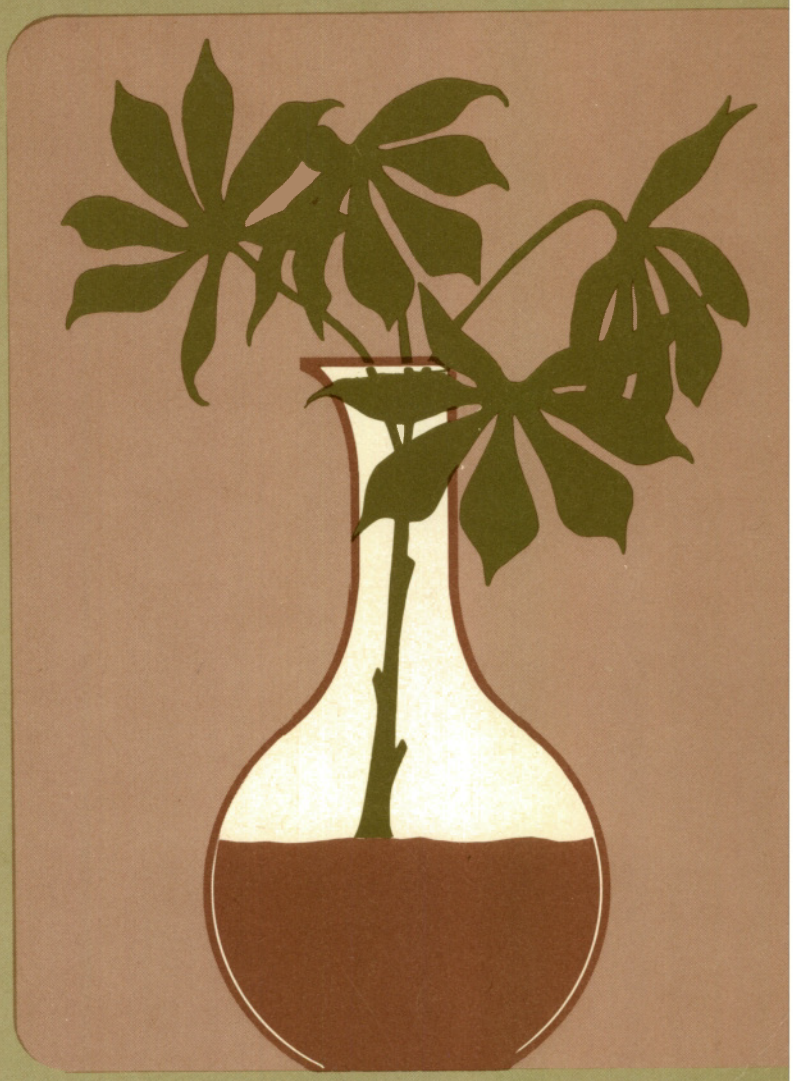
CASSAVA BACTERIAL BLIGHT

report of an
interdisciplinary workshop
held at
IITA, Ibadan, Nigeria,
1-4 November 1976.



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IDRC-096e

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Cassava bacterial blight: report of an interdisciplinary workshop held at IITA, Ibadan, Nigeria, 1–4 November 1976. Ottawa, IDRC, 1977. 36p.

/IDRC publication/. Report of a workshop on the /cassava/ /bacteria/l blight (CBB) /plant disease/ in /Africa south of Sahara/ — discusses the /diagnosis/ and /geographic distribution/ of CBB, influence of shade (/solar radiation/) and /intercropping/ on its incidence, /plant breeding/ for /disease resistance/; /disease control/ efforts in /Nigeria/, /Zaire/ and /Ghana/. Includes /bibliography/s, /list of participants/ and country statements from /Benin PR/, /Congo PR/, Ghana, and /Togo/.

UDC: 633.68 ISBN: 0-88936-143-6

Microfiche Edition $1

IDRC-096e

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Editors: **Gabrielle Persley, Eugene R. Terry, and Reginald MacIntyre**

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Contents

Survival of *Xanthomonas manihotis*, the Cassava Bacterial Blight Pathogen

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Cassava bacterial blight (CBB) caused by *Xanthomonas manihotis* has been reported from Brazil (Bondar 1912), Argentina (Zyngier de Resnik 1968), Nicaragua and Guatemala (Normanha 1971), Venezuela and Colombia (Lozano 1975), Madagascar (Bouriquet 1946), Mauritius (Orian 1947), Nigeria (Williams et al. 1973), Zaire (Williams, personal communication), Malaysia (Bradbury, personal communication), Thailand (Lozano, personal communication), and Taiwan (Leu and Chen 1972).

