Blight of Douglas-Fir: A New Disease Caused by *Dothiorella dothidea*

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ABSTRACT

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Dothiorella dothidea was confirmed, by wound and nonwound inoculation of 7-yr-old seedlings, as the incitant of a twig blight of Douglas-fir. The most conspicuous symptoms were yellowing, wilting, and browning of the needles on individual branches. This was followed by progressive dieback of branches from tip to the central leader and complete defoliation of some branches. The disease was most prevalent in rapidly growing leaders. The primary diagnostic feature was the presence of pycnidia on the leaf scars and bud scales of diseased branches. One to several black, globose to subglobose pycnidia were produced in stroma. Conidia were hyaline, one-celled, and ellipsoid to clavate and measured 18.0–28.0 × 5.3–7.7 μm. Conidia were borne individually on simple conidiophores lining the inner pycnidial wall. On potato-dextrose agar, the fungus grew well between 20 and 37 C, slight growth occurred at 40 C, and the optimum was 30 C. The fungus produced white, cottony mycelium that turned gray and then black with age on potato-dextrose agar. The disease was found in two counties in Kansas.

Additional key words: *Botryosphaeria ribis, Pseudotsuga menziesii*

In 1977, an unusual twig blight of Douglas-fir (*Pseudotsuga menziesii* var. *glauca*) trees appeared in Riley and Johnson counties in Kansas. In a nursery in Johnson County, the disease produced symptoms similar to those incited by *Phomopsis lokoyae* (1) or caused by winter injury. However, numerous isolations from diseased twigs consistently yielded the imperfect *Dothiorella* stage of *Botryosphaeria dothidea* (Moug. ex Fr.) Ces. & de Not. (*Botryosphearia ribis* Gross & Dugg.). This fungus causes stem and branch cankers on a wide variety of woody plants (3–6, 8–10, 12–14) and also a serious twig blight of Arizona cypress (7). Smith (11) showed by stem inoculation that this fungus was pathogenic to 50 plant species, and was considered a wound pathogen. However, Luttrell (6) and Milholland (8) reported that *B. dothidea* also can invade unwounded elm and blueberry stems, respectively.

These studies were initiated to determine the causal organism of the twig blight of Douglas-fir.

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MATERIALS AND METHODS

Isolation and identification. Numerous attempts were made to isolate fungi from diseased Douglas-fir twigs. Diseased twigs were treated with 95% alcohol, then rinsed with sterile distilled water before wood chips (1–2 cm) were cut with a sterile scalpel, placed on 2% water agar plates, and incubated at 26 C for 1 wk. Sections of apparently healthy twigs adjacent to the diseased ones also were sampled. Pycnidia were removed from leaf scars and bud scales of diseased branches and examined in detail.

Growth studies. Unless otherwise stated, single spore isolates of the fungus maintained on potato-dextrose agar (PDA-Difco) were used. The fungus was also cultured on 2% malt agar (MA), potato-dextrose agar prepared from fresh potatoes (PDA-F), corn meal agar (CMA), lima bean agar (LBA), yeast-dextrose-calcium carbonate agar (YDCC), and Douglas-fir infusion agar (DFA). The DFA was made by autoclaving green Douglas-fir needles and straining them through cheesecloth. The strained solution was diluted to 50% with distilled water, and then 20 g of agar was added to 1 L of the diluted solution. On the other hand, our laboratory medium (PDA-F) contained 20 g of agar, 20 g of dextrose, and infusion materials from 50 g of fresh potatoes per liter of distilled water. It was prepared by boiling fresh peeled potato sliced in distilled water for 15 min and filtering through two layers of cheesecloth. The plates were incubated at 26 C under continuous darkness. Cultures were examined regularly for growth and sporulation. The amount of growth at different temperatures was studied on plates of PDA-Difco (pH 5.6) incubated in the dark at 0, 5, 10, 15, 20, 25, 30, 35, 37, 40, and 42 C. Plates were centrally inoculated with 3-mm diameter plugs taken from the edge of actively growing 5-day-old cultures. Five replicates were used per treatment and colony diameters were measured after 7 days.

