Population Dynamics of *Xanthomonas albilineans* in Sugarcane Plants as Determined with an Antibiotic-Resistant Mutant

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ABSTRACT

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Characteristics of a spontaneous mutant of *Xanthomonas albilineans* (GPE 5SR) resistant to streptomycin and rifampicin were not different from those of the wild parental strain (GPE 5) in cultural, biochemical, and immunological properties. The mutant strain was at least as virulent as the parental wild strain and was used as an epidemiological tool for the study of the population dynamics of *X. albilineans*. Populations of *X. albilineans* GPE 5SR and symptom expression of leaf scald were monitored in six sugarcane clones differing in resistance to the disease. The pathogen invaded leaves, stalks, and roots. The apex of the stalk including the apical meristem was invaded. Symptom expression and population size in leaves and shoot apices were correlated under field conditions. In contrast, the pathogen populations in the older portion of stalks increased initially after inoculation and then remained stable for both studied clones, whatever the progress of the disease. Sugarcane inoculated with *X. albilineans* and maintained under greenhouse conditions exhibited few or no symptoms but was colonized by the pathogen. Pathogen populations were highest in susceptible clones. Thus, high population densities of *X. albilineans* in sugarcane are necessary but not always associated with development of disease symptoms. Nevertheless, the correlation between colonization of plants and leaf scald resistance may be useful for screening sugarcane clones for resistance to the disease.

Leaf scald, caused by *Xanthomonas albilineans* (Ashby) Dowson, is one of the most important diseases of sugarcane (interspecific hybrids of *Saccharum*). The disease exists in numerous sugarcane production areas in Africa, Asia, Australia, North and South America, and the Caribbean region (23,25). It has recently increased at alarming rates in the Dominican Republic (J. O. Despradel, *personal communication*), Florida (7), Guadeloupe (28), and Mauritius (2). The reasons for these recent outbreaks are not yet known. Leaf scald is characterized by a latency phase (17,18,22,23). Numerous stalks can be infected with the pathogen for a long time without exhibiting any symptoms of the disease. The symptoms can develop weeks or even months after infection, and the disease can cause severe yield losses (22,23,30).

Leaf scald is managed with varying degrees of success by quarantine of newly introduced plant material (4,14), visual surveys and elimination of diseased plants in the field, planting of healthy seed cane obtained by tissue culture (28) or hot-water treatment (12,31), and use of resistant sugarcane cultivars (33). Identification and detection of the pathogen are important aspects of control, especially when symptoms are not evident. Knowledge of the dynamics of *X. albilineans* populations within the host is of paramount importance for optimum application of diagnostic tests. Presently, screening sugarcane clones for resistance to the disease is based only on symptoms, but symptom production can be erratic. A negative correlation might exist between *X. albilineans* population size and leaf scald resistance that would be useful in screening procedures. Such a correlation was reported for ratoon stunting disease caused by *Clavibacter xyli* subsp. *xyli*, another vascular bacterium of sugarcane (9,10).

Leaf scald has been studied since the beginning of the century, but the interaction between *Saccharum* spp. and *X. albilineans* is not well understood (23,25). The development of pathogen populations inside and outside the host plant during the latency phase and during the development of symptoms has not yet been studied.

Several methods can be used to monitor bacterial populations in the study of microbial ecology (1). Some methods (serological) do not take into account bacterial viability. Therefore, we chose to use a plate-count procedure with a strain of *X. albilineans* marked by antibiotic resistance. In this study, we compared the cultural, biochemical, immunological, and pathogenic characteristics of a wild strain of *X. albilineans* to those of a spontaneous mutant resistant to streptomycin and rifampicin. The antibiotic-resistant strain was then used to examine the density and dynamics of the pathogen populations in sugarcane clones with different levels of resistance to leaf scald disease, and the associated symptomatology under field and greenhouse conditions.

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MATERIALS AND METHODS

Plant material. Leaf scald resistance of the sugarcane clones used in this study was determined previously by measuring disease severity and yield reduction in naturally infected fields and in yield trials (P. Rott and P. Feldmann, *unpublished*). Six sugarcane clones with different resistance levels were used: B 80 08 and Co 64 15 (resistant = no or very few symptoms with no yield losses), R 570 (tolerant = symptoms possible but no yield reduction), B 69 566 and B 69 379 (susceptible = symptoms frequent with accompanying yield losses), and CP 68 1026 (highly susceptible = severe symptoms very frequent with heavy yield reduction). Cuttings used in the greenhouse study came from a primary nursery planted with cuttings from a mother nursery. Cuttings used to start the mother nursery were hot-water treated to eliminate natural infection by *X. albilineans* (31). The field experiment was planted with cuttings from the first ratoon crop of a nursery derived from healthy tissue-cultured plants. Tissue-cultured plantlets were propagated in vitro and transferred to the greenhouse as described by Feldmann et al (13) and termed vitroplants.

***X. albilineans* cultures.** The wild strain of *X. albilineans* (GPE 5) belongs to serovar 1 (24) and was isolated in Guadeloupe from susceptible clone B 69 379 on modified Wilbrink's medium (26) containing sucrose (10 g), peptone (5 g), yeast extract (5 g), K_2HPO_4 (0.50 g), $MgSO_4 \cdot 7H_2O$ (0.25 g), $Na_2SO_3 \cdot 7H_2O$ (0.05 g), benomyl (12.5 mg), agar (15 g), and distilled water (1 L), pH 6.9–7.0. The spontaneous mutant (GPE 5SR) was obtained by successive plating of strain GPE 5 on modified Wilbrink's medium containing first 50 ppm of streptomycin and afterwards 50 ppm of rifampicin. Once obtained, both cultures were stored in sterile distilled water at −20 C. Sugarcane was inoculated with bacteria grown

on the modified Wilbrink's medium without antibiotics.

Biochemical characteristics. Utilization of 95 different carbon sources was tested with Biolog's GN microplates (Biolog, Inc., 3447 Investment Blvd., Suite 3, Hayward, CA 94545). Inoculum was grown for 2 days on modified Wilbrink's medium. Plates were inoculated with a bacterial suspension of 10^9 cfu/ml in sterile saline (0.85% NaCl). Results were read after 24–48 hr incubation at 28 C. Three independent replications were performed.

Immunological techniques. A polyvalent serum reacting with the three known serovars of *X. albilineans* (24) was used in indirect immunofluorescence and dot-blot assays (16,26). This serum was produced in the plant pathology laboratory of CIRAD-CA in Montpellier, France (26).

