PHYTOTOXIC METABOLITES FROM *BOTRYODIPLODIA THEOBROMAE* PAT.

T. S. SURYANARAYANAN
Botany Department, Vivekananda College, India Madras 600004
AND
K. MANI
University Botany Laboratory, Madras 600 005, India

Botryodiplodia theobromae is a common wound parasite with a wide host range[1,2] and is also known to cause post-harvest rot of vegetables[3,4]. This fungus was responsible for a leaf blight disease of *Pandanus odoratissimus* (screw pine) in the city of Madras. In this paper we report the production of some phytotoxic metabolites by *B. theobromae* isolated from the diseased leaves of screw pine.

A single spore isolate of the fungus was used in the study. Erlenmeyer flasks containing 50 ml of liquid Czapek's medium (pH 6·5) were inoculated with a plug of mycelium removed from the margin of a three day old agar culture and incubated at 30° C without agitation for seven days. After this, the culture medium was filtered through four layers of cheese cloth and then through Whatman No. 1 filter paper. The toxins from the culture filtrate were extracted by a modified method of Umetsu *et al.*[5] For the bioassay, healthy leaf or leaf bits of *Pandanus* and other plants were washed with sterile distilled water and incubated in moist chambers. A drop of the culture filtrate or its fractions obtained by extraction was kept on the upper surface of the leaf and the leaf pricked with a sterile needle. A drop of liquid Czapek's medium or water similarly placed served as control. The toxic fraction was further purified by thin layer chromatography using Silica Gel G. The chromatogram was visualised under UV light and the fluorescent zones were eluted in ethanol, evaporated to dryness, redissolved in water and tested for toxicity. Spray reagents were used to identify the chemical nature of the toxins[6].

The culture filtrate induced dark brown lesions with yellow halo on the leaves of *P. odoratissimus*; *Agave* sp.; *Arachis hypogaea*; *Crinum asiaticum*; *Eichhornia* sp.; *Eucalyptus* sp.; *Ficus elastica* and *Pisonia morindifolia* within 48 hr of incubation. The culture filtrate lost this lesion inducing capacity on dialysis against water for 24 hours but not when it was autoclaved at 15 PSI for 15 minutes. These facts suggested that the toxin(s) was not host specific and that it was of small molecular weight. Of the fractions obtained by solvent extraction, only acid fraction I^5 induced lesions on the leaves of *Pandanus* and also on the leaves of all the other plants mentioned above. This fraction on TLC with butanol : acetic acid ; water (3 : 1 : 1) had the lesion inducing principle between Rf 0·65 and 0·8. This band was eluted and rerun with toluene : ethylacetate : formic acid (6 : 1). With this solvent system there were three lesion inducing principles at Rf 0·3, 0·53 and 0·65. Under UV they fluoresced with reddish orange, bright blue and bluish green respectively. The toxic metabolite at Rf 0·53 reacted positively for phenols when tested with spray reagents. The other two toxic metabolites answered for phenols, polynuclear aromatic compounds, coumarin, anthroquinone glycosides and their aglucones, aromatic polycarboxylic acids and aromatic amines. It is interesting that the lesions produced by the fungus on the leaves of *Pandanus* were similar to those produced by its toxins.

Authors are grateful to Dr. R. N. Swamy, Reader, Centre of Advanced Study in Botany, University of Madras, Madras 600 005, for his advice and helpful suggestions.

October 7, 1980.

1. Punithalingam, E., *CMI Descriptions of Pathogenic Fungi and Bacteria*, 1976, No. 519.
2. Bilgrami, K. S., Jamaluddin and Rizwi, M. A., *Fungi of India*, Part I, Today and Tomorrow's Printer and Publishers, New Delhi, 1979.
3. Arinze, A. E., Naqui, S. H. Z. and Ekundayo, J. A. *Int. Biodeterior. Bull.*, 1975, **11**, 41.
4. Jackson, G. V. H. and Gollifer, D. E., *Ann. Appl. Biol.*, 1975, **80**, 217.
5. Umetsu, N., Kaji, J., Aoyama, K. and Tamari, K., *Agr. Biol. Chem.*, 1974, **38**, 1867.
6. Stahl, E., *Thin-layer Chromatography*, George Allen and Unwin Ltd., London, Springer-Verlag