Cassava Bacterial Blight in Taiwan

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Common bacterial blight, the most important cassava disease in Taiwan, was probably present before 1945. The disease is systemic in nature and transmitted primarily by cuttings contaminated with bacteria and secondarily by wind-borne water. Angular leaf spots, wilting, defoliation, gum exudates on leaf lobe, stipule, stem, especially on the latter two, and death of plants are caused by vascular-invading bacterium *Xanthomonas manihotis*, which confines itself to the genus *Manihotis* and shows poor survival ability in the soil. The disease was induced by using bacterial suspension, dipping of healthy cuttings, injecting into young stems, spraying of whole plants, cutting leaves of young plants with contaminated scissors, and pouring into injured roots of young plants.

Cassava *Manihot utilissima* has been cultivated in Taiwan for more than 80 years. At present, 22 000 ha of cassava are grown on the island where the total arable land is 915 000 ha. Cassava is the 8th largest crop in Taiwan next to rice, sweet potato, sugar cane, peanut, soybean, tea, and corn. Cassava is grown mostly on marginal lands in mountain hills and slopes, or in flat areas without irrigation. The total yield is close to 330 000 tons annually with an average yield of 16 tons/ha where the crop is grown for 1–2 years. Most roots are used for starch production and only 20% is made as cut chips for animal feed. Recent starch production reached 65 000 tons and is generally used as adhesive for eel feeding materials, textiles, paper, and paperboards, or as raw materials for producing glucose, antibiotics, and sodium glutamate. The requirement for cassava starch is still growing.

Some parasitic fungi (*Cercospora cassavae*, *Colletotrichum manihoticola*, *Guignardia manihoticola*, *Irpex lacteus*, *Macrophoma cassavae*, *Pellicularia rolfsii*, *Phoma manihotina*, *Phyllosticta cassavae*, and *Sclerotinia sclerotiorum*) of cassava have been reported (Sawada 1919, 1955). These and possibly others are probably present in Taiwan. However, most do not cause serious damage except common bacterial blight (CBB), known as bacterial wilt or "gumming disease" locally. Virus diseases do not cause any damage although occasionally mosaic-like symptoms can be seen on some introduced varieties.

Serious damage from CBB was first noted at Puli, central Taiwan, 1963, though the disease probably existed before 1945 (Mau 1951). Since then the disease has become island-wide, and some fields have been abandoned. The disease was caused by *Xanthomonas manihotis* (Leu and Chen 1972).

Materials and Methods

The diseased plants were collected mostly from Puli and various other areas. The bacteria were isolated from diseased tissues or gum substances present within tissues on potato dextrose agar (PDA) at room temperature or at 26–30 °C in the incubator. After bacterial colonies grew out 1–2 days later, they were further streaked. Colonies which developed from single cells were then transferred to PDA. The culture was renewed by new isolates or sometimes from the culture stored in the laboratory or in a refrigerator or the bacterial suspension was absorbed by small brick pieces and then kept dry in a refrigerator.

Symptoms were described both from naturally infected plants and those of artificially inoculated ones. The materials were also used for histological studies. Both paraffin and free hand sections were observed.

For inoculation, a thick bacterial suspension prepared from the culture on PDA was introduced to cuttings: (1) by dipping the cuttings for 1–2 min, draining and planting; and also for 1–2-month-old plants, (2) by injecting below the apical meristem; (3) by spraying on leaves at dusk; or (4) by pouring the bacterial suspension into an injured root. "Wu-Chi," the most widely cultivated variety, was used exclusively except as otherwise mentioned. Cutting of leaves by contaminated scissors was also tried.

Survival of the bacteria in the soil was determined by planting healthy cuttings into flats prepared: (1) by pouring the bacterial suspension into wet soil; and (2) by mixing soil with sliced diseased plantlets.

For morphological studies, the bacteria were

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shadowed with chromium tungstic oxide and observed by electronmicroscope. The samples were prepared from those inoculated on PDA at 26 °C for 24 h. Physiological studies followed standard methods. Thermal death point was determined by pipetting bacterial suspension into a 1-mm diameter glass tube, sealing the end, and treating in a temperature-adjusted water bath for 10 min.

Results

More than 80% of 150 pieces of tissues grew mucoid and colourless colonies on PDA 1–3 days after isolation. After single cell cultures, more than 30 isolates were tested for their pathogenicity by injecting the bacterial suspension near the apical meristem. No differences in pathogenicity were observed.