Inoculation techniques and experimental designs. Two types of inoculation techniques were used. A decapitation technique, based on the aluminum cap method described by Koike (19), was used for inoculation of the field experiment. Stalks (6 mo old) were cut above the growing point (third dewlap from the top) with pruning shears that had been dipped in a suspension of *X. albilineans* (10^8 cfu/ml). The same inoculum was also sprayed onto the cut end of the remaining stalk. Control stalks were treated with distilled water. The experimental field was planted using a randomized block design. Each plot consisted of three 10.5-m rows of sugarcane (15 plants and about 150 tillers per row). One row was inoculated with *X. albilineans* GPE 5, the center row with distilled water (control), and the remaining row with *X. albilineans* GPE 5SR. Each of three blocks contained two replications of one three-row plot per clone.

Vitroplants were also inoculated by the decapitation technique, with strain GPE 5SR only, 8 wk after being placed into a greenhouse. They were cut approximately 2 cm above ground level, i.e., at the first visible dewlap from the top. The vitroplants were distributed in the greenhouse using a totally randomized design of three replications with 15 plants each.

A cutting inoculation technique was used for the other greenhouse experiment. Single-node cuttings were excised with a knife that had been dipped in a suspension of the strain to be tested (2×10^8 cfu/ml). The cuttings were then soaked in an inoculum of 4×10^7 cfu/ml for 1 hr. Control cuttings were treated similarly but with distilled water. The cuttings were planted in 70 × 70 mm Jiffy Fertil pots containing a mixture of clay soil and peat moss (1:1). To prevent fungal rotting, the cuttings were treated once with 0.25 g/L benomyl (Benlate) when they were watered the first time. The pots were distributed in a greenhouse according to a totally randomized design of three replications of 20 cuttings each.

Disease assessment. Symptoms were recorded and disease severity was calculated according to a modified Australian method (P. W. J. Taylor, *personal communication*). A score ranging from 0 to 5 and corresponding to the severity of symptoms was assigned to each stalk. These data were used to calculate disease severity (*DS*). $DS(\%) = [(1FL + 2ML + 3CB + 4N + 5D)/5T] \times 100$. FL = number of stalks with few pencil-line streaks (one or two, score 1), ML = number of stalks with many pencil-line streaks (more than two per leaf, score 2), CB = number of stalks with leaf chlorosis or bleaching (score 3), N = number of stalks with leaf necrosis (score 4), D = number of dead stalks or stalks with side shooting (score 5), and T = total number of stalks. The percentage of diseased stalks corresponded to the number of stalks with symptoms in relation to the total number of stalks, whatever the severity of symptoms.

Measurement of bacterial population densities. Twenty-five stalks were randomly sampled in all experiments (field and greenhouse). The stalks were separated into five groups of five stalks each, and leaf scald symptoms were recorded before removal of the different sugarcane parts. Bacterial populations were measured in leaves, apices, stalks, and roots. The leaf samples were composed of the middle part of two spindle leaves per stalk. An apex of approximately 1 × 2 cm was obtained from each stalk after removal of the upper external leaves. A single-node section of 5 cm was taken

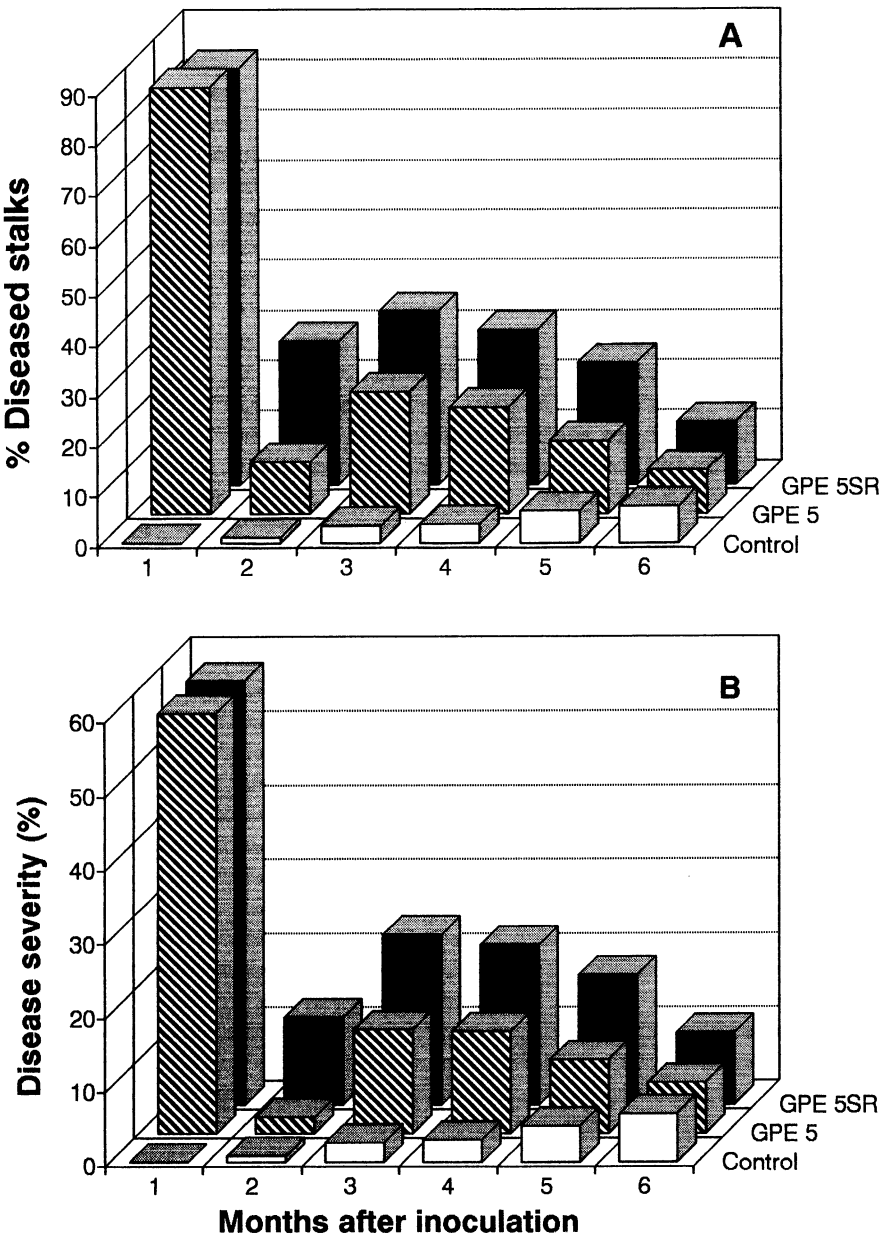

Fig. 1. Disease progress in stalks of susceptible clone B 69 566 inoculated in the field by the decapitation method with *Xanthomonas albilineans* GPE 5 or GPE 5SR. **(A)** % diseased stalks and **(B)** disease severity. Values are the means of six replications of one 10.5-m sugarcane row each.

in the lower third of each stalk. All roots of the primary stalk issued from inoculated cuttings were analyzed. All aerial parts except inoculated leaves were taken from the primary stalk of each vitroplant.