Conidial germination and germ tube growth. To determine the effect of specific temperatures on conidia germination and germ tube development, conidia taken from naturally occurring pycnidia were suspended in sterile distilled water. After thorough shaking, the conidial suspension was sprayed onto 2% PDA in plastic petri plates (100 × 15 mm). Three plates were incubated in continuous darkness at each of the following temperatures: 5, 10, 15, 20, 25, 30, 35, 37, 40, and 42 C. Spore density on the agar surface averaged 1–5 spores/mm^2. Agar-plate temperatures were equilibrated with the chamber air

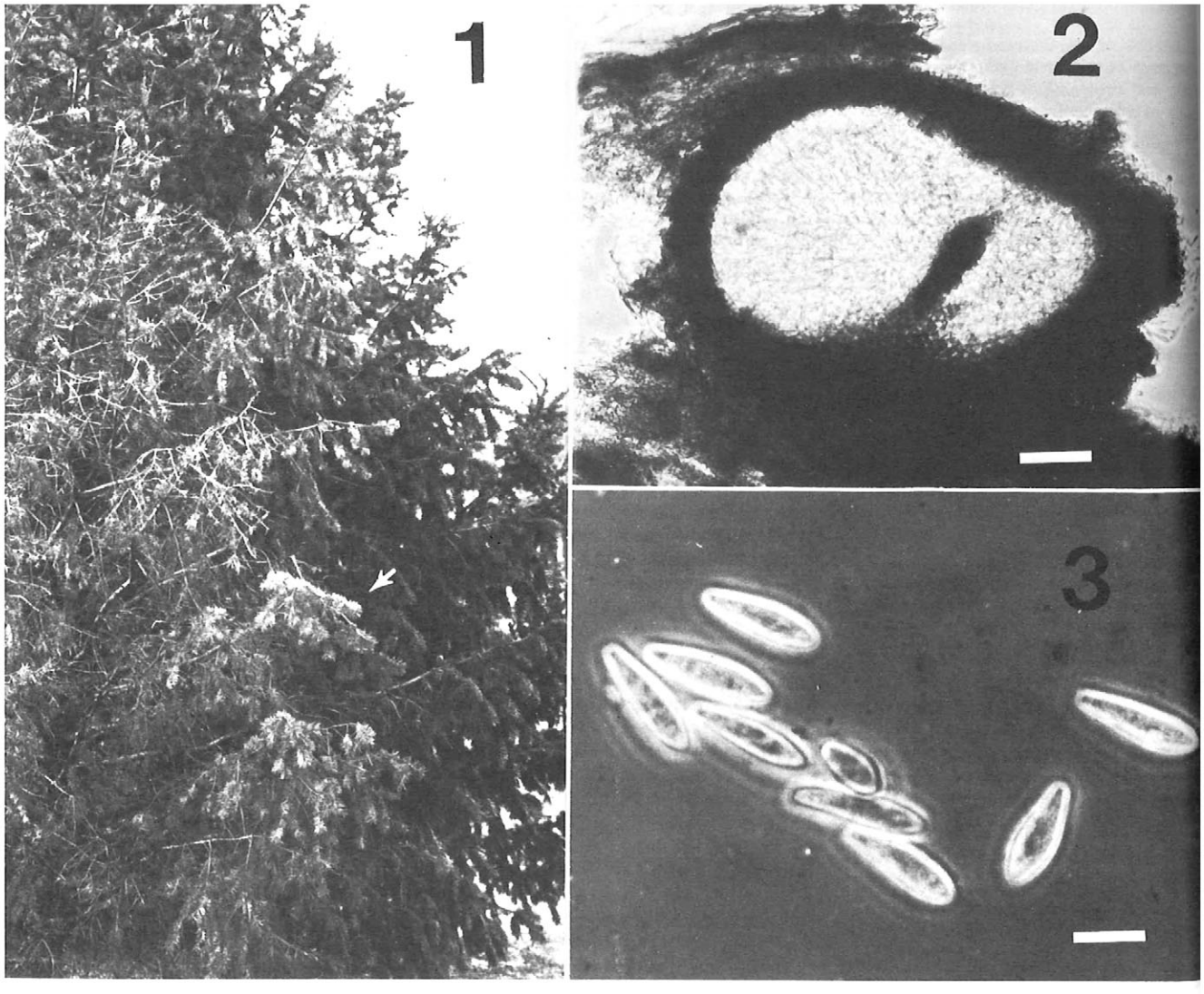
temperatures before seeding the plates. Air temperatures in the chambers were monitored throughout the incubation period. After an 8-hr incubation, plates were opened and 40% formaldehyde was added to kill the spores. Random counts of the germinated and ungerminated conidia (100 per plate) were conducted at ×200. The length of germ tubes (25 per plate) was measured with an ocular micrometer in each of three replications.

