Die-Back of Eucalyptus citriodora Caused by Xanthomonas eucalypti sp.n.

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ABSTRACT

A disease which produces die-back of lemon-scented gum (*Eucalyptus citriodora*) was shown to be caused by a bacterium. The morphological, cultural, and biochemical characteristics of the organism place it in the genus *Xanthomonas* and show that it is distinct from other described species. The name proposed for the pathogen is *Xanthomonas eucalypti* sp.n.

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This note describes a bacterial disease of lemon-scented gum, *Eucalyptus citriodora* Hook., a species which has been widely planted as an ornamental throughout Australia. Three outbreaks have been recorded; the first in the spring of 1963, the second in the summer of 1967, and the third in the spring of 1970. All occurred in suburbs of Sydney and involved a number of avenue and garden trees ranging from 3 to 7 m in height.

Symptoms appeared first as blackened areas on the distal 5-10 cm of the twigs. The blackened areas then increased in size and the terminal buds and last-formed leaves died. In severe cases the trees were completely defoliated. Recovery was generally effected by the production of new growth from epicormic buds situated in the branches (Fig. 1-A).

Bacteria which produced small yellow colonies on glucose-yeast extract-carbonate agar (GYCA) after 48 hr at 28 C were invariably isolated from apparently healthy tissue immediately bordering the blackened areas. Pure cultures of these bacteria in peptone-water were inoculated into lemon-scented gum seedlings 30 to 45 cm high by first wounding the stem immediately above a petiole with a sterile needle and then placing a drop of the culture in the axil. Typical symptoms were produced on all plants (Fig. 1-B, C) accompanied in some cases by two symptoms not previously recorded. These were: (i) blackened areas at the base of the leaf immediately below the point of inoculation; and (ii) small cankers on the stem which contained droplets of resinous exudate (Fig. 1-D).

In a limited host range study, pathogenicity tests were carried out using the following species: *E. maculata* Hook.; *E. haemastoma* Smith; *E. grandis* (Hill) Maiden; *E. saligna* Smith; *E. laevopinea* R. T. Baker; *Leptospermum petersonii* F. M. Bail.; *Lycopersicon esculentum* Mill. 'South Australian Dwarf Red'; *Phaseolus vulgaris* L. 'Epicure'; *Pisum sativum* L. 'Earlicrop'; and *Citrus limon* (L.) Burm. f. 'Lisbon'.

Symptoms appeared on approximately 50% of the spotted gum (*E. maculata*) seedlings but die-back was not extensive. Organisms re-isolated from these seedlings again proved pathogenic on lemon-scented gum. No symptoms appeared on any of the other species within 5 wk after inoculation.

The morphological, cultural, and biochemical characteristics of the 33 isolates obtained from diseased trees and inoculated seedlings were identical. Flagellae were stained by a modification of Fleming's method (4). Apart from lipolytic activity which was determined by Cabral's modification of Starr's method (4) the media used to determine the above characteristics were those employed by Dye (5). The absorption maxima of the yellow pigment were determined by the method described by Starr & Stephens (6).

The bacteria were found to be gram-negative nonsporing rods with rounded ends. They occurred singly or in pairs, single rods ranging in size from 0.5-0.6 μ X 1.3-1.8 μ. They were motile with a single polar flagellum.

Colonies which appeared on GYCA plates after 48 hr at 28 C were 1- to 2-mm diam, yellow, smooth, shining, and convex, with entire margins. On potato-dextrose agar slopes and on potato plugs growth was yellow and mucoid. Yeast extract-salts (YS) broth became turbid and later a yellow ring formed at the surface. No growth occurred in glucose agar stabs covered with a mixture of petroleum jelly and liquid paraffin. Growth occurred in YS broth containing 3% but not 4% NaCl.

Metabolism of glucose was oxidative. Acid but no gas was produced from arabinose, xylose, glucose, fructose, galactose, mannose, ribose, lactose, sucrose, maltose, trehalose, melibiose, cellobiose, raffinose, starch, dextrin, glycogen, and mannitol. No acid was produced from rhamnose, melezitose, inulin, adonitol, sorbitol, dulcitol, inositol, salicin, or α-methyl-D-glucoside.

Acetate, citrate, malate, lactate, propionate, and succinate were utilized but benzoate and tartrate were not. In the case of gluconate, a weak reaction was evident after 17 days at 28 C. Asparagine was not utilized as a sole source of carbon and nitrogen.

Catalase was produced. Gelatin, starch, and aesculin were hydrolyzed. Milk was proteolyzed but no acid was produced. Ammonia was formed from peptone, and H_2S was produced from cysteine and peptone but not from thiosulphate. Nitrate was not reduced to nitrite. Tests for methyl red reaction, indole, acetoin, lipolysis, and tyrosinase activity were negative.

The absorption maxima of the extracted pigment were (435), 453, and 480 nm in benzene; (418), 437, and 465 nm in petroleum ether; and (420), 440, and 462 nm in methanol. Parentheses around an absorption maximum indicate a shoulder.