Each pooled sample of leaves, shoot apices, nodal sections, or roots was weighed and then homogenized in 150–400 ml of 20-mM TBS buffer (2.42 g of Tris, 8 g of NaCl, and 1,000 ml of distilled water, pH 7.5) with a blender for 2 × 30 sec. The blended sugarcane extracts were kept at room temperature without agitation for 2 hr and then filtered through a cloth. The recovered tissues were dried for 4 days at 80 C to determine the dry weight.

The filtrate was used in quantification of the bacteria. Serial dilutions of the extracts were plated in triplicate on modified Wilbrink's medium amended with 50 ppm of streptomycin and 50 ppm of rifampicin. Bacterial colonies were counted after a 7-day incubation at 28 C. The filtrate was also sometimes tested for the presence of *X. albilineans* by immunofluorescence and dot-blot.

Apical meristems from stalks of all sugarcane clones in the field and in the greenhouse (cutting inoculation) were randomly sampled. The meristem approximately 0.5 mm in diameter with no visible leaf primordia was excised under aseptic conditions without direct contact with the sterilizing agent. The meristem and the three or four leaf primordia around the meristem were then separately macerated in 0.5–2 ml of sterile distilled water. Bacterial populations were quantified by dilution plating as previously described.

Statistical analysis. Computer programs of Stat-ITCF 4.0 (ITCF Boigneville, 91720 Maisse, France) were used for data analysis. The parametric analysis techniques could not be applied to untransformed data because the requirements for normality of distributions or homogeneity of variances were not always met. Therefore, the values for disease severity and percentage of diseased stalks were transformed to ranks and then examined by analysis of variance according to Conover and Iman (8) and SAS/STAT User's Guide (29).

RESULTS

Comparison of mutant with wild-type parent. *X. albilineans* GPE 5 and GPE 5SR had the same appearance and the same growth rate on modified Wilbrink's medium. Colonies appeared after 4–5 days of growth at 28 C. Both strains formed colonies that were circular with entire margins, convex, shiny, transparent, honey-yellow in color, and not mucoid. Only strain GPE 5SR was able to grow on the culture medium amended with streptomycin and rifampicin. The time necessary for colonies to appear was similar to the one observed on Wilbrink's medium without antibiotics. Both strains had a similar biochemical profile for the 95 carbon sources studied:

- *Carbon sources used:* Dextrin, Tween 40, Tween 80, N-acetyl-D-glucosamine, L-arabinose, cellobiose, D-fructose, L-fucose, D-galactose, gentiobiose, α-D-glucose, maltose, D-mannose, sucrose, methyl pyruvate, mono-methyl succinate, acetic acid, α-keto-glutaric acid, D,L-lactic acid, succinic acid, bromo succinic acid, alaninamide, L-alanyl-glycine, glycyl-L-glutamic acid, L-serine;
- *Carbon sources not used:* α-Cyclodextrin, glycogen, N-acetyl-D-galactosamine, adonitol, D-arabitol, *i*-erythritol, *m*-inositol, α-lactose, lactulose, D-mannitol, D-melibiose, β-methyl glucoside, D-raffinose, L-rhannose, D-sorbitol, turanose, xylitol, *cis*-aconitic acid, citric acid, formic acid, D-galactonic acid lactone, D-galacturonic acid, D-gluconic acid, D-glucosaminic acid, D-glucoronic acid, τ-hydroxy butyric acid, *p*-hydroxy phenylacetic acid, itaconic acid, α-keto butyric acid, α-keto valeric acid, malonic acid, propionic acid, quinic acid, D-saccharic acid, sebacic acid, succinamic acid, glucuronamide, D-alanine, L-asparagine, L-aspartic acid, L-histidine, hydroxy L-proline, L-leucine, L-ornithine, L-phenyl alanine, L-pyro glutamic acid, D-serine, D.L-camitine, τ-amino butyric acid, urocanic acid, inosine, uridine, thymidine, phenyl ethylamine, putrescine, 2-amino ethanol, 2.3-butanediol, glycerol, D.L-α-glycerol phosphate, glucose-1-phosphate;
- *Carbon sources with variable reaction for the same strain:* Psiose, D-trehalose, α-hydroxybutyric acid, β-hydroxybu-

