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Epiphytic phase of *Xanthomonas campestris* pathovar *manihotis* on aerial parts of cassava

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SUMMARY

Study of the bacterial microflora of the phyllosphere of cassava plants growing in fields previously infected with cassava bacterial blight demonstrated the epiphytic activity of *Xanthomonas campestris* pv. *manihotis*. An epiphytic phase was found to be a normal part of the disease cycle of cassava bacterial blight. In the rainy season, when disease spread occurs, large numbers of the pathogen were present on symptomless leaves, constituting a potential inoculum which could explain the sudden outbreaks and rapid spread of the disease. In the dry season the numbers of the pathogen decreased to undetectable levels, but the presence of *X. campestris* pv. *manihotis* a few weeks before the first new symptoms at the beginning of the next rainy season suggested that the pathogen could survive as an epiphyte throughout the dry season. The role of the endophytic population in the first step of the epiphytic life of the pathogen was also investigated. The capacity of *X. manihotis* to have an epiphytic phase in its disease cycle contributes to the buildup of primary inoculum and to the establishment of the pathogen in the field, and enhances its survival.

Additional key words : *Epiphytic survival, populations dynamics, Congo.*

RÉSUMÉ

Phase épiphyte de Xanthomonas campestris *pathovar* manihotis *sur les parties aériennes du manioc.*

L'étude de la microflore bactérienne de la phyllosphère de plants de manioc cultivés dans des plantations où le dépérissement bactérien est présent nous permet de mettre en évidence la capacité de *Xanthomonas campestris* pathovar *manihotis* d'avoir une vie épiphyte. Cette phase épiphyte est une composante normale du cycle de la maladie.

Pendant la saison des pluies, quand la maladie s'exprime, le pathogène se trouve en quantité importante sur les feuilles sans symptômes, constituant un inoculum qui peut expliquer l'apparition soudaine de la maladie et sa rapide expansion. En saison sèche, le nombre de bactéries pathogènes diminue progressivement jusqu'à des niveaux non décelables par les techniques utilisées. Cependant, la détection du pathogène quelques semaines avant l'apparition des premiers symptômes au début de la nouvelle saison des pluies, suggère que la bactérie peut survivre en épiphyte durant la saison sèche. Le rôle de la population endophyte au cours de la première étape de la vie épiphyte du pathogène est aussi analysé.

La phase épiphyte de *X. campestris* pathovar *manihotis* au cours de son cycle infectieux contribue à la constitution d'un inoculum primaire, à l'installation du pathogène dans le champ et favorise sa survie.

Mots clés additionnels : *Survie épiphyte, dynamique des populations, Congo.*

I. INTRODUCTION

Cassava (*Manihot esculenta* Crantz), a tropical perennial shrub which is one of the major food sources in the People's Republic of the Congo, can be affected by a severe bacterial disease caused by a white *Xanthomonas, Xanthomonas campestris* pv. *manihotis* (Arthaud-Berthet) Starr. In the Congo, where this crop has a growing cycle varying from 10 to 18 months (according to cultivar and growing zone), the symptoms (angular leaf spots, leaf blight) appear after the first rains. They continue throughout the rainy season (from October to April), with leaf wilting, defoliation, tip die-back and, in the case of susceptible cultivars, death of the plant. In the dry season (June to August) the characteristic symptoms of the disease disappear. Only dead stems and dry necrosis reveal the presence of the disease in the fields.

The fact that the disease can suddenly and rapidly cause widespread outbreaks in plantations, raises the problem of the origin of primary inoculum and of its survival. In order to study this aspect of the disease cycle of cassava bacterial blight, the presence of the pathogen on the aerial parts and in the tissues of

cassava plants has been examined. Quantitative variation in the level of *X. c.* pv. *manihotis* populations on cassava leaves was also investigated.

This study was carried out in the People's Republic of the Congo during 1979 and 1980.

II. MATERIALS AND METHODS

Farm plantations distributed in the southern part of the Congo and two collections of cassava cultivars (M'Bé : Bateke region ; Kombé : Pool region) were used for the analysis of the microflora of cassava leaves in different locations. The level of the populations of *X. c.* pv. *manihotis* on the aerial organs of the cassava plant and the quantitative variation in the number of pathogenic bacteria on leaves were monitored in the plantations of Kombé where the rainfall data were collected.

