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Epiphytic Survival of *Xanthomonas manihotis* on Common Weeds in Colombia

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Abstract

Many common weeds are implicated in the epiphytic survival of *Xanthomonas manihotis*, the cassava bacterial blight pathogen, during the dry season in Colombia. Studies using direct immunofluorescence and ELISA techniques established pathogen survival in or on most weeds at below 10^4 cells/leaf disc, approximately. Studies on weed control in CBB-endemic and non-endemic areas are suggested.

Introduction

Cassava bacterial blight (CBB), caused by *Xanthomonas manihotis*, remains one of the most destructive diseases of cassava (11, 12). In CBB endemic areas, cassava plants rapidly become infected under favorable conditions. For this reason, many attempts have been made to determine the means by which *Xanthomonas manihotis* survives from one season to another (3, 9, 12, 14, 15). Methods for CBB control include the use of disease-free and resistant planting materials (11) and removal, burning, and burial of infected material (9). These methods are considered effective because *X. manihotis* is known to attack only plants belonging to the *Euphorbiaceae*. However, although weeds are a common problem in most cassava fields, their importance as a potential source of inoculum, or as an epiphytic host during the dry season, has not been considered. This paper describes the potential of some common weeds on the survival of the pathogen during the dry season in Colombia.

Materials and Methods

Sample Collection

Two sampling areas were selected, one in Carimagua (in the eastern plains of Colombia) and the other at Media Luna (north coast of Colombia), two widely different ecosystems in which CBB epiphytotics occur. Selected common weeds (Table 1) were identified and samples of them were taken

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Table 1. Weed species[a] used in ecological studies on *Xanthomonas manihotis*.

Species	Family	Common name	Other classification[b]
Solanum nigrum americanum	Solanaceae	Hierba mora	N,D,C.
Sida dictyocarpa	Malvaceae	Escoba	N,D,C.
Emilia sagittata	Compositae	Oreja de alce	B,D,C.
Hyptis mutabilis	Labiatae	Botoncillo mastranto	N,M,C.
Amaranthus dubius	Amaranthaceae	Bledo	B,D,C.
Amaranthus espinosus	Amaranthaceae	?	B,D,C.
Melothria sp.	Cucurbitaceae	Pepinillo	B,D,C.
Euphorbia hirta	Euphorbiaceae	Pimpinela	N,D,C.
Conyza canadiense	Compositae	Venadillo	B,D,C.
Centrosema macrocarpum	Labaceae	Centrosema	B,D,ML
Boerhaavia erecta	Nytaginaceae	rodilla de pollo	B,D,ML
Eleucina indica	Gramineae		N,M,C
Digitaria sanguinalis	Gramineae		N,M,C
Cyperus rotundus	Gramineae		N,M,C

a Weeds were identified by E. Escobar, Taxonomist, Universidad Nacional, Facultad de Agronomía, Palmira, Colombia.

b N=Narrow leaved; M=Monocot. weed; C=From Carimagua; D=Dicot. weed; B=Broad leaved; ML=From Media Luna.

once monthly at each location from January to April, periods corresponding with the driest part of the year when CBB symptoms disappear on cassava plants.

Pathogen Detection

Leaf comminution was used for detecting the bacterium. For each sampling, weeds were collected along two diagonals of a cassava field, and each weed was separately put in a plastic bag and used within 24 h. Ten leaf discs/weed obtained along the diagonal of the lamina were removed with a 7 mm diameter flamed cork borer and each sample was comminuted in 5 ml of sterile distilled water. A smear (10 nl) of each crude weed extract and its Kelman's enriched broth (4) was stained with an *X. manihotis* antibody conjugated with fluorescein isothiocyanate (FITC), previously prepared (1, 2). Slides were observed under an U. V. microscope at 1000 magnification. Similarly, crude and enriched weed and cassava leaf extracts were subjected to the enzyme-linked immunosorbent assay technique (6); the enzyme used was horse-radish peroxidase. Results were visually scored as reported previously (4). Another sample of the crude and enriched extract of each weed and control (cassava leaf) was plated on Kelman's tetrazolium chloride agar (10) in order to isolate the pathogen. Verification of the identity of *X. manihotis* was made morphologically and symptomatologically. To relate numbers of cells/microscope field in each weed extract to numbers of cells/ml, a 48 h growth of a pure culture of the pathogen was serially diluted and stained with the fluorescent conjugate. The average number of bright fluorescent cells/10 microscope fields per dilution at 1000 magnification was determined.

