

Seed Capsule Abortion and Twig Dieback of *Eucalyptus camaldulensis* in South Florida Induced by *Botryosphaeria ribis*

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ABSTRACT

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A new disease of *Eucalyptus camaldulensis* (Spanish seed source) seed capsules and twigs was observed in three seed-production plantings in South Florida. Necrosis and abortion of colonized capsules prevented seed production in commercial quantities. Pathogenicity of *Dothiorella eucalypti*, a conidial stage of *Botryosphaeria ribis*, was established after isolation from symptomatic capsules, pedicels, peduncles, twigs, and leaf veins.

Eucalyptus camaldulensis Dehnh. (Spanish seed source) is capable of rapid growth and prolific seed capsule production in South Florida, yet regeneration of this superior hardwood pulp species (2) is limited by seed capsule abortion. Seed crop failures have occurred annually since trees in the several seed-production areas began flowering, and crop failures have resulted from seed capsule necrosis and abortion and twig dieback. Vegetative cuttings have replaced seedlings as a source of *E. camaldulensis* nursery stock for commercial plantings.

Botryosphaeria ribis Gross. & Dug. (3) had been associated with a basal canker of *E. grandis* Hill ex Maiden in South Florida (E. L. Barnard, personal communication). *Dothiorella eucalypti* (Berk. & Br.) Sacc., a conidial stage of *B. ribis*, was isolated from necrotic *E. camaldulensis* seed capsules, pedicels, peduncles, twigs, and leaf veins. Isolation of *D. eucalypti* from symptomatic *E. camaldulensis* prompted an investigation to determine pathogenicity of the fungus.

MATERIALS AND METHODS

Isolation. Fifteen *E. camaldulensis* trees, each constituting a separate Spanish provenance genotype, were selected at one seed production planting, and a minimum of five branches in the upper canopy of each tree was sampled at random. Samples of seed capsules, pedicels, peduncles, twigs, and leaves

tissue samples were surface-sterilized in 0.5% NaOCl for 1 min and incubated on 2% malt extract agar (MEA) for 7 days at 24 C.

Pathogenicity. Succulent stems of 25 1-yr-old seedlings of *E. camaldulensis*, *E. robusta* Sm., *E. torelliana* F. Muell., and *E. viminalis* Labill. were each inoculated aseptically with MEA plugs (0.5 cm diam.) of *D. eucalypti* hyphae isolated from symptomatic *E. camaldulensis* seed capsules. Sections of bark (about 0.5 × 0.5 × 0.1 cm) were removed with a sterile scalpel, inoculated, and wrapped in

were removed from each branch, placed in plastic bags, and stored on ice during transfer to the laboratory. Symptomatic