The number of bacteria on macroscopically healthy apices, first expanded leaves, mature leaves, flowers and fruits was monitored on susceptible cultivar 'Maloenda' in the rainy season. A sample of 10 apices, 10 first expanded leaves, 10 mature leaves, 100 flowers and 50 fruits selected at random were taken from 10 cassava plants and washed according to the method of CROSSE (1959) modified by LUISETTI & PAULIN (1972). Each sample was placed in an Erlenmeyer flask with 500 ml of deionised water and shaken for 4 h at 5 °C. The initial suspension and each dilution (10^{-1}, 10^{-2}, ..., 10^{-5}) were spread on Y.P.D.A. medium (yeast extract 5 g, Difco peptone 5 g, dextrose 5 g, agar 15 g, H_2O 1 000 ml, pH 7.2) with cycloheximide (50 mg/l) (two replications with five Petri dishes per dilution). The initial suspension was also infiltrated with a hypodermic syringe in young leaves of susceptible cultivar 'M'Pembé' for production of angular leaf spots (10 infiltrated spots per leaf).

The variation in the number of epiphytic bacteria on healthy mature leaves of susceptible 'M'Pembé' cultivar was monitored for each year's study at weekly intervals in two diseased fields (6 and 12 months old) as well as in one field (6 months old) which had never shown any symptoms of bacterial disease in the growing area of Kombé. In each field, a sample of 20 leaves was selected at random from the middle of the foliage region of five plants. Each sample was ground in 750 ml of deionised water for one minute (DANIEL *et al.*, 1978). As described above, aliquots (0.05 ml) were distributed on Y.P.D.A. medium. The dilution 10^{-1} was checked by the leaf infiltration method and indirect immunofluorescence technique.

To detect the presence of the pathogen in host tissues (stem, leaf scars, cankers), excised portions of pieces of diseased tissues were crushed in sterile water and allowed to stand 15 mn. The resulting suspension was streaked on Y.P.D.A. medium.

Colony counts and examinations were made after 72 h of incubation at 30 °C. Recognition of bacteria on plates was based on cultural characteristics ; however for each experiment representative cultures of each colony type were checked by indirect immunofluorescence technique with a specific serum and tested for pathogenicity by the leaf-infiltration method.

III. RESULTS

Analysis of the phyllosphere microflora of the cassava plant proved that the pathogenic bacteria *X. c.* pv. *manihotis* could be found on the symptomless aerial organs (apices, first expanded leaves, mature leaves, flowers, fruits) of susceptible cultivar 'Maloenda' growing in infested fields (table 1). *X. c.* pv. *manihotis* occurred on apparently healthy leaves of both susceptible and resistant cultivars growing in different climatic locations (table 2). In all cases, the epiphytic population of *X. c.* pv. *manihotis* was detected on symptomless leaves of cassava plants growing in diseased fields.

In spite of important quantitative variations, the 2 years' study (1979, fig. 1 ; 1980 ; fig. 2) of the levels of the bacterial population on cultivar 'M'Pembé' showed that in January-April (rainy season) the numbers of epiphytic *X. c.* pv. *manihotis* were high and fluctuated between 10^6 and 10^7 bacteria/leaf. Then, with the start of the dry season in mid May, the number of bacteria gradually fell to a low level (10^3-10^4 bacteria/leaf) and subsequently decreased to undetectable levels by the methods used (plating method and leaf infiltration technique). The respective

TABLE 1

Populations of Xanthomonas campestris *pv.* manihotis *on macroscopically healthy organs of cultivar 'Maloenda' in a diseased field.*

Population de Xanthomonas campestris *pv.* manihotis *sur des organes macroscopiquement sains du cultivar « Maloenda » dans une plantation contaminée.*

Organs	X. c. pv. manihotis*
Apices	1.7×10^3
First expanded leaves	1.5×10^5
Mature leaves	5.6×10^6
Flowers	1.2×10^4
Fruits	1.6×10^6

* Number of bacteria per organ.

