Distribution of *Clavibacter xyli* subsp. *xyli* in Stalks of Sugarcane Cultivars Differing in Resistance to Ratoon Stunting Disease

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ABSTRACT

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The spatial and temporal distributions of *Clavibacter xyli* subsp. *xyli* populations in stalks of sugarcane cultivars were studied. Sap containing cells of the pathogen was extracted by centrifugation from stalk sections, and the cells were enumerated using a fluorescent-antibody direct-count procedure. Small but significant differences in mean bacterial populations were detected among sap extracts from nodal and internodal sections of cultivar CP 53-1, depending upon the direction of extraction. Sap extracted in a basipetal direction from nodal tissues excised immediately below leaf scars contained the greatest numbers, otherwise populations in extracts from internodal tissues equalled or exceeded those from nodal tissues. No effect due to direction of extraction was found among populations in sap extracted from internodal tissues. Pathogen populations developed at a faster rate and to a greater extent in internodal tissues of the more susceptible of nine cultivars examined on three successive sampling dates. Larger populations were observed in sap from mature as compared with less mature internodes. As tissues matured, population densities at different internode locations became correlated with yield reduction estimates for the cultivars.

Ratoon stunting disease (RSD) of sugarcane (*Saccharum*, interspecific hybrids) occurs in most sugarcane-producing areas of the world (19) and is caused by *Clavibacter xyli* subsp. *xyli* Davis et al (6,7), a small, xylem-inhabiting, coryneform bacterium that was first reported to be associated with RSD in 1973 (10,16,21). The disease is usually not lethal but often causes significant yield reduction in susceptible cultivars (14,15,23). Immunity to infection by *C. x.* subsp. *xyli* has not been discovered among known cultivars and other genotypes. Stunting is the only overt symptom of RSD but is not characteristic of it alone. Consequently, both disease diagnosis and assessment of cultivar susceptibility are difficult (19). Yield trials in which healthy and diseased plantings are compared constitute the only proven method for determining the effect of RSD on sugarcane cultivars (9,14). However, such trials have been economically impractical in breeding programs. As a result, the relative susceptibility to RSD is known only for a few commercial sugarcane cultivars. Alternative approaches to screening sugarcane genotypes for RSD resistance have been sought.

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Both greater branching of metaxylem vessels (20) and greater resistance to hydraulic conductivity in noninfected nodal tissues (22) were correlated with resistance to RSD in sugarcane, evidence for an anatomical basis for resistance. However, a practical procedure for routine determination of the degree of metaxylem branching has not been developed, and measuring hydraulic conductivity may be useful only when differences between healthy and infected nodal tissues are determined (8,24). Infectivity titrations may provide another means to screen for resistance (12), but the replication of plants required at different inoculum doses for each genotype tested may be prohibitive.

Another approach to screening for RSD resistance is to compare the population density of *C. x.* subsp. *xyli* in different sugarcane genotypes (2,5,11). Gillaspie et al (11) found that relative densities of the pathogen in sugarcane cultivars were inversely correlated with their resistance to injury due to RSD. Bailey (2) subsequently confirmed this relationship and demonstrated that it held when similar locations within leaves and stalks of different cultivars were sampled. Population densities were greater in basal stalk tissues, and in stalks when compared with leaves. Also, populations in stalk tissues increased with plant age (2,11). When both pathogen concentrations in crude juice extracts and yield responses were compared in 20 cultivars with a history of different reactions to RSD, pathogen concentrations were useful in separating those cultivars that were most susceptible from those that were most resistant to RSD. Differences between cultivars intermediate in disease reaction could not be discriminated on the basis of pathogen concentrations (15). However, only the total numbers of bacteria counted after sampling three successive crops of a replicated planting of each cultivar were reported, and information on the quantitative variability of the data was not given.

