

# ***Diplodia* Stem Canker and Die-back of *Casuarina equisetifolia* in Puerto Rico<sup>1</sup>**

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## **INTRODUCTION**

*Casuarina equisetifolia* Linn., the horsetail *casuarina*, is a tree native to tropical Asia and Australia. It is known commonly as Australian pine, also beefwood. The Australian pine was introduced to Puerto Rico presumably from Australia during the latter part of the 19th century, probably between 1880 and 1890. In Puerto Rico, *Casuarina equisetifolia* has been widely grown along the littoral regions of the Island. This pine is very well adapted to the sandy coastal soils and thus used commonly as an ornamental as well as for windbreak and hedge purposes to stabilize shifting sands. It also is planted because its timber is preferred by some people in constructing barns and tobacco sheds.

A large number of these pine trees were killed in 1940 by a die-back disease in the Guánica area (5). Recently, stem canker and die-back were observed on *Casuarina* at the Dorado Beach Hotel golf courses. *Diplodia* species was isolated from affected branches.

The purpose of this paper is to report on the pathogenicity of various isolates secured from Australian pine trees growing in the Dorado Beach area. Also reported are results of studies on the relative resistance of this fungus to the toxicity of various fungicides in vitro.

## **REVIEW OF LITERATURE**

In Florida, the weak parasite, *Diplodia theobromae* has been blamed for die-back of *Aleurites* and *Platanus*, and fruit rot (8). According to Voorhees (8), *Botryodiplodia theobromae* is a synonym for *Diplodia natalensis*. The perfect stage of both is *Physalospora rhodina*. *Diplodia natalensis* was considered the cause of a sycamore canker (8) and the cause of heavy mortality in a fig orchard in Texas (2). Wester et al. (6) found that *Physalospora rhodina* (*Diplodia theobromae*), in inoculation, parasitized *Tilia neglecta* and *T. cordata*. Large (4) in 1948 reported that a black, sunken rot canker, caused by *Physalospora rhodina*, has killed young trees back almost to the ground line in several nurseries of Tung trees. Cooke (3) and Verrall (7) reported

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that *Diplodia natalensis* caused black stain of wood. Alvarez and López (1) reported in Puerto Rico that *Physalospora rhodina* caused serious die-back and stem canker on mango. No data are available in Puerto Rico concerning the cause of canker on *Casuarina equisetifolia*.

## MATERIALS AND METHODS

### DESCRIPTION OF SYMPTOMS

Affected *Casuarina* trees showed much top killing (figs. 1 A, B, C, and D) during dry years. The cankers seldom girdles the trees but consist of long, narrow strips. In the early stage, sunken areas appear on the bark of any part of a tree. Then, the strips extend to the top of the tree (fig. 2). As soon as the strips reach the top, the top dies. The bark is covered with small