After the dipped cuttings sprouted to 1–6 cm or 10 cm high, the leaves wilted and the plantlets died in a few days. Before death, gum was exuded on the stipules and the stem, being white at the beginning and changing to golden yellow and then brown. The gum exudates swelled and turned "spongy" and white when water was available (Fig. 5). The bacteria within "spongy" exudates were thus spread by wind-borne rain to nearby plants. The tissues where gum protruded were sunken, irregular, spindled but enlarged longitudinally, turned to purplish and finally blackened and caused the death of the plants by girdling the stem. The same syndrome was observed when the plant was derived from bacteria-contaminated cuttings.

In larger plants, wilting occurred totally or laterally. When death of plants was not so acute, buds from each node sprouted in situ but then wilted, died, and usually exuded gum. Gum exudate could also be observed on green capsules, but seeds were gum-free. Discoloration of vascular tissues could be observed on the stem and also on vascular tissues of swollen roots (Fig. 6). However, no destruction of root and gum exudates was observed.

The diseased plants could be observed throughout all seasons, although epidemics occur when plants are young and the weather is warm and wet (March–November). In warm winter, gum exudate could be observed on matured tissues of the stem, particularly in Brazil No. 4 variety.

When plants were injected with bacterial suspension, gum usually exuded from the point of injection and then spread to upper parts of stems and stipules. Infection was high and usually reached 100%.

After spraying, wilting and gumming of plants occurred in 10–20 days. About 10% of the inoculated plants left uncovered died, and 90–100% of those covered with plastic bags died after inoculation.

Water soaking symptoms were not observed when very young plants were uncovered, and were not conspicuous even when covered with a plastic bag. However, water soaking lesions were clear when robust growing plants were inoculated and covered with a plastic bag. The lesions developed to angular leaf spots a few days after inoculation (Fig. 3). Gum exudates also occurred from angular spots when humidity was high. Angular leaf spots sometimes coalesced and formed necrotic bands. Gumming on stipules and stems followed and wilt then occurred. Plants grown in rich soil showed recovery after a few leaves wilted and defoliated; however, gum exuded heavily on the stem tissues. Recovery of diseased plants also took place in newly planted fields rotated with summer radish. Heavy manure had been applied and resulted in less disease.

Pouring the bacterial suspension onto the injured root caused wilting and death of the plants 3–4 weeks after inoculation in the summer. Only 1 out of 5 plants was infected in each of two tests. Symptoms developed mostly near apical young tissues even in rather small plants (15–20 cm high).

Leaf blades cut with bacteria-contaminated scissors wilted and defoliated without gumming. The leaves later wilted, sometimes exuding gum on stipules and stems. Plantlets died in 1–2 months after inoculation. However, death occurred faster in some varieties, correlating well with their degree of resistance in the field.

For screening resistant varieties, cuttings of different varieties were dipped into the bacterial suspension and then planted. All 21 tested varieties showed 50–100% infection. On average, 75.2% of plants wilted and died, although 67% of the widely cultivated variety "Wu-Chi" wilted and died. For some other untested varieties, all showed symptoms and high disease incidence including Brazil No. 4.

Host range of the bacterium was confined to cassava. Injections near the apical meristem of the seedlings of tomato, watermelon, lettuce, cucumber, sorghum, and lima bean, and also