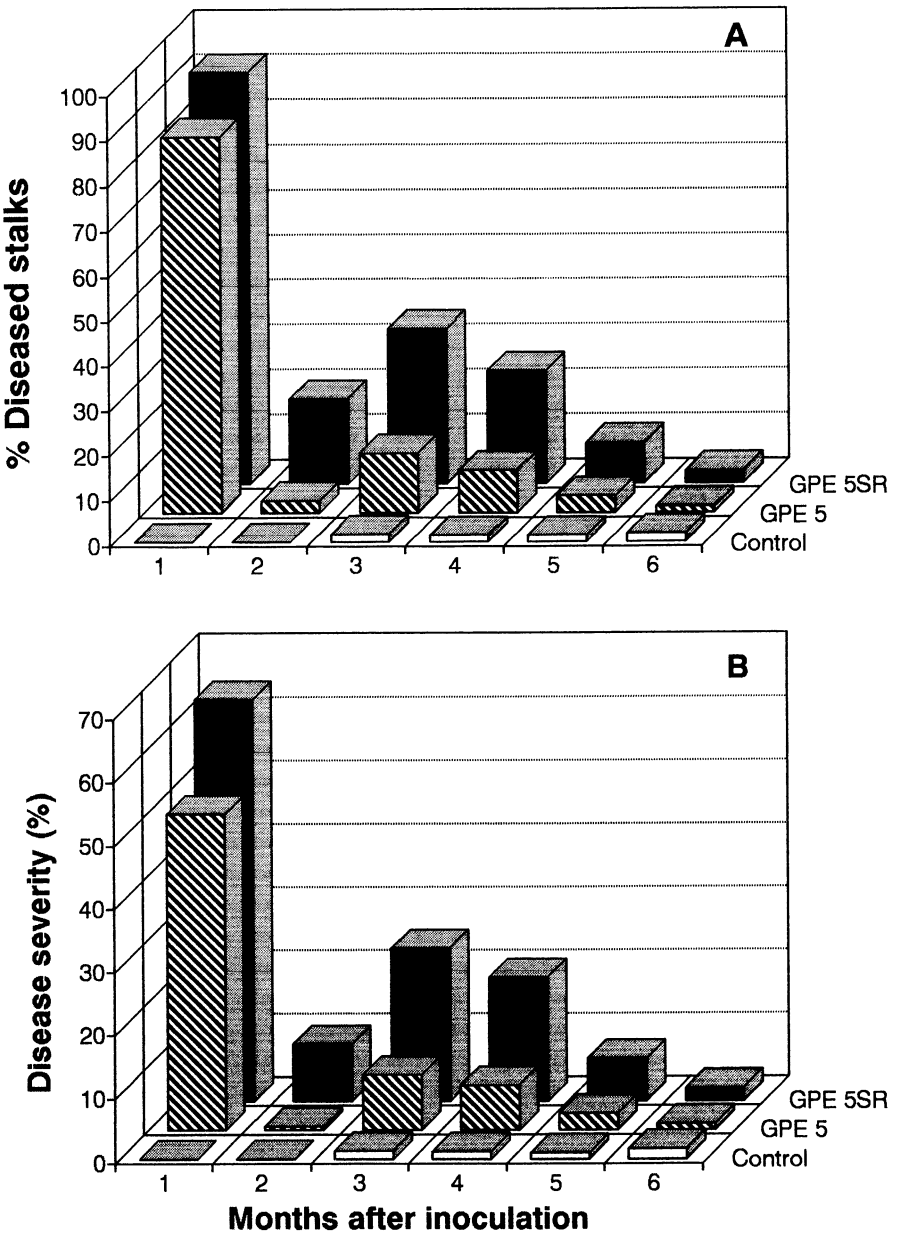

Fig. 2. Disease progress in stalks of tolerant clone R 570 inoculated in the field by the decapitation method with *Xanthomonas albilineans* GPE 5 or GPE 5SR. **(A)** % diseased stalks and **(B)** disease severity. Values are the means of six replications of one 10.5-m sugarcane row each.

tyric acid, L-alanine, L-glutamic acid, glycyl-L-aspartic acid, L-proline, L-threonine, glucose-6-phosphate.

Using immunofluorescence, the titer of the serum (1/6,400) and the mean number of bacteria observed per microscopic field were similar for *X. albilineans* GPE 5 and GPE 5SR. With the dot-blot method, the intensity of the colored reaction was uniform for the serum dilutions between 1/5,000 and 1/640,000, and decreased below 1/1,200,000. The titer of the serum (1/20,000,000) and the threshold for a positive reaction (10^6 cfu/ml) were identical for both strains.

One month after field inoculation by the decapitation method of the tolerant (R 570) and susceptible (B 69 566) clones, the percentage of diseased stalks and disease severity varied, respectively, from 82.9 to 91.4% and from 49.8 to 63.4%. These values decreased greatly 2 mo after inoculation when observations were made on newly developed leaves and not on inoculated leaves which had become senescent (Figs. 1 and 2). The percentage of diseased stalks and disease severity increased between the second and third month after inoculation, whatever the clone and the bacterial strain studied. Thereafter, disease percentage and severity decreased progressively for 3 mo. The plants were then 12 mo old and harvestable. There was no difference between the symptoms caused by *X. albilineans* GPE 5 and GPE 5SR 1 mo and 6 mo after inoculation. At the other dates of disease assessment, the disease percentage and severity attributed to strain GPE 5SR were higher than those for strain GPE 5 for both sugarcane clones (Figs. 1 and 2).

Some control plants of susceptible clone B 69 566 had typical leaf scald symptoms in the field trial (Fig. 1). Their number was lower than the number of diseased inoculated plants. The 14 strains of *X. albilineans* obtained from these diseased control plants were all susceptible to streptomycin and rifampicin, and therefore were different from strain GPE 5SR.

After inoculation of the cuttings by soaking in the greenhouse experiment, the percentage of diseased stalks of the four sugarcane clones varied from 0 (resistant clone B 80 08) to 91.7% (highly susceptible clone CP 68 1026). Disease severity ranged from 0 to 66.3% (Table 1). When considering all clones, the number of diseased plants and disease severity were more pronounced for *X. albilineans* GPE 5SR than for *X. albilineans* GPE 5. This difference between strains was particularly noticed for the tolerant clone (R 570).

Table 1. Percentage of diseased stalks and disease severity ratings for four sugarcane clones 7 wk after inoculation of cuttings with *Xanthomonas albilineans* GPE 5 or GPE 5SR (greenhouse experiment)[x]

Clone	Strain	Diseased stalks (%)	Disease severity (%)
B 80 08	GPE 5	1.7 (5.33) ab	0.3 (5.00) ab
(resistant)	GPE 5SR	0.0 (3.50) a	0.0 (3.50) a
B 69 566	GPE 5	6.7 (8.83) bc	4.0 (10.67) bc
(susceptible)	GPE 5SR	13.3 (13.33) cd	3.7 (11.50) c
R 570	GPE 5	5.0 (9.00) bc	2.0 (9.33) bc
(tolerant)	GPE 5SR	28.3 (17.00) de	16.3 (17.00) d
CP 68 1026	GPE 5	68.3 (20.00) ef	38.3 (20.00) de
(highly susceptible)	GPE 5SR	91.7 (23.00) f	66.3 (23.00) e
Mean		26.9 (12.50)	16.4 (12.50)
Clone effect		**[y]	**
Strain effect		**	*
Interaction		*	(*)
RSE[z]		(2.28)	(2.67)
SEM		(1.32)	(1.54)

[x] Completely randomized design of three replications of 20 plants each. Values in parentheses correspond to the data transformed to ranks. Means in a column followed by the same letter are not significantly different at $P = 0.05$ according to the test of Newmann-Keuls.

[y] ** = Significant at $P = 0.01$; * = significant at $P = 0.05$; (*) = not significant at $P = 0.05$ but significant at $P = 0.06$.

[z] RSE = residual standard error; SEM = standard error of the mean.