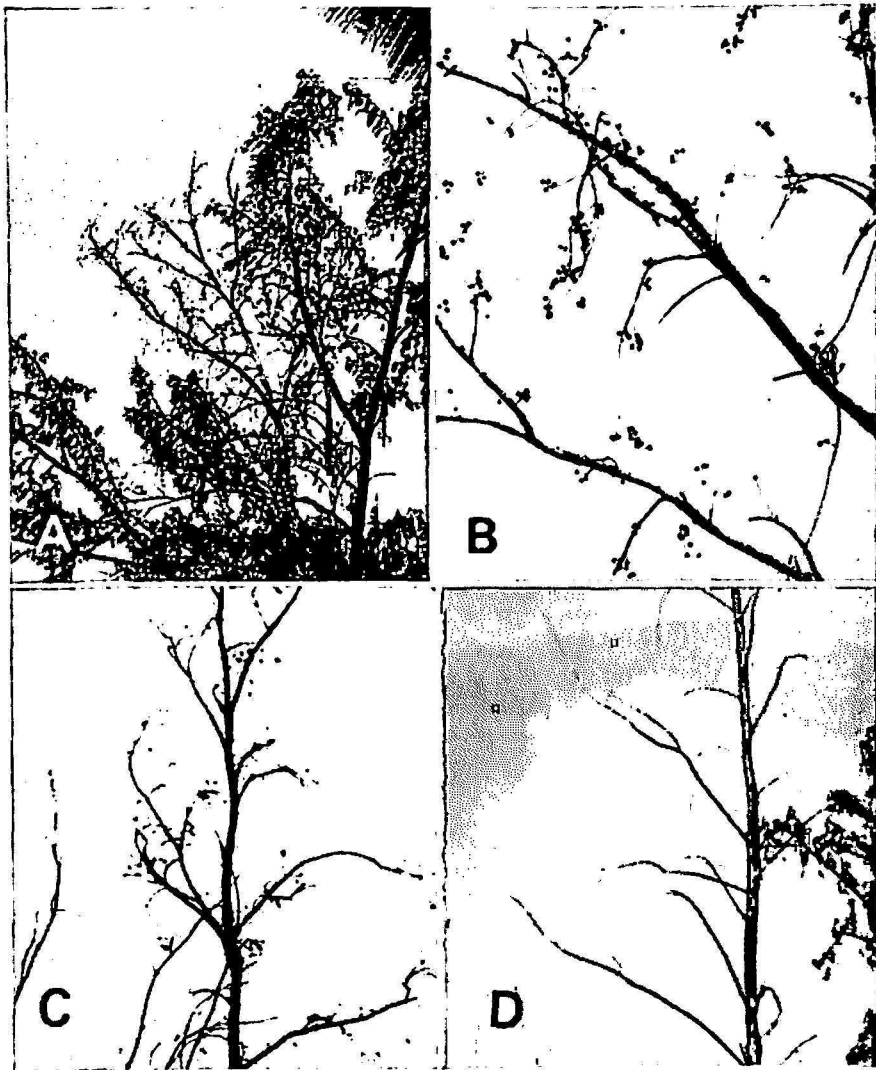


FIG. 1.—Telescopic view of the top killing of *Casuarina equisetifolia* by *Diplodia natalensis*: A and B, early-stage symptoms and C and D, advanced stage symptoms.

black conidia of the causal organism. Later, the bark sloughs off and the wood beneath becomes darkly stained.

#### FUNGUS MORPHOLOGY AND IDENTIFICATION

Diseased branches of *Casuarina* showing cankers were collected in the fall of 1971 from the Dorado Beach Hotel area. *Diplodia* sp. was isolated by plating affected tissue on potato dextrose agar medium. Monosporic cultures were made. Subcultures of the isolates were grown in potato dextrose agar (PDA). The morphology of the fungus was studied by microscopic observation of cultures grown on PDA. Five days after subculturing, dark colonies appeared on the medium. An examination of the pycnospores



FIG. 2.—Cankers of *Casuarina equisetifolia* caused by *Diplodia natalensis*—sunken area appearing on the bark.

and the pycnidia produced by the monosporic colonies revealed that all were typical of the fungus *Diplodia*. Four weeks after inoculation with pycnospores, pycnidia developed either singly or in groups: Pycnidia were papillate or shortbeaked, globose or subglobose, varying in size from 125  $\mu$  to 260  $\mu$  long X 85  $\mu$  to 165  $\mu$  wide, carbonaceous; immature pycnospores were hyaline subguttulate, 1-cell, granular, from 15  $\mu$  to 26  $\mu$  long X 7.5  $\mu$  to 15  $\mu$  wide, subcylindric or oblong, oosing out in coils through the ostioli; pycnidia wall was dark, without stroma, paraphyses present; mature pycnospores were 2-cell, dark, striated, not constricted, darker at the septum (figs. 3, A-C), and measured 21.30  $\mu$  to 25.25  $\mu$  long X 14.91  $\mu$  to 17.04  $\mu$  wide. Dry specimens of the cankers were sent to Dr. M. B. Ellis, Principal Pathologist, Commonwealth Mycological Institute, Kew, England for identification of the species. Dr. Punithalingara of the same Insti-