Since the organism is a nonsporing, gram-negative rod with one polar flagellum, forms a slimy yellow growth on potato and media containing sugars, does not produce acid from salicin, and produces a carotenoid pigment with absorption maxima close to those reported for xanthomonads (6), it belongs to the genus *Xanthomonas* Dowson (3).

A comparison of its morphological, cultural, and biochemical characteristics with those of

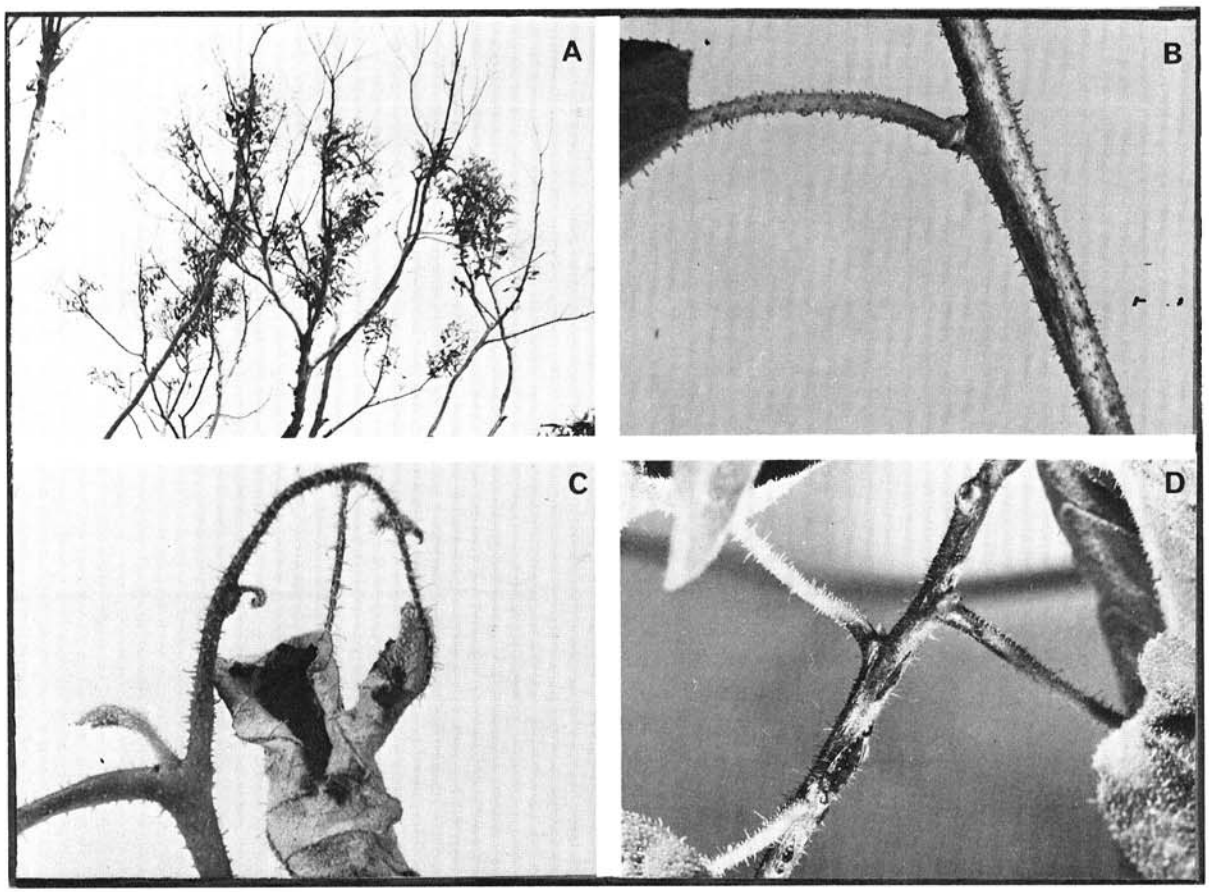

Fig. 1. A) Lemon-scented gum defoliated by *Xanthomonas eucalypti* showing new growth produced by epicormic buds situated in the branches. **B-D)** Symptoms shown by lemon-scented gum seedlings after inoculation with *Xanthomonas eucalypti.* **B)** Blackened area on stem. **C)** Dead apical bud and last-formed leaves. **D)** Stem cankers.

Xanthomonas species listed in Bergey's Manual (1) shows that it differs from all except *X. citri* (Hasse) Dowson, *X. cajani* Kulkarni et al., and *X. sesbaniae* Patel et al. Evidence to show that it is distinct from these species is provided by Burkholder (2) who reported that *X. citri* does not produce acid from lactose, and Dye (5) who found that *X. sesbaniae* tolerates 5% NaCl and that *X. cajani* shows tyrosinase activity.

No record could be found in the literature of an xanthomonad on *Eucalyptus* species or in fact any member of the Myrtaceae. It is considered therefore that the organism is a distinct species and the name *Xanthomonas eucalypti* sp.n. is proposed.

Cultures of *X. eucalypti* (NCPPB 2337 and 2338) have been deposited with the National Collection of Plant Pathogenic Bacteria, Harpenden, England.

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