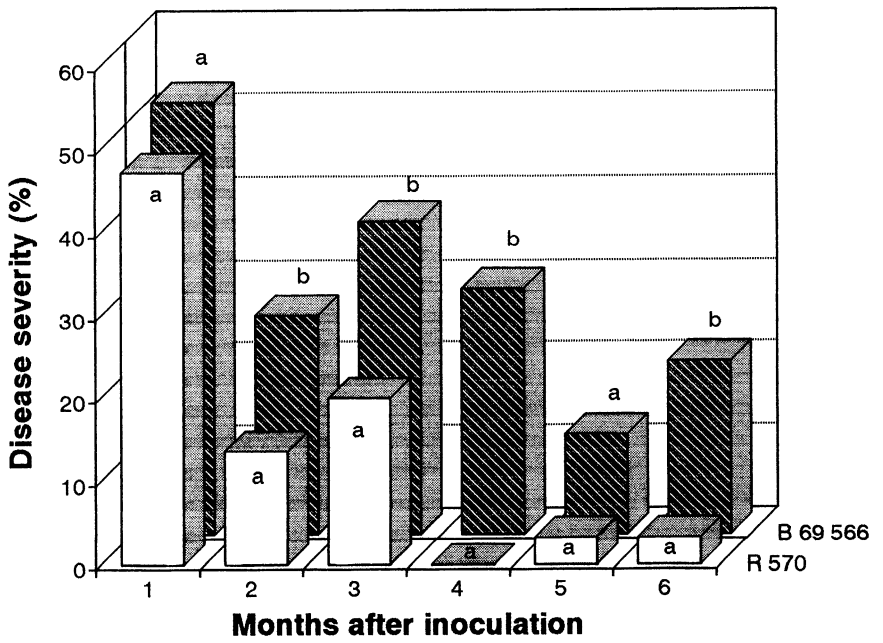

Fig. 3. Disease progress in stalks of two sugarcane clones inoculated in the field by the decapitation method with *Xanthomonas albilineans* GPE 5SR. B 69 566 = susceptible clone, R 570 = tolerant clone. Values are the means of five replications of five stalks each. Within each date, bars with the same letter are not significantly different at $P = 0.05$ according to the test of Newmann-Keuls.

Efficiency of pathogen detection (GPE 5SR). A total of 140 sugarcane extracts (leaves, apices, and basal nodes) were analyzed from the cutting experiment in the greenhouse and the stalk experiment in the field for the presence of *X. albilineans* GPE 5SR by isolation and serological methods. The pathogen was detected in 92, 79, and 38% of the samples by isolation on the selective medium, immunofluorescence, and dot-blot, respectively.

Leaf scald symptoms and population dynamics of the pathogen (GPE 5SR). In the field experiment, 1 mo after inoculation of the susceptible clone (B 69 566) and the tolerant clone (R 570), 25 stalks inoculated with GPE 5SR were sampled. Symptoms were recorded for inoculated leaves. Only leaves which had been inoculated were present at the time. Disease severity was high, and there was no significant difference between the two sugarcane clones (Fig. 3): 47.2% for R 570 and 52.0% for B 69 566. Twenty-one out of 25 (84%) and 19 out of 25 (76%) stalks were diseased for R 570 and B 69 566, respectively. The bacterial population densities were similar for both sugarcane clones in all plant parts analyzed. The populations of *X. albilineans* in the foliar parts (Fig. 4) were more than 10^9 cfu/g of dry matter (cfu/gDM) in the leaves and 10^{10} cfu/gDM in the apices. Standard errors were low, reflecting a good homogeneity between

the different stalks or replications. The bacterial populations in the basal nodes varied between 4.0×10^5 cfu/gDM (R 570) and 4.5×10^6 cfu/gDM (B 69 566).

Two months after inoculation, symptoms were observed on newly developed leaves which had not been inoculated. Disease severity decreased between 1 and 2 mo after inoculation and increased between 2 and 3 mo after inoculation, reflecting the time necessary for the disease to progress from inoculated to noninoculated leaves. Disease severity was highest 3 mo after inoculation and decreased after that time (Fig. 3). Except for 1 and 5 mo after inoculation, when no significant differences were observed, disease severity was significantly higher for the susceptible clone B 69 566 than for the tolerant clone R 570.

The populations of *X. albilineans* varied from 3.8×10^9 cfu/gDM in the leaves and 3.2×10^{10} cfu/gDM in the apices 1 mo after inoculation to low or nondetectable levels 6 mo after inoculation (Fig. 4). Standard errors were low for the two or three initial months, reflecting similar colonization in all apices and leaves of the two sugarcane clones. Standard errors increased greatly for the last 3 mo, which indicated high differences of colonization between samples within a clone. Symptom expression and population size in leaves and apices varied concurrently. In contrast, the pathogen populations in basal nodes of stalks reached more than 10^7 cfu/gDM 2 mo after inoculation and remained at this level for at least 4 mo (Fig. 4). Standard errors were low, reflecting a good homogeneity between the stalks. Whereas disease severity was generally higher for the susceptible clone B 69 566, bacterial population densities were similar for both clones, whatever the sampling date and the analyzed sugarcane part.

In the greenhouse experiment with cuttings, 25 shoots infected with GPE 5SR were sampled per sugarcane clone 7 wk after inoculation of the cuttings. Disease severity ranged from 0% for the resistant clone B 80 08 to 60.0% for the highly susceptible clone CP 68 1026 (Table 2). There was no difference between R 570 (tolerant) and B 69 566 (susceptible). The apex of all clones was colonized by the pathogen, but colonization was much more extensive in susceptible clones. For example, 5×10^6 times more bacteria were detected in CP 68 1026 than in B 80 08. Furthermore, even if disease severity was low (3.2%) for B 69 566 and R 570, bacterial population densities were relatively high: 7.9×10^7 cfu/gDM and 2.0×10^8 cfu/gDM. The roots of the primary stalk of all clones except the resistant one were also colonized by the pathogen. More than 10^8 cfu/gDM were detected for CP 68 1026 (Table 2). Roots of the susceptible clone B 69 566 were less colonized than the roots of the tolerant clone R 570, whereas the population densities in the apex were similar.

In the greenhouse experiment with vitroplants, disease severity of all six sugarcane clones issued from tissue culture was nil 2 mo after inoculation. Disease severity was still nil several weeks later for the remaining plants which were not destroyed for measurement of path-