The rapid spread of this disease across the cassava-growing areas of the world in recent years has highlighted the importance of the survival of the pathogen in considering how it is carried over from one rainy season to another to reinfect new crops.

Some workers have suggested that the movement of CBB-infested soil during cultural operations and the use of infected cuttings as planting stock are partially responsible for the spread of the bacterial blight disease from place to place (Drummond and Hipolito 1941; Lozano 1975). It is therefore important to investigate the survival of the CBB pathogen in these ecological niches to identify the most important factor in the spread of the disease. Possible methods of cultural control arising from the results are also discussed.

Materials and Methods

Soil Survival

The three types of soils used were from the following sites in Colombia: CIAT (pH 6.8), Jamundi (pH 4.2), and Popayan (pH 4.5). All soils were used for cultivating cassava but only Jamundi soil carried plants infected by CBB. Soils were inoculated with a suspension of *X. manihotis* in sterile distilled water to give a concentration of 3.2×10^7 cells/g of soil.

Numbers of *X. manihotis* cells in the soil were estimated by serial dilution and plate count techniques on medium D_5 (Kado and Heskett 1970). Plate counts were made at the time of CBB inoculation into the soils and subsequently at intervals of 7 days. The population was followed in both sterile and nonsterile soil.

Experiments and Results

Survival in Sterile and Nonsterile Soil

The results given in Fig. 1 show that there was a rapid decline of CBB numbers after soil inoculation, the rate being less in sterile than nonsterile soils. Survival was longer in CIAT soil (near neutral) than in Jamundi and Popayan soils (acidic). CBB cells did not survive in Popayan soil.

Field Survival

To determine the vertical distribution of CBB in infested soil, Jamundi soil was collected from a plot from which diseased plants had recently been removed and from a plot carrying diseased plants. Samples were taken at 10-cm intervals to a depth of 50 cm. Samples of plant debris on the soil surface were also taken from the plot of diseased plants.

Results (Table 1) show that bacterial cells were present in the infected plant debris on the soil surface and in the 0–5-cm zone of the plot carrying diseased plants. There were fewer CBB cells in the 0–5-cm zone of the soil from which infected plants had been removed.

Survival at Different pH

The survival of CBB in soils at different pH was recorded in sterilized and nonsterilized Jamundi soils. The pH of each 1-kg sample was raised by steps of 0.5 to pH 7.25 using $CaCO_3$. Water dilutions of CBB cells were added to a concentration of 8.0×10^7 cells/g of soil and bacterial counts were taken immediately and at intervals of 7 days.

There was a general decline of CBB numbers

Table 1. Vertical distribution of cassava blight bacterium (CBB cells/g of debris or soil; mean of three replicates) in soil samples taken from an infected plantation.

	Treatment	
Origin	Infected plants removed	Infected plants present
Surface debris	None	3.7×10^7
Surface soil	0	0
0–5 cm	2.7×10^4	1.9×10^7
Over 5 cm	0	0