TABLE 2

Levels of epiphytic populations of Xanthomonas campestris *pv.* manihotis *on resistant and susceptible cultivars in diseased plantations at different locations.*

Niveaux des populations épiphytes de Xanthomonas campestris *pv.* manihotis *sur des cultivars résistants et sensibles dans des plantations contaminées situées dans différentes zones.*

Location	Cultivar	Resistance	*X. c.* pv. *manihotis**	Other bacteria*
Kombé	MA 547	Resistant	2.9×10^5	3.6×10^7
	MA 109	Resistant	3.0×10^2	2.9×10^5
	MA 514	Susceptible	2.2×10^5	1.7×10^5
	M'Pembé	Susceptible	1.0×10^7	1.0×10^6
M'Bé	Ondzion	Susceptible	3.3×10^6	1.0×10^5
	Nkoh	Susceptible	4.5×10^6	1.3×10^6
	N 10	Resistant	1.1×10^6	9.0×10^5
	N 13	Resistant	4.8×10^6	1.8×10^6
Mindouli	Mouyondzi	Susceptible	3.3×10^4	2.2×10^5
Loutété	Mixed	Susceptible	5.9×10^4	1.4×10^5
Loudima	Mixed	Susceptible	4.1×10^6	1.6×10^7

* Number of bacteria per leaf.

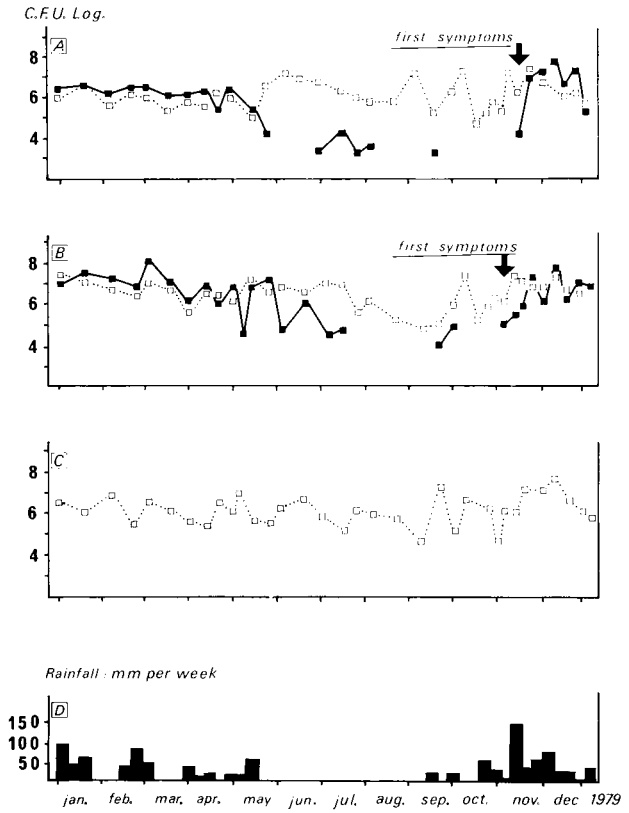

Figure 1

Population levels of epiphytic bacteria isolated from leaves of cultivar 'M'Pembé' and rainfall data for 1979.

Niveaux des populations épiphytes isolées de feuilles du cultivar « M'Pembé » et niveaux hebdomadaires des précipitations pendant l'année 1979.

■ *Epiphytic population of* Xanthomonas campestris *pv. manihotis.*

□ *Other epiphytic bacteria.*

A : diseased field 1

B : diseased field 2

C : healthy field 3

D : rainfall data.