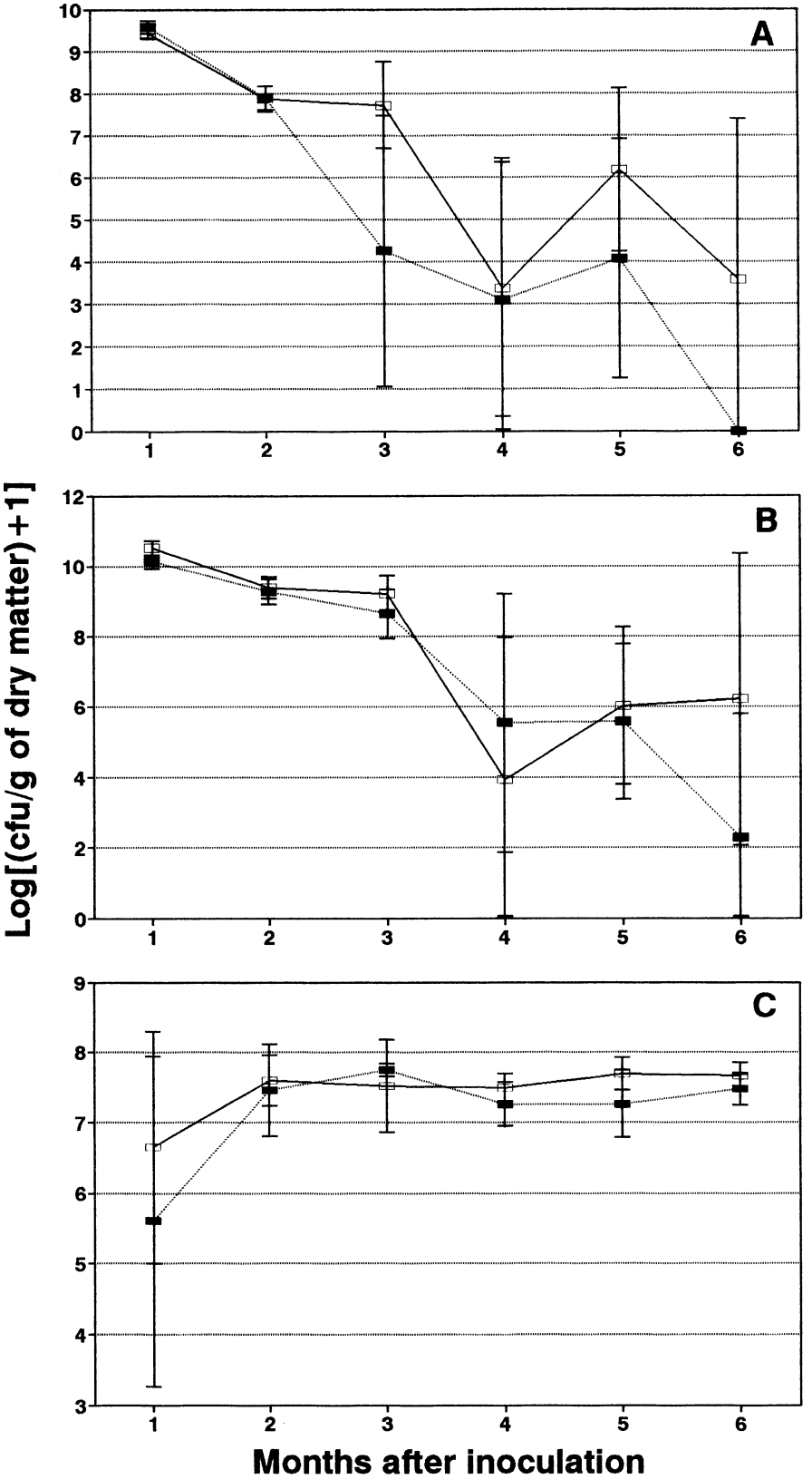

Fig. 4. Population dynamics of *Xanthomonas albilineans* GPE 5SR in two sugarcane clones inoculated in the field by the decapitation method (□ = B 69 566, susceptible; ■ = R 570, tolerant). **(A)** Leaves, **(B)** apices, and **(C)** basal nodes. Values are the means of five replications of 10 leaves (middle part), five apices, or five basal nodes each. Vertical bars denote standard errors.

ogen populations. The reasons for the absence of symptom development in this greenhouse experiment were not known. Nevertheless, all of the clones were colonized by the pathogen, and mean bacterial populations ranged from 50 cfu/gDM for the resistant clone B 80 08 to 6.3×10^7 cfu/gDM for the highly susceptible clone CP 68 1026 (Table 3).

Pathogen detection in the meristem. *X. albilineans* was also found in the apical meristem of diseased plants and in the foliar primordia around this meristem. The pathogen was isolated from the apical meristem of eight sugarcane stalks out of 15 with leaf scald symptoms (Table 4). Bacterial populations ranged from 7 to 900 cfu per meristem. The seven symptomatic plants from which *X. albilineans* was not isolated from the meristem exhibited no symptoms on spindle leaves but had symptoms on older unfolded leaf blades. The pathogen was never detected in the meristem but was detected once in the foliar primordia of 15 infected but symptomless stalks.

DISCUSSION

X. albilineans GPE 5 and GPE 5SR had identical cultural properties on modified Wilbrink's medium, similar profiles for the utilization of carbon sources, and similar immunological properties. Furthermore, strain GPE 5SR appeared at least as virulent as strain GPE 5.

Various techniques can be used to inoculate sugarcane with *X. albilineans* and to assess virulence of the bacterium or the resistance level of the host plant. However, published results are divergent on the efficiency of these methods, especially the decapitation method and the inoculation of cuttings (20,32). In this study, both methods proved to be efficient, resulting in a high proportion of inoculated plants that developed symptoms.

Several methods were assessed to detect the pathogen inside the host. The superior results obtained by isolation on selective medium were probably due to the minimum detection level of this method, which was low (7 cfu/ml) in comparison to those of the immunological techniques: $2–5 \times 10^3$ cfu/ml for immunofluorescence and 10^6 cfu/ml for dot-blot (27).

Even when precautions were taken to use healthy plant material, leaf scald developed in some control plants in the field trial. The hypothesis of accidental transmission of GPE 5SR at the time of inoculation was unlikely because all the strains of the pathogen obtained from these plants were susceptible to streptomycin and rifampicin. Transmission of *X. albilineans* by means other than mechanical is probable but presently unknown in Guadeloupe. Aerial transmission has recently been reported in Mauritius (3).

Very little information is presently available on the dynamics of *X. albilineans* populations inside and outside the host plant. Epidemiological studies of sugarcane leaf scald disease are often limited by the difficulties encountered in isolating the pathogen. Low populations of the pathogen are frequently overgrown or inhibited on nonselective media by other bacteria (6,11). *X. albilineans* is slow growing and cannot be easily isolated on common culture media, especially during the latency phase when symptoms are not visible (6). To overcome these problems, the possibility of using a strain of the pathogen carrying resistance to antibiotics and selective media containing these antibiotics could be advantageous. This technique is all the more promising because it appeared to be more efficient than the immunological techniques that we used. In consideration of the results obtained, especially that strain GPE 5SR was at least as virulent as strain GPE 5, this mutant can be used for studies of population dynamics of *X. albilineans* by specific isolation of the pathogen on selective culture media.