Pathogenicity tests. Three types of inoculations were made on 7-yr-old Douglas-fir seedlings in the greenhouse. They were: 1) defoliated shoots (20 needles were removed per shoot), 2) succulent shoots wounded with a sterile needle, and 3) unwounded shoots. Inoculations were made by spraying a spore suspension (5×10^5 conidia per milliliter) onto the shoots or by introducing aerial mycelium from PDA cultures into stem wounds with a needle. All plants were covered with plastic bags for 48 hr and then placed on a bench in the greenhouse under natural light at 25–30 C. Control plants were wounded but not inoculated with the fungus.

RESULTS

Disease symptoms. In the field, the most striking symptoms of Douglas-fir twig blight were the browning and killing of the stem tips over the entire area of the tree (Fig. 1). In an early stage of infection, needles on an infected branch were yellow or reddish brown. Later on, those infected needles withered suddenly and died; defoliation occurred on some of the dead branches. Severe infection resulted in complete dieback of branches from tip to the central leader. The disease was generally prevalent on rapidly growing leaders where the blight extended as much as 1 m from the tip of the shoot but never reached the hardened main stem. Symptoms near the tip of succulent twigs resembled those caused by winter injury or *P. lokoyae* (1). However, stroma-bearing pycnidia of *Dothiorella dothidea* were evident in the bud scales and leaf scars. Our observation of field samples and artificially inoculated seedlings indicated such fruiting bodies appeared first at the branch tip and later down the twig. On this basis, we concluded that the fungus had grown from the tip, killing the lateral shoots in its downward invasion of the twig.

The pathogen. Fungus isolations from diseased branches commonly yielded a *Dothiorella* sp. Species of *Fusarium, Alternaria, Cladosporium,* and *Epicoccum* were isolated from both diseased and healthy branches. Preliminary inoculations indicated that the *Dothiorella* sp. caused symptoms similar to those observed on Douglas-fir. This fungus was identified as the imperfect stage of *B. dothidea*. Pycnidia were black, stromatic, later erumpent, globose or subglobose, and 215–537 μm in diameter (Fig. 2), with a parenchymatous wall 42–48 μm thick; conidiophores were simple, bearing a single conidium at the tip; and conidia



Figs. 1-3. Foliar symptoms of infection by *Dothiorella dothidea* on Douglas-fir: **(1)** Note dead branches (arrow). **(2)** Section through stroma and pycnidia of *D. dothidea*. Bar represents 50 μm. **(3)** Conidia of *D. dothidea*. Bar represents 10 μm.

were hyaline, clavate to ellipsoid, and 18.0–28.0 × 5.3–7.7 μm (Fig. 3). Hyphae were hyaline, septate, 4 μm in diameter, sometimes brown or dark in old culture. During humid weather, the pycnidia exuded fine cirri. Various tests were conducted, in vitro and in vivo, to produce the perfect state of *B. dothidea*, but the results were negative. This infrequent occurrence of the sexual stage of this fungus in infected host tissue and/or in culture has been reported by several investigators (5,13,17).

Growth studies. The fungus grew well on PDA-Difco, PDA-F, MA, LBA, CMA, DFA, and YDCC. On these media, the mycelium was initially white and cottony; it later turned gray and then black as it matured. Hyphae were hyaline or brown, finely guttulate, and 2–6 μm in diameter. Pycnidia appeared on the third day as primordia, and hyaline conidia were present in the pycnidia a day later. Pycnidia were produced abundantly in cultures incubated in continuous light (fluorescent, approximately 5,380 lux) at 26 C; few or no pycnidia developed in the dark, and those that did remained sterile. Of the different media tested, PDA-F prepared from fresh potatoes provided the best conditions for pycnidial formation. At maturity, pycnidia were covered with fine, grayish, woolly hairs and produced conidia at the tips of simple conidiophores along the inner wall. The conidia were identical to those produced on diseased tissues and frequently emerged in a white droplet. The fungus grew well over the 20–37 C range, with optimum growth at 30 C and slight growth at 40 C. After 7 days mean colony diameters of *D. dothidea* incubated at 0, 5, 10, 15, 20, 25, 30, 35, 37, 40, and 42 C measured 0.0, 0.0, 1.0, 3.40, 4.80, 8.0, 8.70, 6.80, 4.0, 1.0, and 0.0 cm, respectively.