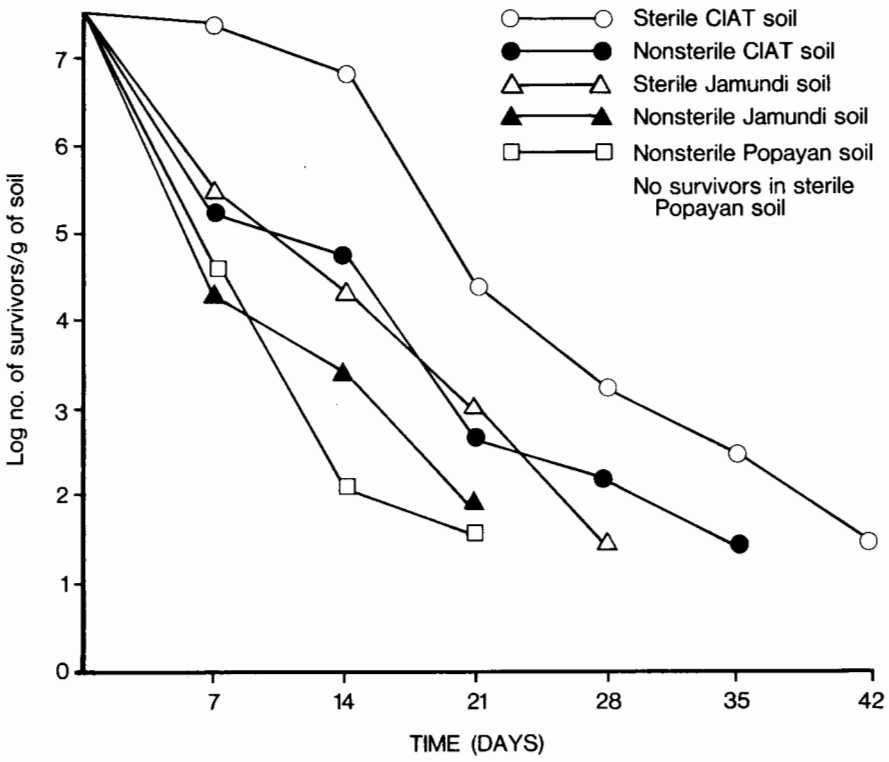

Fig. 1. The survival of X. manihotis in three types of soil.

from the time of inoculation. CBB cells survived a little longer in sterile than in nonsterile soils and better at pH 5.0, 5.5, 6.0, and 6.5 than at pH 4.0, 4.5, 7.0, and 7.25. The optimum pH for CBB survival was 6.0–6.5.

Flooding and Desiccation

The survival of CBB was also determined in flooded and desiccated soils. To each 1-kg soil sample, CBB cells were added up to a concentration of approximately 3.2×10^7 cells/g of soil. Samples for the flooding experiments were packed into plastic tubes (15 cm diameter × 20 cm high), which were submerged in water. Bacterial counts were taken immediately before and after flooding and at intervals of 7 days.

For the studies on survival in desiccated soil, 20-g portions of each soil inoculated to a concentration of 3.2×10^7 CBB cells/g of soil were spread in petri dishes and allowed to dry over anhydrous calcium chloride in a desiccator. In the controls, soils were not desiccated. Plate counts were taken before soil was placed in the desiccator and afterwards at intervals of 7 days.

Results show that CBB did not survive for more than 7 days in flooded and desiccated Popayan and in flooded Jamundi soils. They survived for 14 days in desiccated and in flooded and desiccated CIAT soils. In soils at field capacity CBB cells survived for 21 days in Popayan and Jamundi soils and for 35 days in CIAT soil.

Infectivity of CBB in Soil

Infectivity of CBB in Jamundi soil was studied by using infected soil for leaf-spray inoculation and as a growth medium for healthy cassava cuttings. A sample of 50 g of soil was infected with CBB (3.2×10^7 cells/g of soil) and suspended in 50 ml sterile distilled water. Suspensions were sprayed immediately and at intervals of 7 days on healthy cassava leaves maintained in a mist chamber for 48 h and 25 °C and then in a glass house at 80–90% RH and 25 °C. Uninoculated soil was used for control.

There was a direct relation between concentration of CBB in soil and the number of leaf spots that developed when leaves were sprayed with suspensions of CBB-infested soils. Below a population of 10^3 CBB cells/g of soil, leaf spots did not develop. Using a similar concentration of CBB, more leaf spots occurred using suspensions of bacteria in sterile distilled water than with CBB-infested soil suspension.

Table 2. Disease development in plots after different postharvest treatments of soil/no. of viable cassava blight bacterium (CBB).

Time of replanting after harvest (days)	Debris removed		Debris removed, surface hoed		Debris on surface	
	No. diseased plants	CBB/g soil	No. diseased plants	CBB/g soil	No. diseased plants	CBB/g soil
0	6(24%)	2.7×10^6	3(12%)	1.3×10^5	6(25%)	3.1×10^7
7	7(28%)	2.0×10^3	—	1.3×10^2	16(64%)	2.7×10^5
14	—	—	—	—	21(84%)	1.3×10^5
21	—	—	—	—	25(100%)	2.1×10^4

Growth of Cuttings in Infested Soil

An area of land 25 × 25 m was divided into nine plots of 5 × 5 m, each plot separated by 3 m. Each plot was planted with 100 cassava cuttings at 50 cm spacing along and between rows. Four weeks later plants were inoculated with CBB. Six weeks after inoculation, plants were removed and the plots were treated as follows: (*a*) plants removed; (*b*) plants removed and soil surface hoed; and (*c*) infected plant remains left on the plots.