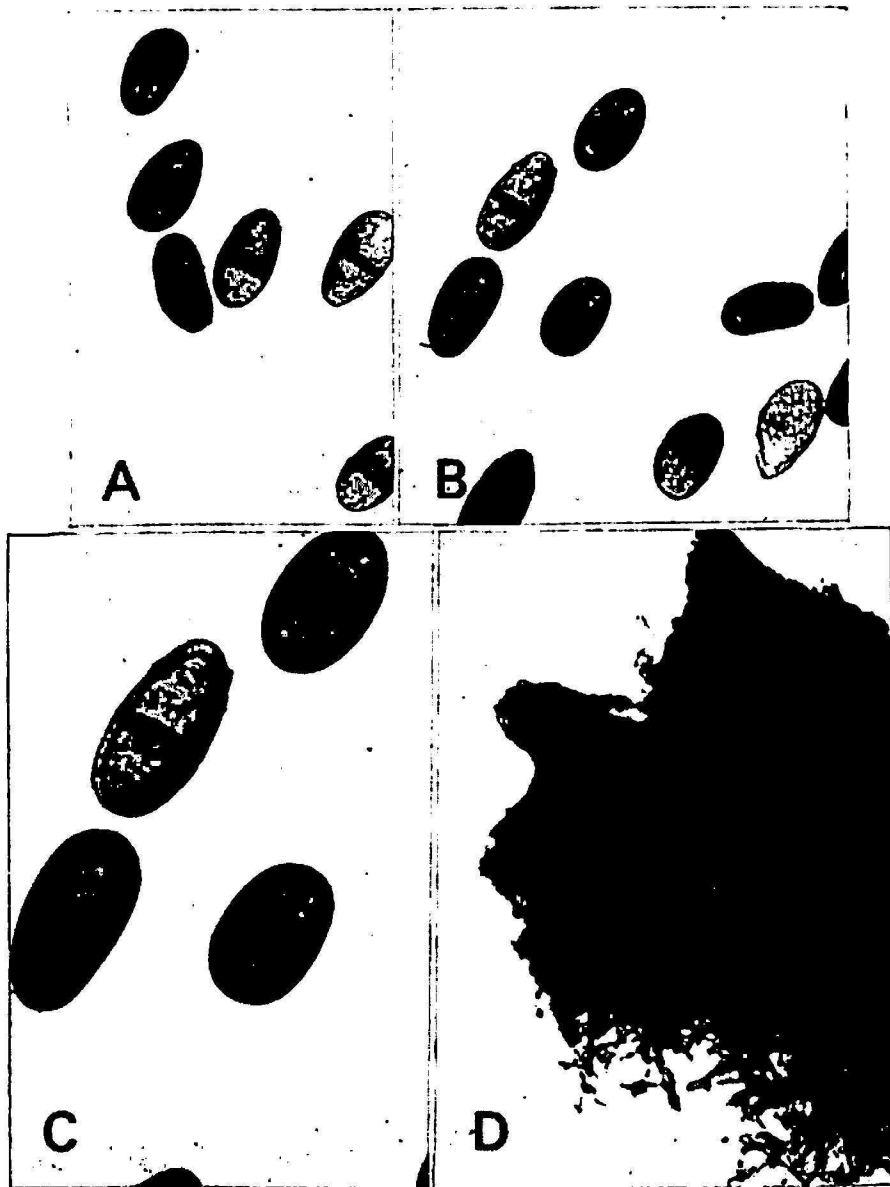


FIG. 3.—Pycnospores and pycnidia of *Diplodia natalensis* from *Casuarina equisetifolia*: A and B, pycnospores (1000X); C, pycnospores (2000X); and D, pycnidia (300X)

tute identified the fungus in the affected tissue of the cankers as *Botryodiplodia theobromae* Pat., a synonym for *Diplodia natalensis*.