In early studies on *C. x.* subsp. *xyli* populations, phase-contrast microscopy was used to enumerate cells of the pathogen in either milled stalk juice (11,15) or in tissue diffusates and vacuum extracts (2). To overcome problems associated with these procedures, a direct-count technique using fluorescent-labelled antibodies was developed (4). Relatively undiluted sap without excessive plant debris was extracted from tissues by centrifugation of stalk sections, and pathogen cells stained with the fluorescent-labelled antibody were collected on membrane filters and enumerated. This direct-count technique was more sensitive, specific, reliable, and rapid than other techniques.

Distributions of the frequency of density estimates for individual *C. x.* subsp. *xyli* populations in sugarcane stalk internodes obtained using the fluorescent-antibody direct-count technique were best normalized by a quartic-root transformation that eliminated the relationship between means and variances (5). Analysis of the quantitative variability among pathogen population density estimates in sap extracted from basal internode tissues demonstrated that variability decreased with time in both the plant and first-stubble crops of cultivars differing in resistance to RSD (5). Furthermore, densities were highly correlated with yield loss for comparisons based on populations at different dates in both the plant and first-stubble crops and for plant crops at two different geographic locations.

The present study was initiated to examine both the spatial and temporal distributions of *C. x.* subsp. *xyli* within stalks of different sugarcane cultivars

relative to the maturity of the tissue sampled and to the correlation between yield loss due to RSD and the population density of the pathogen. Such information is needed to determine if screening procedures for disease resistance can be based on pathogen population densities in sugarcane.

MATERIALS AND METHODS

Plant material and field plot design. Plants infected with *C. x.* subsp. *xyli* were established first in a nursery and then in experimental field plots at Ft. Lauderdale, Florida, as previously described (5). The cultivars included CP 43-47, CP 44-101, CP 53-1, CP 59-22, CP 63-588, CP 65-357, CP 70-1133, CP 72-1210, and CP 74-2005. Briefly, plants presumed to be infected with the F1 strain (7) of *C. x.* subsp. *xyli* as a result of inoculations were propagated as single-node cuttings during December 1982 and grown in pots in a screenhouse until they were transplanted to the field plots in March 1983. The experimental field was planted using a randomized complete block design. Each plot contained a row of five plants (subplots) of a single cultivar. Plots of each cultivar were replicated once in each of three blocks.

Table 1. Mean difference in plant weight between healthy and ratoon-stunting-diseased sugarcane plants in the plant crop of four paired-comparison trials at Canal Point, FL

Cultivar	Mean loss[y] in weight (kg/plant) 1979	1983	1984	Mean (kg)
CP 59-22	ND	8.6*	4.2*	6.4
CP 43-47	5.7* (18)[z]	3.8*	3.8*	4.2
CP 53-1	4.2* (20)	ND	3.6*	4.0
CP 63-588	1.2 (39)	3.5*	3.1*	2.5
CP 72-1210	ND	2.1*	2.2*	2.1
CP 65-357	ND	2.7*	0.8	1.8
CP 44-101	ND	ND	1.8	1.8
CP 70-1133	1.9 (41)	−0.2	0.6	0.9
CP 74-2005	ND	0.9	1.0	0.9
CP 72-2086	ND	−1.2	1.1	−0.1
H 60-6909	ND	ND	−0.4	−0.4

[y] * = Significant loss ($P<0.05$), ND = not done. Published data: 1979 and 1980 data of Dean (9) and 1984 data of Davis et al (5). The mean yield losses for two trials conducted in 1979 and 1980 are given under 1979 for CP 63-588 and CP 70-1133. A significant loss (2.8 kg/plant) was observed for CP 70-1133 in 1979 but not in 1980 (1.1 kg/plant).

[z] Number in parentheses is the total number of replicates; otherwise there were 30 replicates in 1983 and 29 replicates in 1984 for all treatments.

