Bacterial Blight of Cassava in Colombia: Epidemiology and Control

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ABSTRACT

Dispersal by splashing raindrops is the most important means of dissemination of the cassava blight bacterium (a possible strain of *Xanthomonas manihotis*) within localized areas in Colombia. Dissemination from one area to another occurs through propagation of infected plant parts and by means of infested tools. In controlled inoculation experiments in the field, plant-to-plant spread occurred in the direction of prevailing winds, and disease incidence was correlated with amount of rainfall. However, no dissemination occurred when host plants were located at least 15 m away from the inoculum source.

Satisfactory disease control was obtained by excising upper portions of infected plants and allowing the stumps (20-30 cm) to resprout. Effectiveness of this control method was reduced when treating highly susceptible, severely infected cultivars. Rooting excised buds was an efficient method of obtaining healthy planting stock from infected cultivars.

Eight out of 1,293 cassava cultivars tested under greenhouse conditions were resistant to bacterial blight. Resistance was dependent on restriction of penetration and systemic invasion by the pathogen; two cultivars ('M. Col. 647' and 'M. Col. 667') exhibited a hypersensitive response which limited the size of leaf lesions. The use of resistant cultivars remains the most promising method of control of the disease in the tropics.

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A bacterial blight of cassava (*Manihot esculenta* Crantz) causes extensive losses in the American tropics. In Colombia, the disease appears to be caused by a strain of *Xanthomonas manihotis* (Arthaud-Berthet) Starr (12); the taxonomic position of this bacterium is still uncertain. Very few studies have been carried out on dissemination of the pathogen and control of the disease. Amaral (1) suggested that *X. manihotis* spreads from one area to another by infected cuttings or contaminated insects. Others have suggested that this pathogen could be readily spread by movement of soil during cultural operations, or by the use of infested pruning tools (4, 6, 8). The influence of environmental factors on dissemination of the pathogen has not been determined, although leaf spotting was reported to increase during the rainy season (6).

The use of resistant cultivars for control of bacterial blight was first suggested by Gonçalves (8), and several field-resistant cultivars from Brazil have been reported (4, 9). The use of clean propagating stock (4, 6, 9), crop rotation on a 4- to 5-yr cycle (4), avoidance of pruning in infected areas (9), and removal or destruction of diseased plants (6, 9), have also been suggested for control of the disease.

Most of the available information on epidemiology and control of cassava blight is based on field observations, or on analogy to other bacterial diseases, not on actual experimentation. The purpose of these investigations, therefore, was to determine the primary means of spread of the pathogen after controlled inoculations in the field, and to develop control procedures based on the results of greenhouse and field tests.

MATERIALS AND METHODS. – Cassava cultivars were grown in the greenhouse from 8- to10-cm-long stem pieces planted in sterilized sandy soil in 15.2-cm (6-in) diam black plastic bags. Plants were grown for 45 days at 30 C ± 4 C and 80% relative humidity (RH). Inoculations were carried out with distilled water suspensions of bacteria (10^9 cells/ml) obtained from the 48-hr growth of isolate 4.26L on triphenyltetrazolium chloride (TZC) medium (11). Plants were inoculated by either puncturing the stem or spraying the foliage. Stems were inoculated at the third and fourth leaf axil from the top by forcing a sharp needle into the stem through a drop of bacterial suspension. The foliage of each plant was sprayed to runoff with bacterial suspension by means of a DeVilbiss atomizer connected to an air pump at 68.94×10^3 N/m^2 (10 lb/in^2) pressure. To provide high relative humidity after inoculation, a 25.4 - mm (1.0 in) water layer was maintained directly underneath the plants on the bench.

To follow dissemination of CBB in the field, 240 cassava cuttings (clone M. Col. 1) were planted in rows spaced 1 m apart in a rectangular plot at an isolated location at the ICA Experimental Station, Nataima (Espinal), Colombia. When plants were 53 days old, one plant at each corner and one at the center of the plot were stem-inoculated. The incidence of disease in the plot was determined at 2-wk intervals for 3.5 mo. Once plants in the area were 100% infected, four plots (20 plants each, clone M. Col. 1) were established on each side of the original plot so that the closest new plants were 5, 10, 15, and 20 m from infected plants. The incidence of disease in these new plots was determined periodically up to 3 mo after planting.