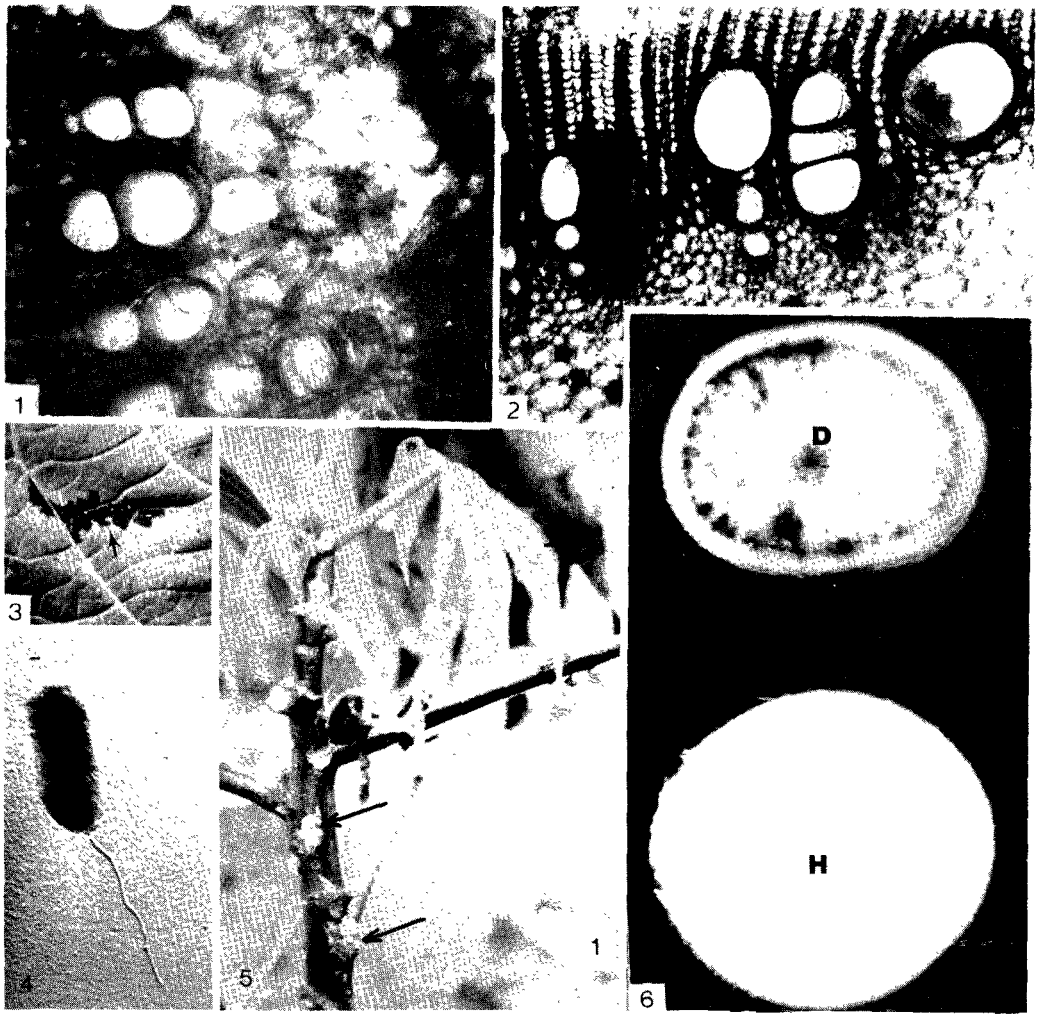

Fig. 1 Free-hand section shows black-coloured substances filling some of the integrated vessels. Fig. 2. Free-hand section shows black-coloured substances filling vessels and paranchymatous cells. Fig. 3. Angular leaf spots on cassava leaf; note gum exudate on the spots (inaicated by arrow). Fig. 4. Polar uniflagellated cells of Xanthomonas manihotis. Fig. 5. Gum exudate showing "spongy" area. Fig. 6. Vascular bundles turned black in root of diseased plant (D) but white in healthy plant (H).

into trees (*Bischofia trifoliata, Codiaeum variegalum*, and *Euphorbia pulcherrima*) all failed to show symptoms. Negative results were also obtained by pouring the bacterial suspension into the injured roots of the above-mentioned seedling plants.

Ability of the causal bacterium in the soil to induce the disease showed that either 3 out of 9 or 2 out of 12 plants wilted and died respectively, soon after cuttings were planted in the flat. The bacterial suspension was poured and mixed with soil and infected plants were chopped and mixed with the soil. However, if the cuttings were planted 1 or 2 weeks later, no symptoms appeared. The experiences in the field also demonstrated that not all replanted cuttings on the rogued sites of the wilted plants showed symptoms.

CBB could be transmitted primarily by bacteria-contaminated cuttings and secondarily by wind-borne water carrying bacteria. However, some insects may transmit the disease, such as bees.

Losses caused by CBB differed from field to

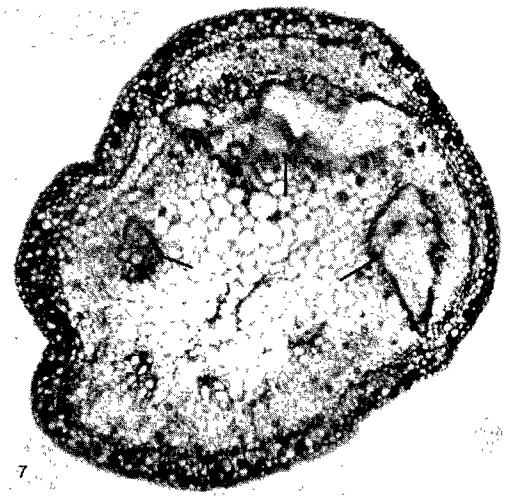

Fig. 7 Paraffin section shows "pocket" in the stipules.

field. In the most severe cases, the field had to be abandoned. Some 30% loss was experienced where the disease was endemic. Control measures such as precaution in selecting healthy cuttings and roguing of the young diseased plants were practiced.

Histological studies revealed that vessels were attacked, with brownish black substances filling some of them and nearby parenchymatous cells in the vicinity of the pith (Fig. 2). In phloem cells similar substances were also observed. Some vessels disintegrated and dissolved (Fig. 1), presumably by enzymatic action. The dissolution of the tissues was initially along outer vessels but then expanding and forming a pocket. Several pockets were observed in the same cross section (Fig. 7). As the pocket enlarged, the epidermis erupted and gum substances containing polysaccharides and the bacteria were excreted. Pith tissues were free from infection and remained intact (Fig. 1–2).