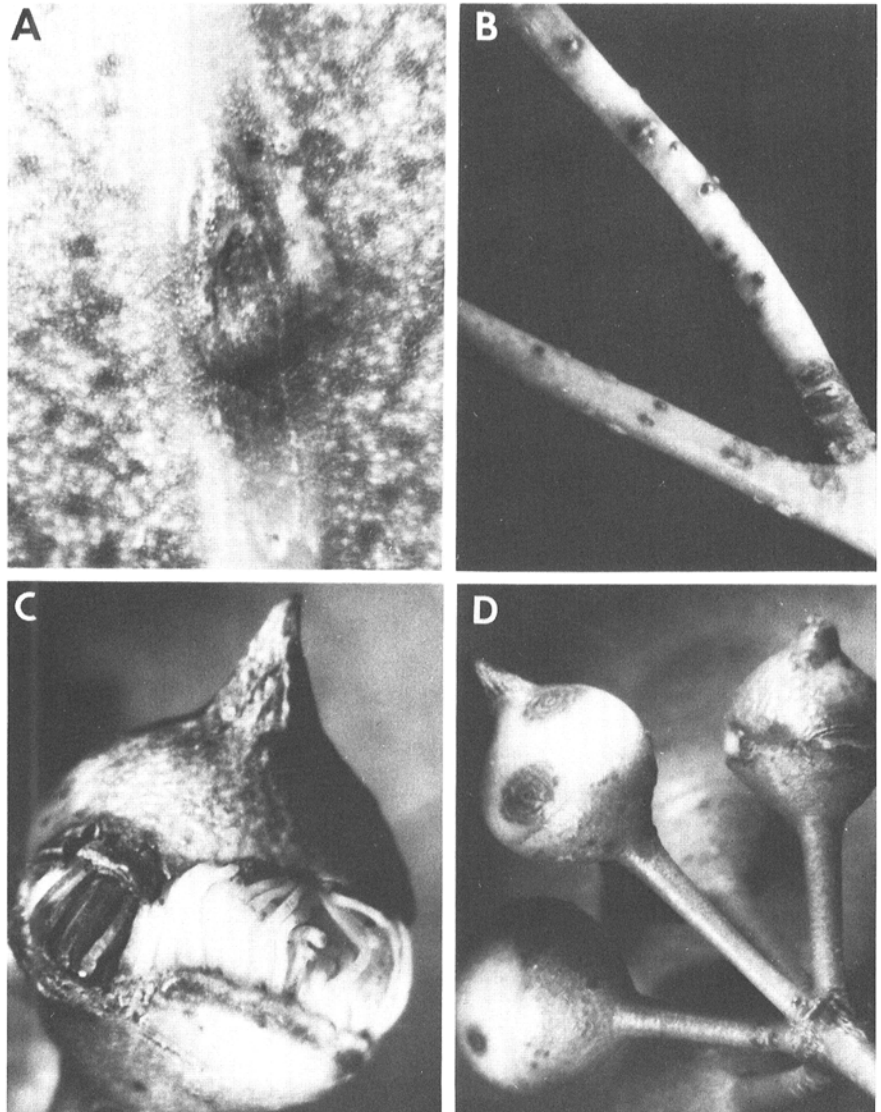


Fig. 1. Circular, reddish purple lesions induced by *Botryosphaeria ribis* on *Eucalyptus camaldulensis* (A) leaf midvein, (B) peduncle, and (C and D) seed capsules and pedicels.

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sterile gauze. Seedlings were incubated under greenhouse conditions. After 2 wk, the inoculation sites were flame-sterilized with 80% ethanol and tissue samples were

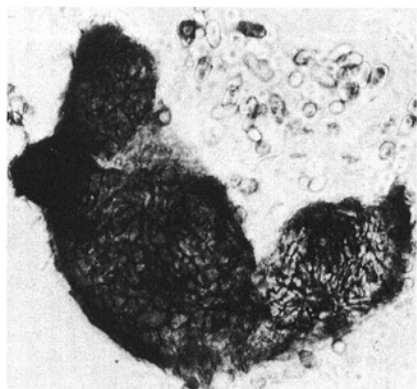


Fig. 2. A ruptured pycnidium of *Dothiorella eucalypti* exhibiting single-celled conidia ($\times 100$).

excised from the canker margins and placed on MEA at 24 C.

RESULTS AND DISCUSSION

Small (1–5 mm diam.), circular, reddish purple lesions were frequently observed on field samples of *E. camaldulensis* seed capsules, pedicels, peduncles, and leaf midveins (Fig. 1A–D). *D. eucalypti* was the only fungus isolated from symptomatic tissue, and the isolates typically produced grayish black hyphal colonies that covered the surface of a petri dish within several days. Pycnidia were formed infrequently in culture and the single-celled conidia were similar in size (17–24 \times 25 mm) to those of *D. eucalypti*, as described by da Camara (1) (Fig. 2). The identity of *D. eucalypti* isolated from symptomatic tissue was confirmed by E. Punithalingam (3) of the Commonwealth Mycological Institute, Kew, Surrey, England.