Evaluation of control methods. – The following procedures were followed in attempts to control the disease:

– 1) Pruning infected plant parts. – Six-month-old cassava plants, cultivar 'Llanera', planted in two plots

of 200 plants each, were spray-inoculated as described previously. Three months later, when heavy infection was evident, all plants in one plot were pruned back to a 20 to 30 cm high stump. All plant debris was removed and burned. Pruning knives were disinfested with 5% formaldehyde before each plant was pruned. Pruned plants were allowed to sprout and the new shoots were observed weekly for disease symptoms for 6 mo.

– 2) Bud rooting and indexing. – Because the pathogen does not invade mature woody tissues of most cassava cultivars, much of the branch and stem tissues of infected plants remains healthy. Materials for propagation, apparently free of the disease, were obtained from unlignified, partially lignified, and highly lignified stems as follows: (i) Five-cm-long stem pieces containing two or three axillary buds, and (ii) individual buds removed from leaf axils. These buds were planted individually in peat pots and placed in the greenhouse at 28 C and 70-80% RH. After rooting, they were transplanted into 6-inch pots, and disease symptoms were recorded periodically during an additional 2-month period.

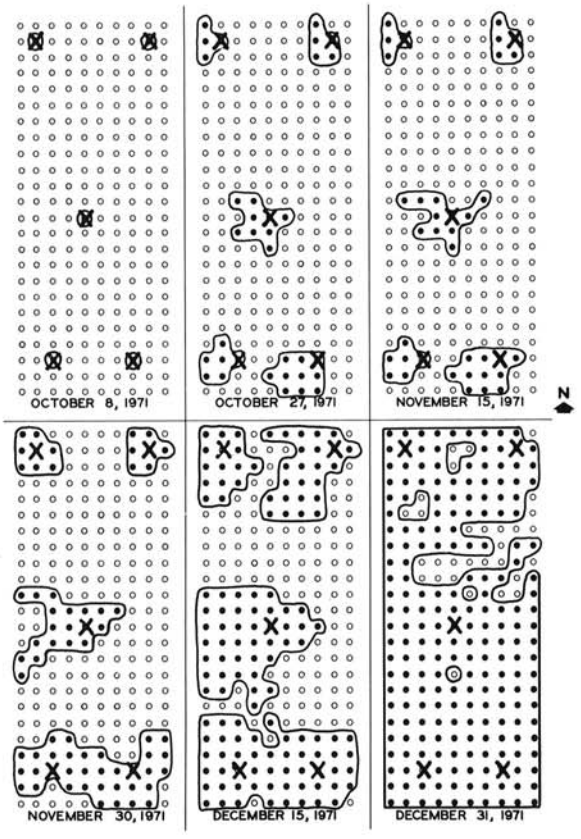

Fig. 1. Spread of Cassava bacterial blight (CBB) in the field from initial sources of infection (X) in experiments conducted between September 15 and December 31, 1971. Black dots indicate the position of infected plants at each 15-day period after inoculation.