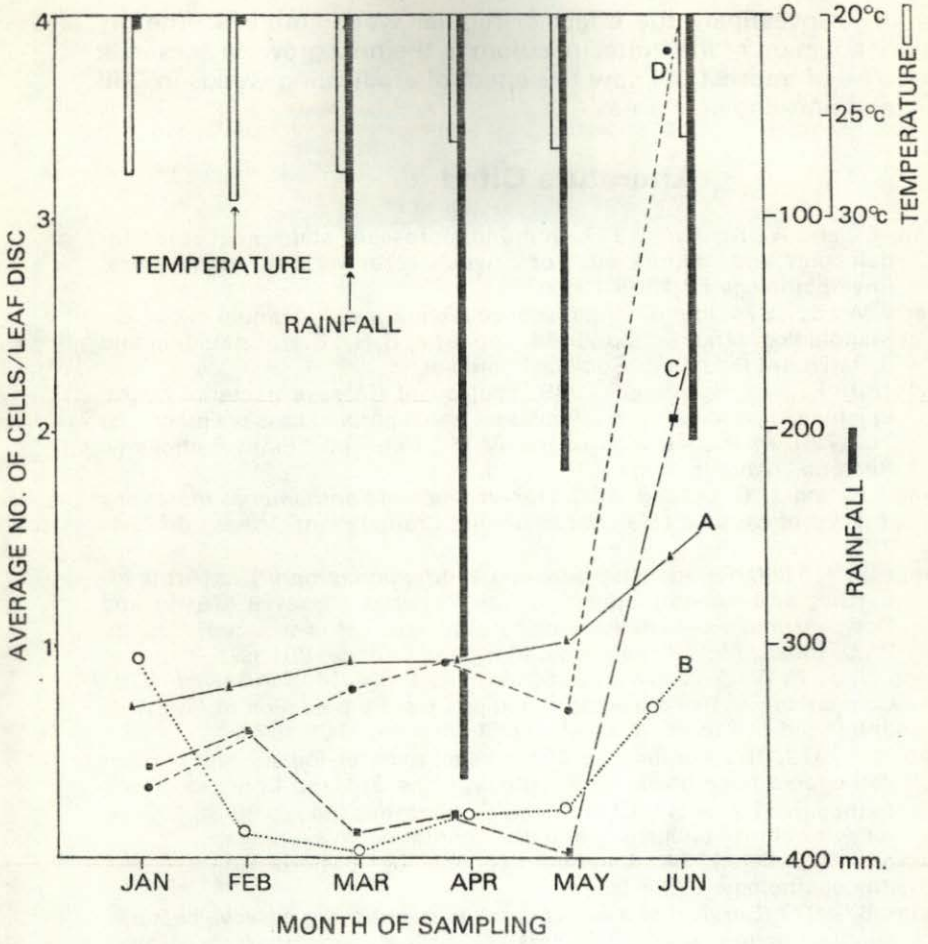
Results and Discussion

When tested with a known concentration of *X. manihotis* in suspension, the fluorescent antibody technique detected with certainty cell concentrations of 10^5 cells/ml whereas the ELISA technique detected 10^4 cells/ml. During most of the period under study, broad-leaved (or mostly dicotyledonous) weeds carried the bacterium at concentrations probably below 10^4 cells/leaf disc, as on symptomless cassava leaves, possibly because they remained more succulent. The population of the pathogen on or in weeds remained steady throughout the sampling period, and this made detection of the pathogen in crude extracts difficult. However, Kelman's broth enrichment of crude extracts plus the immunofluorescence or ELISA technique (4) made pathogen detection easier. The two techniques were reliable in showing whether crude extracts carried the pathogen (Table 2). With the advent of the rainy season, in March, the pathogen population increased to 10^5 cells/leaf disc on symptomless cassava leaves but remained below this level on most weeds sampled (Fig. 1). Differences in cell counts of *X. manihotis* surviving on different weeds before and after enrichment were presumably an indirect reflection of population trends in/on the weed samples, assuming that comminution of leaf discs did not promote or inhibit bacterial growth. High cell counts were most often detected on *Amaranthus dubius* while graminaceous weeds rarely carried the pathogen.

Table 2. Effect of enrichment technique on the detection of *X. manihotis* on weeds.

Weed species	Unenriched leaf extract		Enriched leaf extract	
	I.F.[a]	ELISA	I.F.	ELISA
S. nigrum americanum	±[b]	–	+	+
S. dictyocarpa	–	–	–	+
E. sagittata	+	–	+	+
H. mutabilis	+	+	+	+
A. dubius	+	+	++	++
Melothria sp.	±	+	+	+
E. hirta	–	–	–	–
C. canadiense	–	–	+	+
C. macrocarpum	–	–	–	–
C. nitida	–	–	–	–
Cissampelos sp.	±	–	+	+
E. indica	–	–	–	–
D. sanguinalis	–	–	–	–
C. rotundus	–	–	±	–

[a] IF = immunofluorescence test; ELISA = enzyme-linked immunosorbent assay.
[b] ++ = strongly positive reaction; + = positive reaction; ± = weakly positive – = negative reaction.