Each plot was divided into four subplots. These were replanted with another 100 healthy glasshouse-grown plants: 25 on the day of clearing of plots, and 25 at intervals of 7 days up to 21 days after clearing. Plants were scored for symptoms of disease at weekly intervals. Counts of CBB in soil samples (from the 0–5-cm zone) from each plot were also taken on each occasion. Table 2 shows the number of plants that developed disease symptoms when planted in cleared plots that had previously supported infected plants. The data suggest that, after 7 days, there was insufficient CBB in soil in which diseased plants were removed to infect leaves but that where debris was left on the soil surface, numbers remained high enough to cause infection for up to 21 days. Correspondingly, in treatments (*a*) and (*b*), no more plants became infected after 7 days whereas in plot (*c*) numbers of infected plants continued to increase so that by 21 days all were infected.

Discussion

In naturally infested soils, CBB seems to be restricted to the 0–5-cm zone. This is the part of the soil that is usually disturbed during cultural practices and suggests that numbers of CBB bacteria in soil can be reduced considerably by loosening and exposing the soil surface for a period of time before replanting in infested soil. Leu and Chen (1972) did not observe infected plants on planting cassava cuttings in fields from which infected plants had just been cleared. However, if good control is to be achieved, the infected plant debris on the soil surface of infected plots must be cleared and burned.

Generally, survival of CBB in soil was poor (21–28 days) but was longer in near-neutral soil than in acid soils. Planting cassava in acid soils or soils of low pH may be a way of reducing the risk of infection through soil splashes.

Survival of CBB in sterile soil was longer than in nonsterile soil. This is because xanthomonads lack competitive ability as saprophytes. Hence in sterile soil where all the microbial competitors have been eliminated, CBB survived longer. The introduction of antagonistic microorganisms to infested soil may be useful in keeping CBB numbers low and reduce risk of infection of healthy plants. The lack of surviving cells in sterile Popayan soil may be due to the fact that substances toxic to CBB were released from the volcanic soil during sterilization.

Both flooding and desiccation decreased the longevity of CBB in soil. However, flooding has its attendant problems in that it may drastically alter soil structure and ecological balance in favour of harmful indigenous soil-borne pathogens. Also, it may leach essential minerals from the soil. By planning periods of cultivation so that the dry season falls between one cropping season and another CBB can virtually be eradicated through natural soil desiccation.

A problem arising from soil infestation by CBB is that plants become infected from soil splashes during rainstorms. Fortunately, this is important only when the soil is heavily infested with CBB and contains more than 10^4 CBB cells/g. Again regular loosening of the surface soil to expose it to the sun and dry air is likely to be useful in keeping the population of CBB in soil low, hence the incidence of disease resulting from soil splashes will be low.

Ikotun (1976) has shown that *X. manihotis* cells survived for up to 24 mo in dried bacterial exudate and for more than 30 mo in dried infected cassava stems. These results are similar to those obtained by Terry (1974) in which *X. manihotis* survived

and retained infectivity after 22 mo of dry storage at room temperature.

Comparison of survival times of CBB in bacterial exudate and host tissues indicates that the soil is not a favourable niche for survival. The most important niches in the survival of CBB that aid carryover of viable and infective cells from one cropping season to another are the bacterial exudate and the host tissues. *X. manihotis* therefore belongs to group A of Buddenhagen's classification (1963) of pathogens whose soil phase is one of a rapid decline in numbers. Populations are developed mainly in the host, and survival is mainly in the host plants and their remains. As CBB does not form spores, it is at a disadvantage and has to survive in a niche that offers protection, such as exudate and host tissues. It is known that CBB cells are surrounded by an extracellular heteropolysaccharide (Ikotun, unpublished data). This slimy substance is similar to that produced by *X. phaseoli* (Leach et al. 1957) that confers protection on the bacterial cells against toxic chemicals, radiation, and desiccation.

These results emphasize the importance of bacterial exudates and infected host plant materials in the survival and carryover of CBB from one cropping season to another. Due to the long periods of survival of CBB in exudates and plant materials, it is important to remove dead infected plant parts from the field and burn or bury them to prevent disease carryover. It is also important to use clean planting material to prevent the establishment of the disease in a new plantation.

Acknowledgments

The author is grateful to the International Development Research Centre (IDRC) for providing funds for this research and to the Centro Internacional de Agricultura Tropical (CIAT), Cali, Colombia, for the provision of working facilities.

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