Table 2. Population densities of *Clavibacter xyli* subsp. *xyli* in sap extracted either acropetally or basipetally by centrifugation from nodal and internodal stalk tissues of sugarcane cultivar CP 53-1

Stalk section[y]	Direction of sap extraction	No. samples	Mean population density[z] ($\log_{10}$ cells/ml)
	Experiment 1		
Below leaf scar	Acropetal	47	8.71 b
Above leaf scar	Basipetal	45	8.37 c
Lower internode	Acropetal	45	8.80 ab
Upper internode	Basipetal	45	8.86 a
	Experiment 2		
Below leaf scar	Basipetal	45	9.03 a
Above leaf scar	Acropetal	48	8.61 c
Lower internode	Basipetal	45	8.93 b
Upper internode	Acropetal	45	8.88 b
	Experiment 3		
Below leaf scar	Acropetal	34	8.17 c
	Basipetal	33	8.65 a
Above leaf scar	Acropetal	32	8.21 c
	Basipetal	34	8.37 b
	Experiment 4		
Internode, split	Acropetal	30	9.03 a
	Basipetal	30	9.06 a

[y] In experiments 1–3, the lower portion of 3–4 stalks composed of 16 nodes was cut transversally into sections of four equal lengths between leaf scars. In experiment 4, single sections from the lower 10 internodes of four stalks were split longitudinally for paired comparisons.

[z] Means followed by the same letter within each experiment were not significantly different (Waller-Duncan k-ratio t test; $k = 100$).

Sampling procedures. In an initial set of four experiments using nursery plants of cultivar CP 53-1, the concentration of pathogen cells in sap extracts from nodal and internodal tissues of stalks was examined with respect to the direction of sap extraction by centrifugation. In the first two experiments, three stalks containing 16 mature nodes were cut transversely into a series of smaller sections. Sections delimited by leaf scars of successive nodes were further subdivided into four sections, two nodal and two internodal, of approximately equal length. In the first experiment, sap was extracted by centrifugation from adjacent ends of each pair of nodal or internodal sections. In the second experiment, sap was extracted from opposite ends of each pair. In the third experiment, equivalent sections consisting of the lower and upper portion of each nodal region were cut from four stalks, each with 16–17 nodes, and sap was extracted alternately from the basipetal or acropetal end of corresponding sections from within each stalk. In the fourth experiment, single internodal sections were excised from between the lower 10 nodes of three stalks, and each section was split longitudinally into two equal halves. Sap was then extracted from opposite ends of each pair of split sections.

The field plots were sampled to study the spatial and temporal distributions of *C. x.* subsp. *xyli* in internodal tissues of stalks without regard to the direction of extraction from these tissues. One stalk, the most mature stalk available, was taken from each plant during each of three sampling periods at approximately 6-wk intervals. Sampling was initiated in the latter one-third of the growing season after pathogen cells had been detected successfully in most cultivars. Stalks were harvested within 7–10 days of each other during each sampling period. Thus, the mean sampling dates were 18 October, 2 December, and 22 January.

Sap was extracted, usually within 3 hr after harvesting stalks, by centrifugation of internode sections at 8,300 *g* for 10 min (6,7). The third, seventh, eleventh, and fifteenth internode aboveground of each stalk was sampled. Extracts were stored at 4 C if examined on the day of extraction, otherwise they were stored frozen at −20 C and usually examined within a week. To reduce the number of extracts examined, 0.1-ml samples of each individual sap extract were pooled by treatment. Thus, pooled samples consisting of sap from five stalks were obtained for each combination of block, cultivar, internode location, and sampling date. To determine the effect of pooling samples on estimation of population densities, another 0.1-ml sample of each sap extract from third internodes was examined individually, and the density estimates obtained for pooled samples

were compared with the corresponding mean density estimates for individual samples.

Measurement of pathogen populations. Populations of *C. x.* subsp. *xyli* were enumerated using the fluorescent-antibody-direct-count-on-filters (FADCF) technique (4). Briefly, equal volumes, usually 0.1 ml, of a sap sample and an appropriately diluted fluorescein isothiocyanate-conjugated rabbit immunoglobulin G, specific for *C. xyli*, were mixed and incubated at room temperature for 30 min. Stained pathogen cells in each sap sample were then collected on a membrane filter and observed using epifluorescence microscopy at 1,000×. Cells were counted in 15 fields or until approximately 150 cells were counted, whichever came first. The concentration of cells per millileter of sap was then calculated.