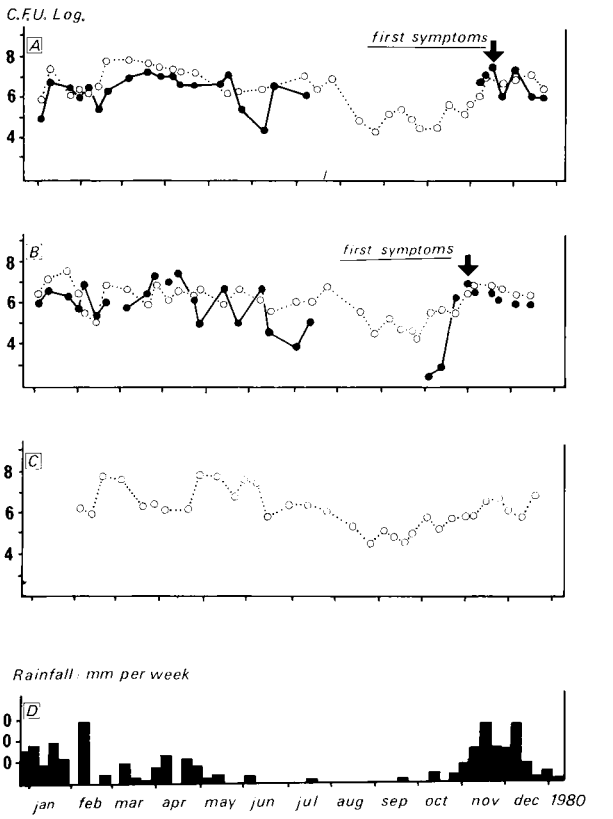

Figure 2

Population levels of epiphytic bacteria isolated from leaves of cultivar 'M'Pembé' and rainfall data for 1980.

Niveaux des populations épiphytes de bactéries isolées de feuilles du cultivar « M'Pembé » et niveaux hebdomadaires des précipitations pendant l'année 1980.

■ *Epiphytic population of* Xanthomonas campestris *pv. manihotis.*

□ *Other epiphytic bacteria.*

A : diseased field 4

B : diseased field 5

C : healthy field 6

D : rainfall data.

threshold of these methods was 10^2-10^3 bacteria/leaf (according to the level of the population of saprophytic bacteria) and about 10^4 bacteria per leaf (number of cells necessary to induce the watersoaked lesions by infiltration of leaves of 'M'Pembé' cultivar). Thus in 1979 (fig. 1), the pathogen was present until the end of July (field A) and mid July (field B), i.e. 3 months after the last rain, whereas in 1980 (fig. 2, field A and B), the epiphytic population was undetectable in early July (last rain : 9 July). It was found again in 1979, after the first rain in September, i.e. 45 days before symptom appearance (fig. 1, field A and B). However, we noticed that one week (field A), and two weeks later (field B), the levels of population of *X. c.* pv. *manihotis* fell again to undetectable levels until the appearance of the symptoms (31 October). Subsequently, the level varied between 10^5-10^6 bacteria/leaf. In 1980, the pathogen was monitored later (fig. 2, field B : 5 October ; field A : 5 November) and was present at low levels (10^3-10^4 bacteria/leaf) three weeks before the first symptoms, and as in 1979, the numbers increased rapidly to a maximum between 10^5-10^6 bacteria/leaf.

On the basis of these results, the highest level of the epiphytic population of *X. c.* pv. *manihotis* occurred during the rainy season, after it had decreased drastically to an undetectable level at the end of the dry season (August, September). Regarding the other bacterial components of the microflora during the same period, they reached in the rainy season about the same levels as those noticed for *X. c.* pv. *manihotis* (10^5 to 10^6 bacteria/leaf). At the beginning of the dry season in May-June, a progressive reduction in the number of bacteria was also observed. This decrease in population was less striking than in the case of the pathogen, and finally stabilized at 10^4-10^5 cells/leaf, which was higher than that of the pathogen. When the new rainy season began, the saprophytic population increased to reach a maximum at about the same level as that of the pathogen.

In the healthy fields, the levels and variations in the population of epiphytic bacteria were similar to those noticed in diseased fields (fig. 1, field C ; fig. 2, field C). In the dry season we also noticed a slight reduction in the number of bacteria but it remained fairly high (about 10^5 bacteria/leaf). Throughout the

growing cycle of cassava, none of our experiments revealed the presence of *X. c.* pv. *manihotis* in the epiphytic microflora of healthy fields.

The population level of the bacterial microflora (both pathogen and saprophytic did not seem to be influenced by age of the plants (6 and 12 months).

Throughout the dry season, isolations made on diseased stems have shown the presence of the pathogen in the vascular tissues, in the leaf scars, in the dry cankers and in the dried up part of stems (table 3).