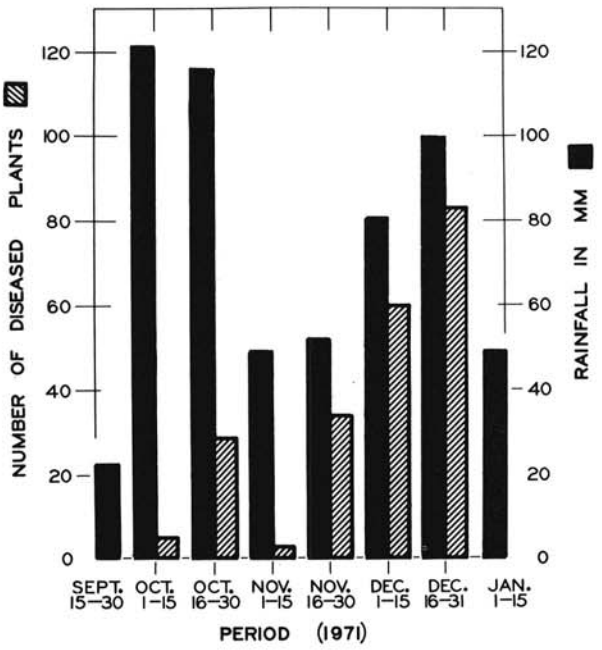

Fig. 2. Spread of cassava bacterial blight (CBB) in the field from initial sources of infection. Relation of total rainfall (mm) and number of newly diseased plants in each 15-day period. Data are not cumulative.

– 3) Testing for resistance in cassava clones. – To search for possible sources of resistance, 1,293 cassava clones (obtained from the Cassava Collection at CIAT, Cali, Colombia) were grown in the greenhouse from rooted stem pieces in 15.2-cm (6-in) diam plastic bags containing sterilized sandy soil. When plants were 45 days old, 10 plants of each clone were both stem- and spray-inoculated with isolate 4.26L, as described previously.

Disease indices were calculated 25 days after inoculation based on visual estimates of: (i) wilting; (ii) dieback; (iii) gum exudation; and (iv) leaf spotting. Each symptom was rated on a 0 to 5 scale in which 0 = no symptoms and 5 = maximum symptoms. Accordingly, a plant with maximum symptoms for each category would show wilting of all leaves, necrosis and gum exudation along the entire stem, and extensive necrosis of leaf blades due to coalescence of numerous spots. Values for leaf spotting were determined only on the fully-expanded leaves of each plant, because young leaves are resistant to penetration by the pathogen.

The average index for each symptom category for each clone was multiplied by 5. The highest possible total value, therefore, was 100. Resistance or susceptibility was designated arbitrarily according to the following scale: VS = Very susceptible (80-100); SS = Susceptible (60-80); MS = Moderately susceptible (40-60); RR = Resistant (20-40); HR = Highly resistant (1-20); II = Immune (no infection).

Five plants of each of the cultivars initially

selected as highly resistant were reinoculated with isolates 5.27L, 9.31L, or 13.35L (12). Plants were maintained in the greenhouse and symptoms were rated as described before.

RESULTS. – *Dissemination of the pathogen.* – Preliminary experiments in the greenhouse indicated that the pathogen could be disseminated by spraying distilled water at 68.9 X 10^3 N/m^2 (10 lb/in^2) pressure on a diseased plant so that the splatter could reach a healthy plant located 30 cm away. Results of controlled inoculations in the field also suggested that dissemination in cassava plantations is due to the splashing of wind-driven rain droplets. Initially, spread was greatest in a southerly direction, the direction of the prevailing winds in the area (Fig. 1). Disease incidence was correlated with amount of rainfall in the area at any given period. There was good statistical correlation (r = +0.49) between total rainfall and number of newly diseased plants in each of six 15-day periods (Fig. 2).

Once plants in the original plot were 100% infected, spread of the bacterium occurred to young plants on adjoining plots 5 or 10 m away. However,, no dissemination to plants growing at 15 or 20 m from the inoculum source was observed (Table 1) even though total rainfall during this 60-day period was 207 mm. Plots located to the south of the inoculum source had the highest number of infected plants. For instance, in the southern 5 m plot there were 17 (out of 20) infected plants versus 7 (out of 20) plants in the northern 5 m plot.

To determine whether dissemination could occur via cuttings normally used for propagation, 100 cuttings each from mature and immature stems were obtained at random from plants in a heavily infected cassava planting. Surveys indicated that 85.7% of the plants in the plot became infected. Each of these cuttings was planted in a 15.2-cm (6-in) diam plastic bag containing sterilized sandy soil, and rooted in a growth chamber at 30 C, 60-80% RH and 8-h photoperiod at 12,912-19,368 lx (1,200-1,800 ft-c). Two months after rooting, 25% of the plants originating from immature tissues and 8% of those from mature tissues showed characteristic symptoms of the disease (Fig. 3).