Maturity measurements. The sugar content of stalks increases as they mature (25), and stalks are commonly considered mature by industry standards when the distribution of sugar is uniform throughout the stalk (17). The sugar content and percent of total dissolved solids of stalk tissues are highly correlated. Because the percent of total dissolved solids (brix) in expressed stalk juices can be determined readily with a refractometer, this method is commonly used to determine maturity (17). In the present study, a drop of crude juice was expressed, using pliers, from internode tissues adjacent to those internode tissues sampled for population density estimates, and the brix of the drop was determined with a hand-held refractometer (model 10100, American Optical, Buffalo, NY) calibrated in terms of percent sucrose.

Statistical analysis. Individual population density estimates were transformed to their quartic root before statistical analysis (5). The relationship between population density estimates for pooled sap samples and the mean estimates for the corresponding individual samples were then examined by regression analysis using the REG or General Linear Models (GLM) procedures of computer programs by SAS (Statistical Analysis Systems, SAS Institute, Inc., Cary, NC). The same procedures were also used to examine the correlation between population density estimates and brix and the correlation between population density estimates and yield reduction due to RSD. Pathogen population densities for cultivars obtained with pooled sap samples were compared with the mean yield-loss estimates for the same cultivars on the basis of internode location and sampling date. Mean yield-loss estimates for the cultivars examined (Table 1) were obtained from published (5,9) and unpublished data for replicated, single-stool plots of paired healthy and RSD-diseased plants. The plants used in the 1983 and 1984 yield studies and in the present study were vegetatively propagated from the same nursery plants as described previously (5).

RESULTS

Pathogen population densities in nodal and internodal tissues. Densities of *C. x.* subsp. *xyli* cells in sap extracts from nodal and internodal stalk tissues varied according to the tissue extracted and the direction of extraction, although the magnitude of the variation was not great (Table 2). The greatest densities were found in sap extracted in a basipetal direction from nodal tissues below leaf scars. When sap was extracted in an acropetal direction from comparable nodal tissues or in either a basipetal or acropetal direction from nodal tissues above leaf scars, pathogen concentrations were not consistantly different. Pathogen concentrations in sap extracts from internodal tissues were not affected by the direction of extraction and were consistently greater than those from nodal tissues, except when nodal tissues from below leaf scars were extracted in a basipetal direction.

Effect of pooling samples. After repeated sampling, *C. x.* subsp. *xyli* was not detected in 11 of 135 plants originally presumed to be infected. These apparently noninfected plants were distributed among cultivars CP 53-1 (3 plants), CP 59-22 (2 plants), CP 65-357 (2 plants), and CP 74-2005 (4 plants). Since sap from these plants was mixed with sap from infected plants during the pooling of samples, these dilutions were taken into account during calculation of population density estimates.

A highly significant ($P < 0.0001$) linear regression was obtained when the quartic roots of population density estimates for pooled sap samples from internodal tissues were regressed on the means of quartic-root transformed estimates for the corresponding individual samples. The regression equation ($R^2 = 0.89$) was $Y = 12.84 + 0.95X$. Both the slope and intercept of the equation were significantly different from zero ($P < 0.001$). However, the slope was not significantly different from unity ($P > 0.164$), indicating that population densities in pooled samples were consistently overestimated by 12.84, the quartic root of 2.7×10^4 cells/ml.

Spatial and temporal distribution of the pathogen. Populations of *C. x.* subsp. *xyli* in different internode tissues generally increased with time in each of the nine cultivars examined. However, the rate of increase differed among