X. albilineans is a pathogen that colonizes vascular tissues of sugarcane (21). It was known to develop in the stalk and in the leaves, but in this study there is evidence that the bacterium also invades roots and the stalk apex, including the

Table 2. Disease severity and bacterial populations in four sugarcane clones 7 wk after inoculation of the cuttings with *Xanthomonas albilineans* GPE 5SR (greenhouse experiment)[x]

Clone	Disease severity (%)	Log_{10} [(cfu/g dry matter)+1]	
		Apex	Roots
B 80 08 (resistant)	0 (4.5) a	3.0 (3.0) a	0 (5.0) a
B 69 566 (susceptible)	3.2 (10.1) b	7.9 (9.6) b	1.8 (7.6) a
R 570 (tolerant)	3.2 (9.4) b	8.3 (11.4) b	4.5 (11.4) b
CP 68 1026 (highly susceptible)	60.0 (18.0) c	9.7 (18.0) c	8.3 (18.0) c
Mean	16.6 (10.5)	7.2 (10.5)	3.6 (10.5)
Clone effect	**[y]	**	**
RSE[z]	(2.91)	(2.43)	(2.81)
SEM	(1.30)	(1.10)	(1.25)

[x] Completely randomized design of five replications of five samples each. Values in parentheses correspond to the data transformed to ranks. Means in a column followed by the same letter are not significantly different at $P = 0.05$ according to the test of Newmann-Keuls.
[y] ** = Significant at $P = 0.01$.
[z] RSE = residual standard error; SEM = standard error of the mean.

Table 3. Bacterial populations in the aerial part of six sugarcane clones 2 mo after inoculation of vitroplants with *Xanthomonas albilineans* GPE 5SR (greenhouse experiment). Disease severity of all sugarcane clones was nil[x]

Clone	Resistance level	Log_{10} [(cfu/g dry matter)+1]
B 80 08	Resistant	1.7 (5.5) a
Co 64 15	Resistant	2.7 (7.2) a
B 69 566	Susceptible	6.4 (14.2) b
R 570	Tolerant	6.0 (18.1) bc
B 69 379	Susceptible	7.6 (22.2) c
CP 68 1026	Highly susceptible	7.8 (25.8) c
Mean		5.4 (15.5)
Clone effect		**[y]
RSE[z]		(4.95)
SEM		(2.21)

[x] Completely randomized design of five replications of five samples each. Values in parentheses correspond to the data transformed to ranks. Means in a column followed by the same letter are not significantly different at $P = 0.05$ according to the test of Newmann-Keuls.
[y] ** = Significant at $P = 0.01$.
[z] RSE = residual standard error; SEM = standard error of the mean.

Table 4. Presence of *Xanthomonas albilineans* GPE 5SR (Xa) in the meristem or the foliar primordia around the meristem of sugarcane with respect to symptom expression

Number of samples	Leaf scald symptoms	Xa in the foliar primordia	Xa in the meristem
8	+	+	+[z]
4	+	+	−
3	+	−	−
1	−	+	−
14	−	−	−

[z] 7-900 cfu/meristem.

apical meristem. As a result of this colonization, cleaning up sugarcane clones by meristem tip culture alone, i.e., without thermotherapy, appears hazardous.

Leaf scald is mainly spread in infected cuttings and transmitted on infested cane knives or mechanical harvesters. Aerial transmission has also been reported recently in Mauritius (3). The pathogen does not appear to survive long in soil, but transmission through the soil has been suspected (23). The fact that the bacterium invades the roots supports the hypothesis that transmission can occur via the soil or through root-to-root contact of sugarcane stalks. Other studies have also shown that *X. albilineans* GPE 5SR can be isolated from rhizosphere soil of contaminated sugarcane (P. Klett and P. Rott, *unpublished*).

After inoculation of sugarcane stalks in the field, the intensity of leaf scald symptoms and their progression were directly correlated with pathogen population densities in the leaves and the apices. The presence of symptoms was related to the presence of large populations of the pathogen in these aerial parts of sugarcane. Few or no symptoms were related to low or undetectable levels of bacteria. Nevertheless, and even though populations were similar, one clone (B 69 566) had more severe symptoms than the other (R 570). Furthermore, high populations of the pathogen were detected in the apex of sugarcane shoots issued from inoculated cuttings and exhibiting nearly no symptoms. Likewise, many of the bacteria were also found in the aerial part of vitroplants of susceptible clones without any symptoms of the disease. In view of these results, high bacterial densities in the apex or in the aerial plant part are necessary but not always associated with the development of leaf scald symptoms. Characteristics of pathogenicity other than colonization probably play an important role at this stage of disease development. Molecules like albicidin, a phytotoxin produced by *X. albilineans* (5), could be one of these factors.

Unlike pathogen populations in the leaves and apices, those in the basal nodes reached a plateau early and remained stable, whereas the progression of foliar symptoms deviated more over time. Disease expression was transient in numerous individuals of the tolerant and susceptible clones, although the clones were colonized by *X. albilineans*. These plants constitute asymptomatic carriers, and this situation strengthens the need for efficient diagnostics for asymptomatic infection (6,11). Basal nodes appear to be the best location to assay for the pathogen, but other parts of the stalk should be assessed. In another study (6), internodes 62.5% up from the base of the stalk were found to be the best to sample for *X. albilineans*.

Colonization of sugarcane was also closely related to the level of resistance to leaf scald disease. In this way, bacterial population densities were lower for the resistant clones compared to the susceptible ones. Similar results were obtained for ratoon stunting disease, another vascular bacterial disease of sugarcane (9,10). Restricted bacterial colonization in xylem appears to be involved in the resistance of sugarcane to leaf scald disease, as was demonstrated for vascular diseases of other plants (15). This characteristic of sugarcane resistance to leaf scald should be studied in more clones and in different parts of the host plant. The population levels of *X. albilineans* in the basal nodes and other aerial parts of sugarcane were indistinguishable in a susceptible vs. tolerant clone. Other characteristics probably played a role in disease progress in these clones. Analysis of bacterial populations in sugarcane should allow us to breed and screen for resistance to leaf scald using not only the parameter "symptoms," which can be erratic, but also the parameter "level of colonization."