A = *AMARANTHUS DUBIUS*
B = *CONYZA CANADIENSE*
C = *EMILIA SAGITTATA*
D = *MANIHOT ESCULENTA* (M Col 22, CONTROL)

Fig. 1. Population dynamics of *Xanthomonas manihotis* on three weeds during the dry season.

The present findings confirm the results of previous workers that *X. manihotis* survives epiphycally on cassava leaves (3, 14). For the first time, weeds were implicated as an epiphytic survival base. In the epidemiology of foliar pathogens, survival of a few cells on vegetation and on symptomless plants may have a far-reaching significance (7, 8). Many studies have established that only one bacterial cell is required to initiate an infection (13, 16). In a situation where all host materials are eliminated, this study shows that weeds may provide a base for inoculum survival. Since weeds are so plentiful in cassava fields and survive remarkably well throughout the driest period when host materials are not available, it would be

interesting to investigate the effect of regular weed control in the dry season on reduction of the initial inoculum in the next growing season. It might also be of interest to know the effect of eradicating weeds in CBB endemic and non-endemic areas.

Literature Cited

1. Allan, E. and A. Kelman. 1977. Immunofluorescent stain procedures for detection and identification of *Erwinia carotovora* var. *atroseptica*. Phytopathology 67:1305-1312.
2. Cherry, W. B. 1974. Immunofluorescence techniques. *In* Manual of Clinical Microbiology 2nd. Ed. pp 29-44. Lennette, E.H., E.H. Spaulding and J.P. Truant (Eds.) Am. Soc. for Microbiol.
3. Daniel, J. F. and B. Boher. 1979. Ecology of Cassava bacterial blight: Epiphytic survival of *X. manihotis* on aerial parts of cassava plants. pp 762-770. *In* Proceedings of the IV Int. Conf. on "Plant Pathogenic Bacteria," Angers, France.
4. Elango, F. and J. C. Lozano 1980. Transmission of *Xanthomonas manihotis* in seed of cassava (*Manihot esculenta* Crantz) Plant Disease 64:784-786.
5. Elango, F.N. 1980. Relationship between *Xanthomonas manihotis* (Arthaud-Berthet and Bondar) Starr and *Xanthomonas cassavae* (Wiehe and Dowson), and some epidemiological studies on cassava Bacterial Blight. Ph.D. thesis, McGill University, Montreal, Canada. 301 pp.
6. Ellens, D. J., P. W. de Leeuw, P. J. Straver and J. AM. M. Van Balken 1978. Comparison of five diagnostic methods for the detection of Rotavirus antigen in calf feces. Med. Microbiol. Immunol. 166:157-163.
7. Goto, M. 1972. The significance of the vegetation for the survival of plant pathogenic bacteria. *In* Proceedings of the 3rd. Int. Conf. on "Plant Pathogenic Bacteria," 1971. Maas-Geesteranus (Ed.) pp 39-53. Centre for Agricultural publishing and documentation, Wageningen.
8. Hayward, A. C. 1974. Latent infections by bacteria. Annu. Rev. Phytopathology. 12:87-97.
9. Ikotun, B. 1977. Survival of *Xanthomonas manihotis,* the cassava bacterial blight pathogen. pp 24-27. *In* Persley, G., E. R. Terry and R. Mac Intyre (Eds.) Cassava bacterial blight-report of an interdisciplinary workshop, IITA, Ibadan, IDRC 096e.
10. Kelman, A. 1954. The relationship of pathogenicity in *Pseudomonas solanacearum* to colony appearance on a tetrazolium medium. Phytopathology 44:693-695.
11. Lozano, J. C. and R. Booth 1974. Diseases of cassava (*Manihot esculenta* Crantz). PANS 20:30-54.
12. Lozano, J. C. and L. Sequeira 1974. Bacterial blight of cassava in Colombia. Epidemiology and control. Phytopathology 64:83-88.
13. Perembelon, M.C.M. 1972. A quantitative method of assessing virulence of *Erwinia carotovora* and *Erwinia carotovora* var. *atroseptica* and susceptibility to rotting of potato tuber tissue. pp 299-303. *In* Maas-Geesteranus (Ed.). Proc. of the 3rd. Int. Conf. on "Plant Pathogenic Bacteria," 1971. Centre for Agricultural publishing and documentation, Wageningen.
14. Persley, G. J. 1979. Epiphytic survival of *Xanthomonas manihotis* in relation to the disease cycle of cassava bacterial blight. pp 772-776. *In* Proceedings of the IV Int. Conf. on "Plant Pathogenic Bacteria," Angers, France.

15. Terry, E. R. 1974. A mode of survival of *Xanthomonas manihotis,* the cassava bacterial blight pathogen. Nigerian Soc. of Plant Protection. Occasional publication No. 1:19 (Abstr.).
16. Thyr, B. D. 1968. Bacterial Canker of tomato: inoculum level needed for infection. Plant Disease 52:741-743.