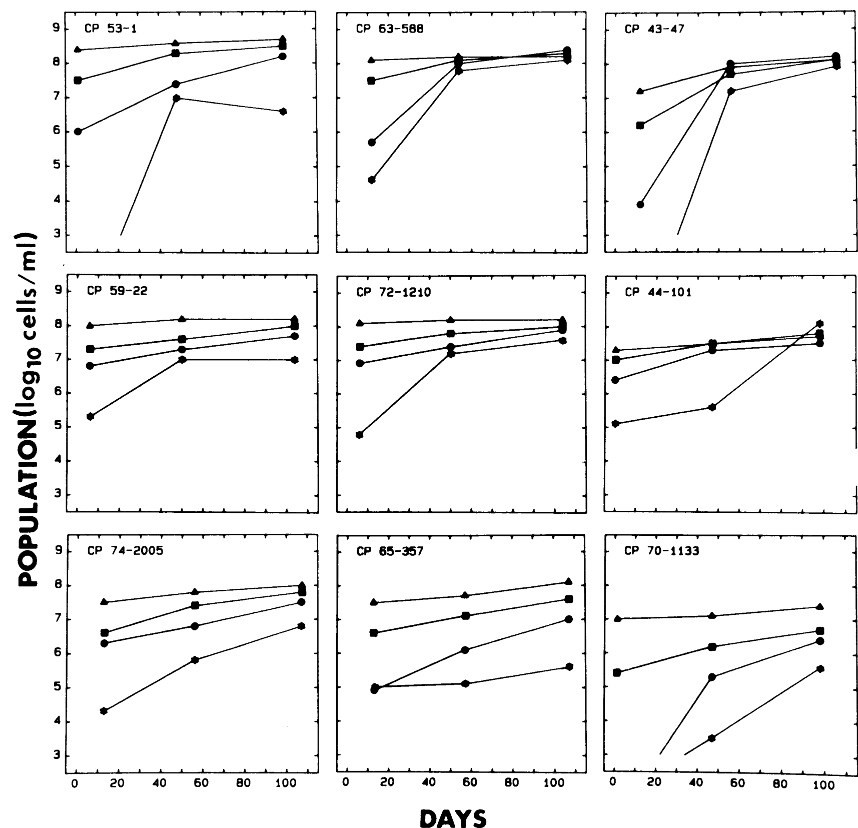

Fig. 1. Spatial and temporal distribution of *Clavibacter xyli* subsp. *xyli* in nine sugarcane cultivars. The mean cell concentrations of the pathogen were determined for sap extracts from the third (▲), seventh (■), eleventh (•), and fifteenth (★) internode locations aboveground in stalks on three dates at approximately 6-wk intervals during the latter one-third of the growing season of a plant crop. Each data point represents the mean cell concentrations of three pooled sap samples from five stalks of different plants taken on a given day after the initial sampling date, 13 October 1983.

internodes of some cultivars (Fig. 1). Initially, populations were greatest in the older portion of stalks, although populations in the younger portion of stalks of some cultivars approached those in the older basal stalk tissues as the growing season progressed. This convergence was generally due to a decline in the rate of population development in older stalk tissues. Populations in the third internode tissues of some cultivars had attained their maximum densities before the first sampling date and remained at approximately this level throughout the time period sampled.

Pathogen population density relative to host tissue maturity. For the most part, the brix of crude juice extracts increased with time (Fig. 2), as did the population density of the pathogen. When density estimates for pooled sap samples were regressed on the mean brix for the corresponding internode tissues, highly significant ($P < 0.0001$) linear or quadratic regressions were obtained for all cultivars. However, since the intercept parameters for the regression equations were not significantly different from zero ($P > 0.05$), except for cultivars CP43-47, CP 74-2005, and CP 70-1133, and because population densities or brix values below zero are not realistic, another set of regression analyses was performed with the regressions forced through the origin. These linear regressions were highly significant ($P < 0.0001$) for all cultivars, and an additional significant ($P < 0.05$) reduction in error was observed when a quadratic regression model was fitted to the data for CP 43-47, CP 59-22, CP 63-588, CP 65-357, CP 70-1133, and CP 74-2005 (Fig. 3). The regression equation and corresponding R_1^2 (3) value for each cultivar was as follows: CP 53-1, $Y = 8.00X$, $R_1^2 = 0.83$; CP 59-22, $Y = 0.45X + 0.19X^2$, $R_1^2 = 0.53$; CP 43-47, $Y = 7.52X - 0.10X^2$, $R_1^2 = 0.86$; CP 63-588, $Y = 8.41X - 0.13X^2$, $R_1^2 = 0.76$; CP 72-1210, $Y = 4.42X$, $R_1^2 = 0.56$; CP 44-101, $Y = 4.61X$, $R_1^2 = 0.38$; CP 74-2005, $Y = -0.09X + 0.18X^2$, $R_1^2 = 0.57$; CP 65-357, $Y = 0.37X + 0.15X^2$, $R_1^2 = 0.53$; and CP 70-1133, $Y = -1.42X + 0.21X^2$, $R_1^2 = 0.64$.