The bacterium is rod-shaped with round ends, uniflagellated at polar (Fig. 4), no capsule, gram-negative but tended to be positive when aged. Average measurement was 0.9–1.5 × 1.8–3.2 μm.

The bacterium grew poorly on nutrient agar, circular, convex, entire, and filiform on agar stroke, no odour, without fluorescence, milky white in colour, and sticky. On PDA surface, growth was excellent, milky white, mucoid, but when stable the growth was only along the stable line. On liquid medium, growth was membranous and tended to be ring-form when shaken and left standing overnight. Temperature range was 14–36 °C, with an optimum at 30–34 °C. Thermal death point was 52 °C. Growth was favoured between pH 6.7–8. No growth occurred at 3, 3.5, and 4% sodium chloride solution but did with 2 and 2.5% solution.

Very weak growth and a slight acid production were obtained with dextrose, sucrose, D-xylose, D-fructose, arabinose, and cellobiose. Lactose gave no growth, and the starch was hydrolyzed. In litmus milk, growth was slow and a slight reduction occurred. Liquefaction began on the ninth day. Indol was not formed even being cultivated for 15 days, but hydrogen sulfide formed in 6–7 days (opposite results were obtained by C. T. Chen, from different isolates; personal communication 1975). Nitrates could not be used. Catalase reaction was positive; also positive for cytochrome oxidase; Voges-Proskauer reaction was negative.

No visible mutants, or changes in pathogenicity, were noted. Older (1 month to several months) cultures in the laboratory, and recultures (1–4 years), all induced the same degree of disease incidence as those of freshly isolated cultures.

Discussion

There have been four bacterial diseases in cassava in the world (Elliott 1951). Symptomatology of the disease and morphology and physiology of the bacterium all indicated that the disease studied was caused by *X. manihotis*, thus the name common bacterial blight was applied. CBB has been reported in Central and South America, and some parts of Africa (Lozano 1975). Detailed studies on this disease have been reported (Lozano and Sequeira 1974a, b).

Since no resistant germ plasm is present in Taiwan, we are unable to control the disease by using resistant cultivars. Through the courtesy of CIAT, we introduced some cassava seeds including those with resistance from crosses with M Col 647 in August 1975. Seedlings were raised and screening for resistance is under way. Recommended control methods such as using bacteria-free planting materials, roguing the diseased plants, rotation with other crops, avoiding the overlapping of the 1- and 2-year-

old crops, are all difficult to practice effectively, except roguing. Distribution of the bacteria-free planting materials is scheduled to be planted by using the tip rooting method for local varieties.

Microbial gum composed of D-glucose, D-mannose, D-glucuronic acid, acetic acid, and pyruvic acid (Chen and Tsou 1974) is produced by *X. manihotis* in a sucrose medium. It is not surprising that bacteria are used to produce gum, such as *X. campestris*, the causal agent of black rot disease of crucifer, used to produce Xanthan gum (Rogovin et al. 1961). The gum probably protects the live bacteria when it is dry and releases the bacteria when it is wet.

This systemic bacterial disease is the biggest problem for cassava growers in Taiwan at the present time.

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Factors Affecting the Incidence of Cassava Bacterial Blight in Africa

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Cassava bacterial blight (*Xanthomonas manihotis*) is a widespread and damaging disease in Africa. Its severity in Africa varies with locality and climatic conditions. Factors that may affect its severity are soil type, climate, cultural practices, and varieties. Distribution and economic importance of CBB in Africa, and results of epidemiological studies, are included.

Cassava bacterial blight (CBB), caused by *Xanthomonas manihotis*, is a widespread and damaging disease in several countries of South America, Africa, and Asia (Lozano and Booth 1974). In Africa, it was first reported in Nigeria (Williams et al. 1973) and subsequently in Zaïre (Hahn and Williams 1973), Cameroon (Terry and Ezumah 1974), and Ghana and Togo (Persley unpublished data).

The extent of damage caused by CBB varies with locality and climate. The regions most severely affected are probably Zaïre and mid-western and eastern Nigeria. In West Africa, it is more prevalent during the rainy season (April–September).

The epidemiology of a disease may be affected by several, often interrelated, factors including soil type, climate, cultural practices, and crop variety.

The following terms are used according to definitions proposed by the Federation of British Plant Pathologists (1973): *Incidence* — frequency of occurrence of disease, expressed as the proportion of plants affected in a given population; *Severity* — intensity of disease in an individual plant expressed as a rating on a numerical scale.

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