TABLE 3

Detection of Xanthomonas campestris *pv.* manihotis *in cassava tissues throughout the dry season.*

Détection de Xanthomonas campestris *pv.* manihotis *dans les tissus de plantes de manioc pendant la saison sèche.*

		Number of isolations	Positive isolations
Stems	Living part	170	113
	Dried up part	70	20
Leaf scars		90	25
Cankers		55	12

IV. DISCUSSION

Study of the phyllosphere microflora of susceptible and resistant cassava plants growing in diseased fields showed that *X. c.* pv. *manihotis* can live as an epiphyte on aerial parts of cassava plants (apices, first expanded leaves, mature leaves, flowers, fruits). This confirmed our preliminary results (DANIEL & BOHER, 1978) and those of PERSLEY (1978). The absence of *X. c.* pv. *manihotis* in fields which were free from disease demonstrates that this bacterium is not a normal component of the epiphytic microflora of the phyllosphere of cassava. From an epidemiological standpoint, we should not speak of the epiphytic life of the pathogen (as defined by LEBEN, 1965) but of an 'epiphytic phase'. This epiphytic activity connected with a parasitic phase is a normal part of the disease cycle of cassava bacterial blight. The fact that the epiphytic population of *X. c.* pv. *manihotis* was detected on resistant cultivars indicates that, for the cultivars tested (table 2) resistance was not related to the inhibition of epiphytic activity of *X. c.* pv. *manihotis.*

Our data suggest that epiphytic activity is sporadic, since, during the last part of the dry season, we could not detect the pathogen. However, taking the sensitivity of methods used into account, the pathogen is likely to be present at the end of the dry season below detectable levels. This would explain some temporary disappearance of the pathogen and the origin of the epiphytic population of *X. c.* pv. *manihotis* detected a few weeks before evidence of the first symptoms.

In the rainy season the parasitic and epiphytic phase occurred simultaneously. A few weeks after detection of the epiphytic population, the lesions appeared and produced inoculum, which, taking the epiphytic growth into account, explains the high level of the epiphytic population throughout that period. In the dry season, when no developing lesions were found, the pathogen managed to survive in low number as an epiphyte on foliage and/or in the host tissues.

The epiphytic phase of *X. c.* pv. *manihotis* has important implications in the epidemiology of the disease by serving to build up an inoculum on aerial parts and by enhancing the survival of the pathogen. The incidence of climatic factors on the general growth of epiphytic bacterial populations (CROSSE, 1963 ; LEBEN, 1965 ; LUISETTI & PAULIN, 1972) suggests the importance of ecological interactions on the severity of cassava bacterial blight. Epiphytic growth of bacterial pathogens is generally favoured by high humidity and warm temperature (LEBEN, 1965). These conditions that prevail in the rainy season contribute to build up an inoculum on the foliage and the rainfall provides the conditions necessary for its dispersal by windsplash aerosols from plant to plant (LOZANO & SEQUEIRA, 1974*b* ; BROWN, 1942 ; WALKER & PATEL, 1964). This phenomenon favours establishment of the pathogen in the field, increasing the opportunity for infection and explaining the sudden widespread out-breaks of bacterial blight following rain in fields where no symptoms were obvious.

The presence of the pathogen in host tissues throughout the dry season indicates that this endophytic population may also contribute to the disease cycle. In previously diseased fields at the beginning of the new growing season, before the manifestation of angular leaf spots and blight, we detected, on young unlignified sprouts developed at the junction of dead and healthy woody stems, micro-lesions where bacterial exudates could ooze out. Similar observations were made in young plantations (1 to 2 months old) grown from infected stem cuttings. This supports the suggestion that infected host tissues constitute a reservoir of bacteria, which may be disseminated from their survival site to the aerial parts of the cassava plant where epiphytic multiplication occurs. The mechanism of movement of bacteria from the cutting to the sprouts is unknown and is an area that merits investigation.

The common occurrence of *X. c.* pv. *manihotis* in stem cuttings (LOZANO & SEQUEIRA, 1974*b*) suggests that infected cuttings are probably the most important source of inoculum in new plantations. In previously diseased fields, the epiphytic population of the pathogen already present on aerial parts and/or the endophytic population found in diseased parts of the plant constitutes the source of inoculum necessary for new infections at the beginning of the rainy season. The role of other potential sources of inoculum (debris, seed, soil, insects, weeds) in association with the epiphytic phase of *X. c.* pv. *manihotis* remains to be investigated.

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