Relationship between pathogen population density and yield reduction. Linear regressions of density estimates on yield losses were significant ($P < 0.05$) for the third internode location on all three sampling dates (Table 3). Similarly, the regressions for the seventh and eleventh internode locations were significant on the last two sampling dates. The correlation between pathogen population densities and yield reductions was greatest for the third internode and for the last two sampling dates. A quadratic regression model was not a significant ($P > 0.05$) improvement over the linear regression model.

DISCUSSION

In sugarcane stalks, the vascular system is composed of discrete vascular bundles containing both xylem, which *C. x.* subsp. *xyli* inhabits, and phloem tissues. The vascular system runs longitudinally, without branching, throughout the internodal region until a portion of it branches into leaves, root primordia, and buds at the nodes (1). Teakle et al (22) demonstrated that the numbers of vascular bundles continuing through the nodal regions of stalks without branching were greater in sugarcane cultivars that are more susceptible to RSD. Bailey (2) found that populations of *C. x.* subsp. *xyli* were consistently larger in diffusates from the nodal regions of stalks than from internodal regions. We observed that pathogen populations in the highly susceptible cultivar CP 53-1 were larger in only the basipetal region of nodes where branching of the vascular system

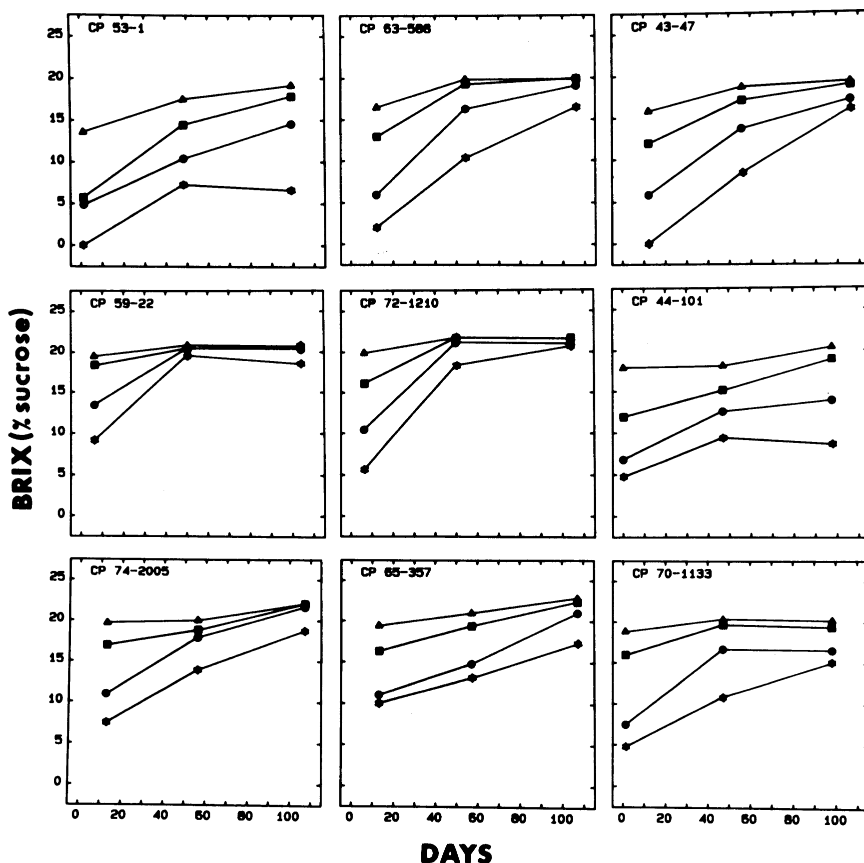

Fig. 2. Spatial and temporal maturation of nine sugarcane cultivars as indicated by the brix (% sucrose) of expressed sap from different stalk locations. Each brix value represents the mean of 15 determinations. The mean cell concentrations of the pathogen were determined for sap extracts from the third (▲), seventh (■), eleventh (•), and fifteenth (★) internode locations aboveground in stalks on three dates at approximately 6-wk intervals during the latter one-third of the growing season of a plant crop. Each data point represents the mean cell concentrations of three pooled sap samples from five stalks of different plants taken on a given day after the initial sampling date, 13 October 1983.

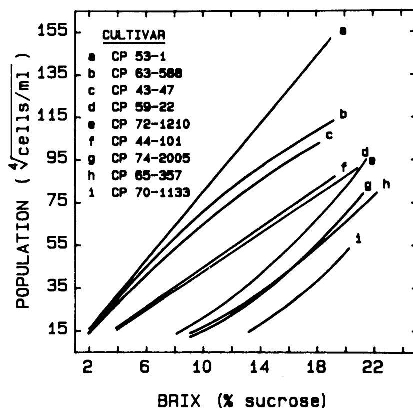

Fig. 3. Comparison of *Clavibacter xyli* subsp. *xyli* population densities with tissue maturity in stalk internodes. The brix of crude juice extracts was used as an indication of tissue maturity. Extracts from stalk internodes three, seven, 11, and 15 from the ground were examined on three different dates for nine sugarcane cultivars. The population densities in pooled sap extracts from the internodes were regressed on the mean brix for crude juice extracts from the same internodes. Linear or quadratic regressions were forced through the intercept for each cultivar examined. The plots represent predicted density values within the range of values actually observed.