To determine whether dissemination could occur by means of infested pruning knives, 150 plants of cassava cultivar M. Col. 1 were grown in the greenhouse at 30 C for 45 days and their stems were wounded by cutting with infested knives (machetes) either at the base, the middle, or the tip of the plant. The incidence of infection 3 mo after wounding indicated that CBB can invade the host plant through such wounds, but that penetration through young stems is more likely than through mature, old stems (Table 2).

Bud indexing. – A high percentage of healthy plants was obtained from bud cuttings removed from infected plants and rooted. From 150, 5-cm, entire stem cuttings and 100, 5-cm half-stem cuttings, only 11 (7.3%) and 7 (7%) plants, respectively, showed disease symptoms 2 mo after rooting. Rooting of such stem cuttings was near 100%. Rooting of individual axillary buds was less successful, but the 23 plants obtained from such buds remained healthy. Green, young stem cuttings could also be rooted, but because of the high susceptibility of such tissues to the disease, the chances of obtaining disease-free plants were lower (only 12.2% from 90 cuttings remained healthy).

TABLE 1. Dissemination of cassava bacterial blight (CBB) to cassava plants located at different distances from the inoculum source

Distance from inoculum source (m)	Number of plants/plot	Infected plants/plot[a] (no.)	(%)
5	20[b]	12	60
10	20	5	25
15	20	0	0
20	20	0	0

[a] Average number of infected plants 60 days after planting.

[b] Number of plants in each of four plots, each plot located on one side of the inoculum source.

Control by pruning infected plant parts. – One month after infected plants were heavily pruned in the field, only 12 out of the 200 plants exhibited symptoms of the disease on the new shoots. Within 2 mo after pruning, five additional plants (8.5% total) showed disease symptoms. As infected plants appeared, they were uprooted, removed from the field, and burned. By 6 mo after pruning, no additional diseased plants had appeared. The control plot (unpruned) remained 100% infected.

Pruning was also carried out on infected cultivars with different levels of resistance. Ten very susceptible (213 plants), 10 susceptible (267 plants), seven moderately susceptible (152 plants), and three resistant (37 plants) cultivars were spray-inoculated in the field when the plants were 3 mo old. All plants became infected, but to different degrees. Six mo later, all plants were heavily pruned as described in Methods.

Most of the resprouts from the very susceptible cultivars (76%) had symptoms of the disease 6 mo after pruning, as compared with 36% infection in the susceptible, 16% in the moderately susceptible, 16% in the moderately susceptible, and 9% in the resistant ones (Fig. 4). The results indicate that the effectiveness of this control measure depends largely on the level of resistance.

Varietal resistance. – The levels of resistance or susceptibility of 1,293 different Colombian cassava cultivars to infection following artificial inoculation with isolate 4.26L, were determined. Eight cultivars were classifed as resistant (Table 3); of these, 'M. Col. 647' and 'M. Col. 667' were the most resistant. Even those cultivars classified as susceptible ('M. Col. 282,' 'M. Col. 707,' and 'M. Col. 803') in our scale had fewer leaf spots per leaf than the very susceptible cultivar 'Popayan'. Generally, the performance of these cassava cultivars in the field was

Fig. 3. Dissemination of cassava bacterial blight (CBB) by infected vegetative propagating stock. Left: healthy sprout from a healthy stem cutting. Right: diseased sprout from an infected stem cutting.

correlated with the resistance ranking obtained by artificial inoculation in the greenhouse. The amount of leaf spotting alone appeared to be as good an index of resistance as all other characteristics combined. However, resistant cultivars M. Col. 647 and M. Col. 667 had a relatively high number of leaf spots per inoculated leaf, but the spots were very small and did not enlarge, apparently because of a hypersensitive response to infection.