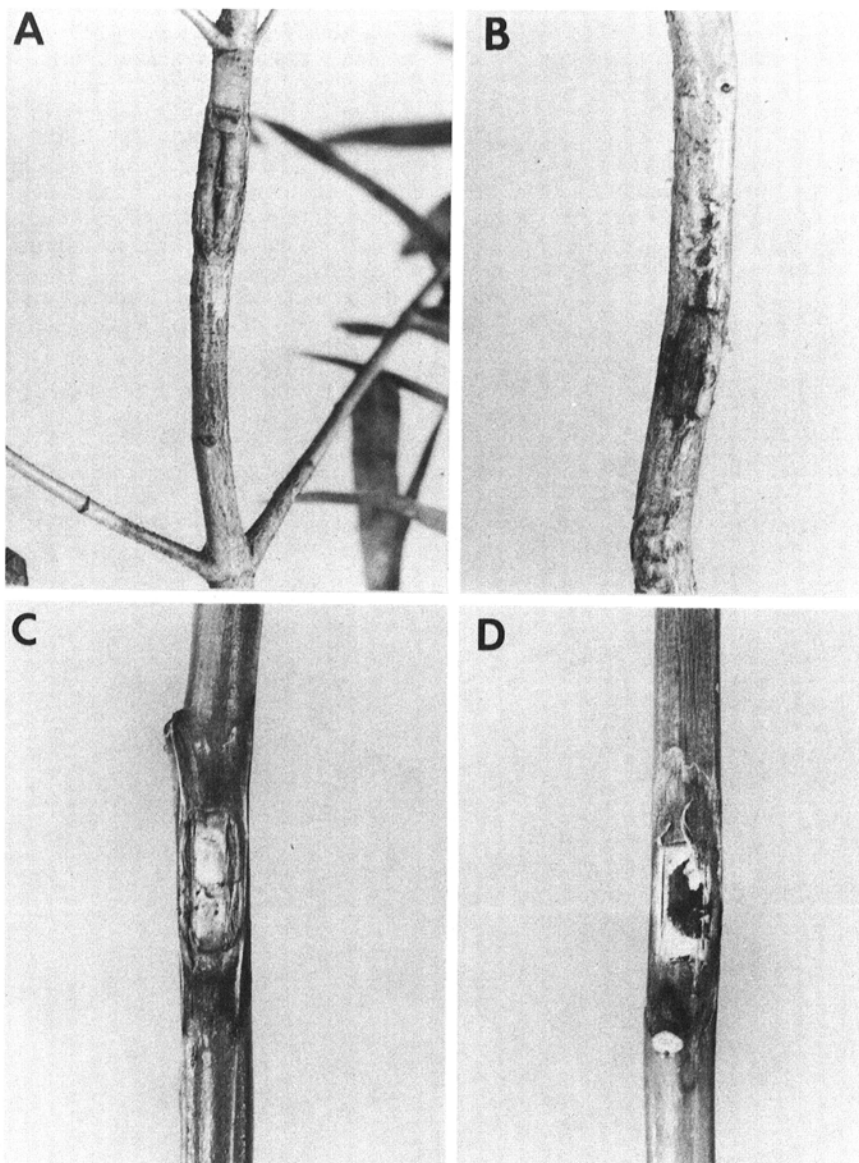


Fig. 3. Inoculated 1-yr-old stems of (A) *Eucalyptus viminalis*, (B) *E. torelliana*, (C) *E. camaldulensis*, and (D) *E. robusta* exhibiting cankers induced by *Botryosphaeria ribis*. Callus production at the canker margins was greatest among *E. viminalis* and *E. torelliana*, whereas cankering was most severe among *E. camadulensis* seedlings.

Examination of staminal filaments underlying the circular lesions on the seed capsule surface revealed similar circular, reddish purple staining indicative of *D. eucalypti* colonization (Fig. 1C,D). Necrosis of the seed capsules from colonization of floral parts by *D. eucalypti* frequently preceded fertilization and caused necrosis, abortion, and premature shedding of colonized capsules. Colonization of seed capsules by *D. eucalypti* progressed through the pedicels and peduncles, causing twig dieback.

Seedlings of the four *Eucalyptus* species inoculated with *D. eucalypti* were susceptible, and the pathogen was frequently isolated from the darkened, sunken canker margins (Fig. 3), eg, *E. torelliana* (88%), *E. viminalis* (80%), *E. robusta* (96%), and *E. camaldulensis* (100%). Control seedlings inoculated with MEA plugs exhibited wounds that were rapidly covered with callus tissue and lacked the darkened, sunken tissue associated with *D. eucalypti*. Disease severity, eg, canker length, was greatest when *E. camaldulensis* seedlings were inoculated with isolates of *D. eucalypti* isolated from colonized *E. camaldulensis*. *E. viminalis* and *E. torelliana* exhibited prolific callus development at the canker margins and appeared much more tolerant to *D. eucalypti* colonization than *E. camaldulensis* or *E. robusta*.

Colonization of *E. camaldulensis* twigs and floral structures by *D. eucalypti* has resulted in the curtailment of seed production in South Florida for commercial plantings. The three seed-production plantings have been abandoned because of disease impact; only one tree appears resistant to *D. eucalypti* and continues to yield viable seed (T. Gheary, U.S. Forest Service, personal communication). The specificity for resistance to *D. eucalypti* has also been observed at a nearby *E. robusta* seed production planting where one tree of 750 was severely colonized, eg, lesions on seed capsules, pedicels, peduncles, and twigs and branch and stem cankers. Future *E. camaldulensis* commercial nursery stock production in South Florida will depend on vegetative propagation of genotypes that are susceptible to *D. eucalypti*. A reevaluation of different *E. camaldulensis* provenances may uncover a variety of genotypes resistant to *B. ribis*. Incorporation of the resistant selections into seed orchards would provide an uninterrupted supply of seed for commercial plantings.

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