was most extensive. However, centrifugal extraction of *C. x.* subsp. *xyli* cells from these tissues was impeded unless the cells were extracted in a basipetal direction. Presumably, extraction in this direction avoids constrictions to the flow of xylem sap, such as vessels ending and narrower xylem vessels associated with the branching of vessels (1). Differences due to the direction of extraction might be more pronounced in resistant cultivars because branching of metaxylem vessels in the nodal region of their stalks is most extensive (20), but this was not investigated. Although significant differences in pathogen densities were observed, the overall ranges of densities were small. Consequently, internodal tissues were selected for further study because the orientation of internodal sections during centrifugal extraction was not important, and the population in internodal sections should be representative of those in adjacent tissues.

Individual sap extracts were pooled in this study to reduce the number of samples while still obtaining accurate estimates of pathogen population densities. Pooling of leaf samples in a study on epiphytic bacteria resulted in an overestimation of population size due to the lognormal distribution of individual populations (13). Likewise, because the frequency distribution of individual populations of *C. x.* subsp. *xyli* is often not normally distributed (5), pooling sap samples resulted in an overestimation of the density of *C. x.* subsp. *xyli* populations in sap extracts. Nevertheless, a strong linear relationship was found between estimates for pooled samples and corresponding mean estimates for individual samples. We assume that the relative physical distributions of the pathogen in sugarcane stalks of different cultivars are probably well represented by the data from pooled sap samples, even though the populations may not have been totally represented in sap extracts because an undetermined portion was probably not extracted.

As previously reported (2) and also observed in this study, populations of *C. x.* subsp. *xyli* first develop a greater density in older sugarcane stalk tissues. Populations were near their maximum densities in the lowest internode sampled (third internode) when samples were first taken in the present study, and relatively small changes in densities for this internode location were detected throughout the remainder of the study. Population densities in younger internodes approached those in older internodes as the season progressed, but rarely exceeded those in older internodes within a given cultivar. Thus, the population dynamics of the pathogen in younger internode tissues probably reflected what had already taken place in older tissues. Presumably, population size in younger tissues would have reached levels equivalent to that of older tissues had the growing season been longer.