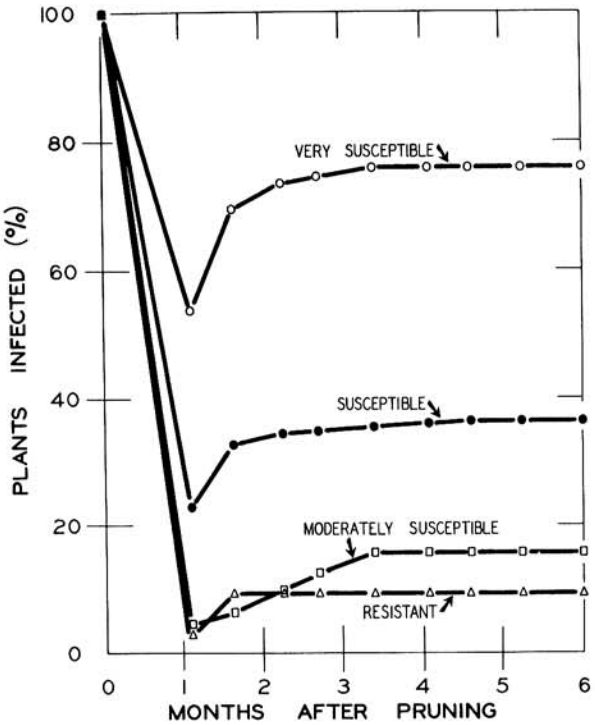

Fig. 4. Effect of pruning infected cultivars having different levels of resistance to cassava bacterial blight (CBB). Plants were pruned 6 mo after they were spray-inoculated with a bacterial suspension (10^9 cells/ml) of isolate 4.26L.

DISCUSSION. – Although our work on dissemination of CBB lacked extensive replication throughout different seasons, the results suggest that splash dispersal is probably the chief means of dissemination of CBB from plant to plant. This is analogous to the situations described for angular leaf spot of cotton (3) and halo blight of beans (18). Rainfall provides the conditions necessary for mobilization, distribution, and penetration of inoculum, and it is probably the most important environmental factor affecting bacterial blight disease of cassava plants. This assumption is supported by the apparent correlation between amount of leaf spotting and the amount of rainfall during 1971. Wind-driven rain droplets appear to determine the direction of spread of the pathogen during the growing season. Our results indicate that infection was more prevalent in the direction of prevailing winds, but the effects of wind-driven rain and water congestion of intercellular spaces of predisposition to CBB infection remain to be determined.

CBB can be disseminated from one area to another by means of infected cuttings used for propagation of cassava. Dissemination of bacterial pathogens by this means is common in crops which are normally propagated by vegetative means (10, 13, 14). In Colombia, this method of dissemination is particularly important because there are at present no restrictions on movement of cassava cuttings throughout the country.

TABLE 2. Infection of cassava cultivar M. Col. 1 following pruning with infested knives (machetes)

Stem tissue wounded	Number inoculated[a]	Number infected[b]	%
Top	27	27	100
Middle	32	9	28
Base	82	9	11

[a] Average of three replications.

[b] Disease readings were recorded 3 mo after inoculation. Control plants, pruned with disinfested knives, showed no infection.

Dissemination of CBB by means of infested tools must be common, since we obtained a high incidence of infection after cuts were made with infested machetes. This method of dissemination is probably most important during harvesting and propagation, because these operations require extensive cutting. Disinfestation of machetes is an obvious control measure that could be applied (17).

Insects have been suggested as possible agents for dissemination of CBB (1), but no evidence has been presented to support this suggestion. If insects do transmit the pathogen in Colombia, they must not be able to move for long distances, because no dissemination occurred to plants located farther than 10 m from an inoculum source. It is unfortunate that

TABLE 3. Disease severity indices of 21 cassava cultivars 30 days after spray- and stem-inoculation with CBB isolate 4.26L