PATHOGENICITY TESTS

Healthy *Casuarina* seedlings from the Dorado nurseries were brought into the greenhouse for inoculation studies. Two were inoculated with spore suspension of *Diplodia natalensis* using a puncture technique. Immediately after inoculation, the site of inoculation was covered with a tape to prevent infection caused by secondary organisms. The surface of the bark was sterilized with 70-percent alcohol previous to the inoculation. Non-inoculated seedlings were left on a bench under a shed covered with a 70-percent

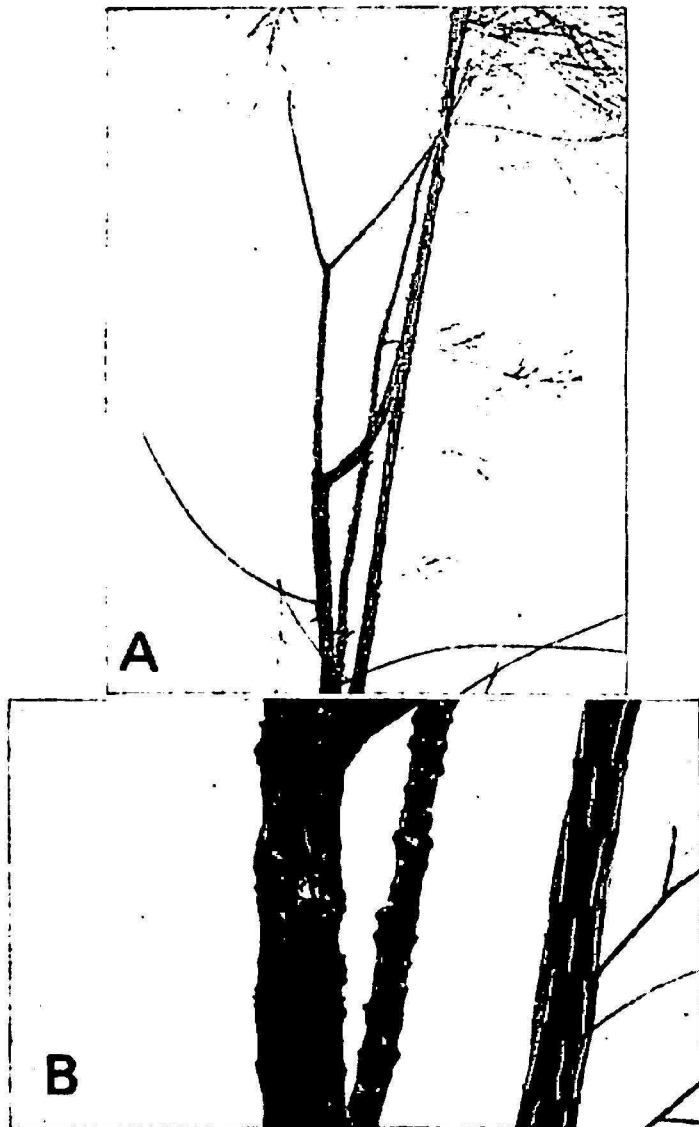


FIG. 4.—Seedlings of *Casuarina equisetifolia* 3 months after inoculation with *Diplodia natalensis*: A, die-back of the top and B, canker formation.

Saran cloth. The temperature in the shed was maintained at approximately 65° to 75° F. Three months after inoculation, a sunken area appeared on the bark (figs. 4, A, B). The strip extended gradually to the top. As soon as the strip reached near the top, the top wilted and died. The causal organism was reisolated from the cankers. No sunken area nor cankers appeared in the non-inoculated seedlings.

#### IN VITRO FUNGICIDE TESTS

Five chemicals, Dithane M-45, Thiodan (Endosulfan), Captan, Benlate, and benzoic acid were tested in the laboratory at 0.025- 0.125-, 0.25-, 0.50- and 1.0-percent concentration (active ingredient) for their toxicity against *Diplodia natalensis*. *D. natalensis* was grown on potato dextrose agar me-

dium, to which was added a 10-mm. paper disc containing varying concentrations of Dithane M-45, Thiodan, Captan, Benlate and benzoic acid. Paper discs containing no fungicides were added to the plates and used as controls. The plates were then kept in the incubator at 30° C. The relative diameter of the inhibiting zone by the various fungicides was measured at the end of a 72-hour incubation period. As shown in figure 5, Benlate appeared to be the most effective fungicides for inhibiting mycelial growth of *D. natalensis*.