During the time period studied, both population density of the pathogen and brix of crude juice extracts were highly correlated and, for the most part, increased with the age of the tissue sampled. Thus, brix measurements might be used as an indicator of when to sample pathogen populations when attempting to predict the response of genotypes to RSD. The correlation between pathogen population density and brix is evidence that the accumulation of dissolved solids in stalk tissues might lead to increased multiplication of *C. x.* subsp. *xyli.* However, if growth of pathogen populations is dependent upon the maturation of xylem tissues, then brix and pathogen population density might only be indirectly related.

In the present study, we established that spatial as well as temporal distributions of *C. x.* subsp. *xyli* in sugarcane stalks can have significant effects on the prediction of susceptibility or resistance of different cultivars to RSD based on pathogen population densities. For such predictions, useful density estimates can be obtained by sampling a specific internode location within the basal portion of stalks as plants reach maturity, because the correlation between yield reduction due to RSD and population densities of the pathogen improved under these conditions. Another advantage to sampling more mature tissues is that pathogen population densities are greater and, therefore, pathogen cells are easier to detect and enumerate. Also, pathogen population densities generally changed the least in older, more mature internodes, evidence that temporal effects may not be as critical if sampling is confined to these tissues. A direct-count technique for estimating the density of *C. x.* subsp. *xyli* populations may have been advantageous because parameters affecting cell viability, such as temporal effects, were probably not as important as they would have been if a colony-count technique had been used.

In the present study, the correlations between *C. x.* subsp. *xyli* population density and yield reduction would have probably been greater if cultivars more resistant to RSD had been used. As such, cultivars H 60-6909, CP 29-116, and CP 72-2086, which typically harbor relatively small populations of *C. x.* subsp. *xyli* (5,15), were originally included in the study but later eliminated from further consideration because too few of these inoculated plants became infected. Correlations between yield reduction and pathogen population density may also have been limited by intrinsic factors, such as host tolerance to RSD. Tolerance to RSD among sugarcane genotypes might prove to be the major factor limiting the accuracy of a screening procedure for RSD resistance based on measuring pathogen population densities. Such tolerance cannot be identified on the basis of pathogen population density alone because it is a relative measurement among host genotypes, based on the amounts of damage to the genotypes in response to equal levels of infestation by the pathogen at the same stage of host development (18). Although not specifically studied, differences in tolerance to RSD apparently exist. For example, in this study and others (5,11,15), cultivar CP 53-1 has consistently contained the largest populations of *C. x.* subsp. *xyli* when compared with other highly susceptible cultivars. However, yield losses sustained by CP 53-1 have been less than those observed for some of these cultivars, such as CP 59-22 (5) and

Table 3. Correlation between *Clavibacter xyli* subsp. *xyli* population densities (cells/ml)$^{1/4}$ in pooled sap extracts and yield loss in plant weight (kg/plant) due to ratoon stunting disease in nine cultivars differing in resistance to ratoon stunting disease[y]

Sampling date	Internode location	No. samples[z]	Correlation coefficient (R)	P
18 October 1983	3	27	0.49	0.0101
	7	25	0.36	0.0768
	11	20	−0.03	0.9080
	15	8	0.16	0.7097
2 December 1983	3	27	0.56	0.0022
	7	27	0.48	0.0122
	11	25	0.42	0.0379
	15	27	0.29	0.1959
22 January 1984	3	24	0.52	0.0089
	7	27	0.49	0.0097
	11	27	0.38	0.0482
	15	25	0.13	0.5499

[y] Mean yield-loss data given in Table 1 was used in this analysis.
[z] Pathogen population densities in pooled sap extracts were determined for each cultivar and internode combination in three plots. Density data was considered missing when the pathogen was not detected in a sample.

F 36-819 (15). Similar situations among cultivars more resistant to RSD are also evident in these studies. Nevertheless, based on this study and other studies (2,5,11,15), screening for resistance to RSD on the basis of pathogen population densities appears feasible, if an appropriate protocol for sampling pathogen populations can be established.

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