Cassava cultivar	Disease indices				Total index	Number of leaf spots/leaf	General evaluation
	Die-back	Wilting	Gum exudation	Leaf spot			
M. Col. 282	15	15	15	20	65	4.0	SS[a]
M.Col. 350	11	10	10	17	48	6.6	MS
M.Col. 353	8	8	10	15	41	10.1	MS
M.Col. 558	10	11	10	20	52	3.8	MS
M.Col. 642	0	6	0	15	21	3.1	RR
M.Col. 647	0	5	0	5	10	20.2	HR
M.Col. 667	5	5	0	5	15	11.0	HR
M.Col. 707	17	15	15	25	72	5.0	SS
M.Col. 800	10	13	10	20	53	5.2	MS
M.Col. 803	15	17	15	20	67	4.0	SS
M.Col. 808	0	7	5	15	27	4.4	RR
M.Col. 853	13	15	10	20	58	5.8	MS
M.Col. 866	11	10	15	20	56	8.5	MS
M.Col. 952	10	10	10	25	55	6.4	MS
M.Col. 1060	10	11	10	20	51	5.4	MS
M.Col. 1073	5	8	5	15	33	2.4	RR
M.Col. 1079	5	7	5	15	32	4.2	RR
M.Col. 1080	6	10	10	15	41	5.6	MS
M.Col. 1137	10	10	10	20	50	5.8	MS
M.Col. 1155	5	9	5	20	39	3.0	RR
M.Col. 1184	6	5	5	20	36	8.4	RR
Popayan (CK)	25	25	25	25	100	139.3	VS

[a] VS = very susceptible, SS = susceptible, MS = moderately susceptible, RR = resistant, HR = highly resistant.

data on the types of insects that visit cassava plantings is lacking.

Control of bacterial diseases by removal of infected plant parts has been attempted previously (2, 10), but has met with varying levels of success. In the case of CBB, severe pruning of most aboveground parts of the plant was very successful. Severe pruning can be performed on cassava plants without apparent serious economic loss, because roots are the most valuable part of the plant and pruned plants generally sprout back vigorously. However, it is possible that severe pruning affects the overall nutritional value, as well as the quality and yield of cassava roots.

Pruning would be most effective if the following conditions are met: (i) the infected cultivar is moderately resistant to the disease; (ii) pruning is carried out within 3 mo after infection first occured; and (iii) pruning is carried out during the dry season to avoid further dissemination of the pathogen by splashing raindrops or infested soil. A practical, large-scale application of pruning as a method of control was successfully carried out at CIAT Experimental Station, Cali, Colombia. Following an epiphytotic at the International Cassava Collection in which 75% of the 2,500 cultivars planted there became infected, pruning was performed in September 1971. Although there was great variability in the susceptibility of cultivars in the collection, the pathogen was practically eradicated by this procedure; 9 mo after pruning only a few infected plants (0.01%) were found.

The cassava bud rooting and indexing method described here could be used to obtain disease-free stocks, either from promising breeding material or as a routine method for propagation of certified propagules of cassava. At present, the grower in Colombia is inevitably forced to use "seed" stock which may carry the bacterium, and this probably constitutes the primary source of inoculum for dissemination of the pathogen, Cassava plantings in many areas in Colombia, which were free of CBB until recently, are now highly infested because of the introduction of infected vegetative material from other locations.

The finding of varietal resistance to CBB under greenhouse conditions confirms previous reports based on field observations in Brazil (4, 7, 8, 9, 15, 16). Most resistant cultivars had a significantly lower number of leaf spots per leaf than the susceptible control. This type of resistance may depend purely on structural features of leaf tissues. In most resistant cultivars, only the younger tissues are invaded even after wounding, suggesting the presence of inhibitory substances that affect growth of CBB in the mature tissues. Cultivars M. Col. 647 and M. Col. 667, on the other hand, exhibited a typical hypersensitive reaction when infected; a reaction similar to that reported in certain pepper cultivars infected by *X. vesicatoria* (5).

The conditions under which resistance to CBB was tested in the greenhouse were highly favorable to the pathogen. For this reason, the resistant cultivars selected can be expected to exhibit a greater degree of resistance in the field. Tests are now under way to

determine the performance of these clones in the field. Resistant cultivars were not from common geographical areas and, because there is very little information on the systematics of the species, it is not possible to determine at present whether these clones are taxonomically related. The commercial value of these clones is also unknown, but, at least, they may constitute a valuable source of resistance for breeding purposes. Resistant cultivars remain our best hope for a practical, long-range control of the disease.

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