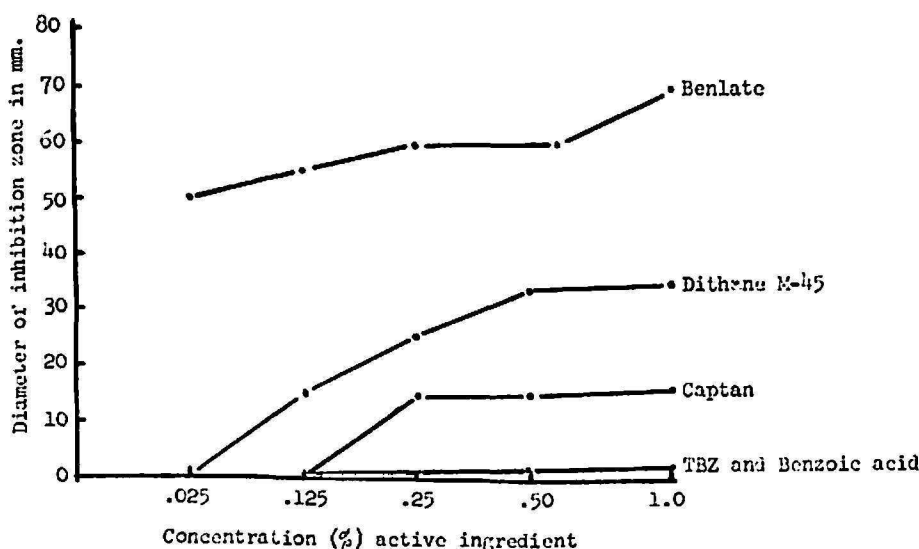


FIG. 5.—Effect of various fungicides (Benlate, Dithane M-45, Captan, TBZ and benzoic acid) on mycelial growth of *Diplodia natalensis* from *Casuarina equisetifolia*.

### DISCUSSION AND CONCLUSION

The results obtained in these investigations indicate that *Diplodia natalensis* causes stem canker and die-back of *Casuarina equisetifolia*. These results agree with the findings of Voorhees (7) on *Aleurites* and *Platanus*.

*Diplodia* canker and die-back has not been reported previously on the Australian pine; thus this constitutes the first report on the incidence.

Although Alvarez and López (1) reported the perfect stage of the fungus, *Physalospora rhodina*, causing severe die-back and canker on mango in Puerto Rico, no perfect stage has been observed on *Casuarina*.

### SUMMARY

*Diplodia natalensis* was isolated from the diseased cankers of *Casuarina equisetifolia* in Puerto Rico. A stem-puncture technique was employed to inoculate *Casuarina* seedlings. Sunken areas or cankers appeared on the bark 3 months after inoculation. The causal organism was reisolated. Among the fungicidal chemicals tested (Dithane M-45, Thiodan, Captan,

Benlate and benzoic acid), Benlate seemed to be the most promising for inhibiting mycelial growth of *D. natalensis* in vitro.

#### RESUMEN

Se aisló el hongo *Diplodia natalensis* de canchros en árboles de *Casuarina equisetifolia*. Se inocularon arbolitos saludables de *Casuarina* punzando el tallo. Tres meses después de la inoculación aparecieron canchros o áreas hundidas en la corteza. El organismo causativo de las lesiones se aisló de nuevo. Entre los fungicidas que se probaron para su control (Dithane M-45, Thiodan, Captan, Benlate y ácido benzoico), el Benlate pareció ser el más eficaz para impedir el crecimiento del micelio de *D. natalensis* in vitro.

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