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LITERATURE CITED

1. Andrews, J. H. 1986. How to track a microbe. Pages 14-34 in: Microbiology of the Phyllosphere. N. J. Fokkema and J. Van Den Heuvel, eds. Cambridge University Press, Cambridge.
2. Autrey, L. J. C., Saumtally, S., Dookun, A., and Médan, H. Studies on variation in the leaf scald pathogen *Xanthomonas albilineans* (Ashby) Dowson. Proc. Int. Soc. Sugar-Cane Technol. In press.
3. Autrey, L. J. C., Saumtally, S., Dookun, A., Sullivan, S., and Dhayan, S. Aerial transmission of the leaf scald pathogen, *Xanthomonas albilineans*. Proc. Int. Soc. Sugar-Cane Technol. In press.
4. Baudin, P. 1984. Quarantaine de canne à sucre à Montpellier, France. Agron. Trop. 39:262-267.
5. Birch, R. G., and Patil, S. 1987. Correlation between albicidin production and chlorosis induction by *Xanthomonas albilineans*, the sugarcane leaf scald pathogen. Physiol. Mol. Plant Pathol. 30:199-206.
6. Comstock, J. C., and Irey, M. S. 1992. Detection of the sugarcane leaf scald pathogen, *Xanthomonas albilineans*, using tissue blot immunoassay, ELISA, and isolation techniques. Plant Dis. 76:1033-1035.
7. Comstock, J. C., and Shine, J. M., Jr. 1992. Outbreak of leaf scald of sugarcane, caused by *Xanthomonas albilineans*, in Florida. Plant Dis. 76:426.
8. Conover, W. J., and Iman, R. L. 1981. Rank transformations as a bridge between parametric and nonparametric statistics. Am. Stat. 35:124-129.
9. Davis, M. J., Dean, J. L., and Harrison, N. A. 1988. Distribution of *Clavibacter xyli* subsp. *xyli* in stalks of sugarcane cultivars differing in resistance to ratoon stunting disease. Plant Dis. 72:443-448.
10. Davis, M. J., Dean, J. L., and Harrison, N. A. 1988. Quantitative variability of *Clavibacter xyli* subsp. *xyli* populations in sugarcane cultivars differing in resistance to ratoon stunting disease. Phytopathology 78:462-468.
11. Davis, M. J., Rott, P., and Dean, J. L. Selective isolation of *Xanthomonas albilineans*, causal agent of leaf scald disease. Proc. Int. Soc. Sugar-Cane Technol. In press.
12. Egan, B. T., and Sturgess, O. W. 1980. Commercial control of leaf scald disease by thermotherapy and a clean seed programme. Proc. Int. Soc. Sugar-Cane Technol. 17(2):1602-1606.
13. Feldmann, P., Féréol, L., and Rott, P. 1989. Les cultures in vitro: un nouvel outil au service du progrès agricole en Guadeloupe. II. Canne à sucre. Bull. Agron. Antilles Guyane 9:79-81.
14. Gillaspie, A. G., Jr. 1989. Sugarcane quarantine. Pages 333-339 in: Diseases of Sugarcane. Major diseases. C. Ricaud, B. T. Egan, A. G. Gillaspie, Jr., and C. G. Hughes, eds. Elsevier Science Publishers B.V., Amsterdam.
15. Grimault, V., and Prior, P. Evidence that bacterial spread in xylem is involved in the bacterial wilt resistance of tomato. Proc. Int. Conf. Plant Pathol. Bact., 8th. In press.
16. Hampton, R., Ball, E., and De Boer, S., eds. 1990. Serological Methods for Detection and Identification of Viral and Bacterial Pathogens: A Laboratory Manual. American Phytopathological Society, St. Paul, MN.
17. Hayward, A. C. 1974. Latent infection by bacteria. Annu. Rev. Phytopathol. 12:87-93.
18. Hughes, C. G. 1978. Diseases of sugarcane. A review. PANS 24:143-159.
19. Koike, H. 1965. The aluminum-cap method for testing sugarcane varieties against leaf scald disease. Phytopathology 55:317-319.
20. Koike, H. 1972. Testing sugarcane varieties for leaf scald disease resistance. Proc. Int. Soc. Sugar-Cane Technol. 14:909-919.
21. Martin, J. P., Carpenter, C. W., and Weller, D. M. 1932. Leaf scald disease of sugarcane in Hawaii. Hawaii. Plant. Rec. 36:145-196.
22. Martin, J. P., and Robinson, P. E. 1961. Leaf scald. Pages 79-107 in: Sugarcane Diseases of the World, vol. 1. J. P. Martin, E. V. Abbott, and C. G. Hughes, eds. Elsevier Publishing Company, Amsterdam.
23. Ricaud, C., and Ryan, C. C. 1989. Leaf scald. Pages 39-58 in: Diseases of Sugarcane. Major Diseases. C. Ricaud, B. T. Egan, A. G. Gillaspie, Jr., and C. G. Hughes, eds. Elsevier Science Publishers B.V., Amsterdam.
24. Rott, P., Arnaud, M., and Baudin, P. 1986. Serological and lysotypical variability of *Xanthomonas albilineans* (Ashby) Dowson, causal agent of sugarcane leaf scald disease. J. Phytopathol. 116:201-211.
25. Rott, P., Chatenet, M., and Baudin, P. 1988. L'échaudure des feuilles de canne à sucre provoquée par *Xanthomonas albilineans* (Ashby) Dowson. I. Synthèse bibliographique. Agron. Trop. 43:236-243.
26. Rott, P., Chatenet, M., Granier, M., and Baudin, P. 1988. L'échaudure des feuilles de canne à sucre provoquée par *Xanthomonas albilineans* (Ashby) Dowson. II. Diagnostic et spectres d'hôtes de l'agent pathogène en Afrique tropicale. Agron. Trop. 43:244-251.
27. Rott, P., Chatenet, M., Granier, M., and Baudin, P. 1988. Recognition and detection of *Xanthomonas albilineans* and *Clavibacter xyli* subsp. *xyli* by indirect immunofluorescence and enzyme immunoassays. Agron. Trop. 43:158.
28. Rott, P., and Feldmann, P. 1991. Les maladies de la canne à sucre en Guadeloupe: situation actuelle et méthodes de lutte mises en place. Pages 90-94 in: Rencontres internationales en langue française sur la canne à sucre. Actes de la première rencontre. Montpellier, France. AFCAS, Nogent-sur-Marne.
29. SAS Institute. 1989. SAS/STAT User's Guide. Version 6 ed. Cary, NC.
30. Spence, J. A. 1957. The incidence of leaf scald disease of sugarcane in the West Indies. Proc. Meet. Br. W. Indies Sugar Technol. 57:140-146.
31. Steindl, D. R. L. 1971. The elimination of leaf scald from infected planting material. Proc. Int. Soc. Sugar-Cane Technol. 14:925-929.
32. Thompson, V. 1983. Developments in leaf scald studies in Jamaica. Jamaican Assoc. Sugar Technol. J. 43:70-77.
33. Walker, D. I. T. 1987. Breeding for resistance. Pages 445-502 in: Sugarcane Improvement Through Breeding. D. J. Heinz, ed. Elsevier Science Publishers B.V., Amsterdam.