Conidial germination and germ tube growth. Conidia readily germinated within 1 hr in water in Van Tiegham cells and on water agar. Usually only one germ tube was produced per conidium; however, two germ tubes (one from each end) were occasionally evident. The hyaline, nonseptate conidia became septate after germ tube formation. Conidial germination and rate of germ tube elongation both were maximal at 30 C and did not occur at either 10 or 42 C.

Pathogenicity tests. Positive results were obtained when wounded or unwounded stems were inoculated with mycelium or sprayed with conidial suspension of *D. dothidea* (Table 1). The stage of plant growth at inoculation affects infection and disease development; succulent twigs were usually girdled at the point of inoculation within 7 days and later died. Unwounded shoots also became infected when sprayed with conidial suspension (5×10^5 spores/ml). Pycnidial stromata formed on the infected tissue within 3 wk after inoculation. In all pathogenicity tests, *D. dothidea* was reisolated from diseased tissues. All control plants were symptomless and free of the fungus.

Table 1. Results of inoculation with *Dothiorella dothidea* on Douglas-fir seedlings

Type of inoculation	Inoculum	Infection (%)
Unwounded succulent shoots	Conidia[a]	5/6[b] (80.33)
	Control	0/4 (0)
Wounded succulent shoots	Conidia	8/8 (100)
	Mycelium	8/8 (100)
	Control	0/6 (0)
Defoliated shoots	Conidia	8/8 (100)
	Mycelium	8/8 (100)
	Control	0/5 (0)

[a] Inoculum suspension = 5×10^5 conidia per milliliter.
[b] Number infected/number tested.

DISCUSSION

D. dothidea, the imperfect stage of *B. dothidea*, is a wound parasite with a wide host range. However, this pathogen invades unwounded stems of blueberry (8) and elm (6). Recently, Weaver (15) reported that conidia of *D. dothidea* infected bark tissues through lenticels and induced peach gummosis. Our studies showed that *D. dothidea* is pathogenic on Douglas-fir and conidial inoculations were successful in both wounded and unwounded stems. The avenue for penetration and infection on unwounded stems is unknown. However, the pathogen may possibly enter through natural openings such as lenticels or small cracks or through wounds caused by insects. It is more likely that the leaf scars from needle abscission due to stress problems on the tree may be the site for fungal penetration. Milholland (8) found that penetration of unwounded blueberry stems by *D. dothidea* occurs through open stomata.

The morphologic characteristics of isolates of *D. dothidea* from Douglas-fir are similar to those described for isolates from other hosts (6,14,16,17). The relationship of temperature to mycelial growth and conidial germination of the Douglas-fir isolates agrees, in general, with that for other isolates (8,14,17). However, the Douglas-fir isolates grew fairly well at 37 C and made slight growth at 40 C but not at 42 C. The fungus was not previously reported to grow at temperatures above 38 C (14). This broad range for growth may explain the persistence of *D. dothidea* when high temperatures occur in Kansas. None of the isolates of *D. dothidea* from Douglas-fir were chromogenic. Others (14,16,17) have concluded that pathogenicity of *D. dothidea* is not correlated with chromogenesis.

In Kansas, fungal infections are likely to occur early in the growing season when the new vegetative shoots arise; however, the dieback of larger shoots occurs during the end of summer when the temperature is fairly high. Extended droughts are fairly common in the Midwest in the summer and are probably directly involved in the etiology of *D. dothidea* on Douglas-fir. Christ and Schoeneweiss (2) reported that exposure of birch seedlings to controlled water stress, freezing stress, and defoliation stress increases susceptibility of stems to attack by *D. dothidea*. White spore masses containing the conidia exuded from the pycnidia after rainy periods. If spores are repeatedly exuded from individual pycnidia, infection could occur more than once per season.

The source of inoculum of the fungus on Douglas-fir in urban areas and nurseries is not known. Inasmuch as the disease was not detected earlier and because *D. dothidea* has a wide range of hosts, the fungus may have been introduced into Kansas on planting stocks, eg, apples, blueberry, and elm; all have been reported as host plants for the fungus (3,6,17).

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