Distribution and Incidence of Ratoon Stunting Disease in Louisiana Sugarcane

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ABSTRACT

Damann, K. E., and Hollier, C. A. 1991. Distribution and incidence of ratoon stunting disease in Louisiana sugarcane. Plant Dis. 75:568-571.

Different incidences of ratoon stunting disease (RSD) were found by alkaline-induced metaxylem autofluorescence (AIMA) in stalks of sugarcane cultivar CP 76-331 released to Louisiana growers in the fall of 1984. The canes released in the eastern and western sugarcane growing areas were the culmination of 14 yr of selection and increase from a common source. The frequencies of diseased stalks assayed in the plant cane crop (1984) and first ratoon crop (1985) were 0 and 3% in the eastern area and 15 and 33% in the western area, respectively. The RSD increases from plant cane to first ratoon crops resulted from mechanical transmission of the disease. The asymmetric distribution between areas reflects the different levels of infection among the original seed sources. Frequencies of diseased stalks and infested fields in commonly grown cultivars assayed in 1986 were: CP 65-357, 33 and 75%; CP 74-383, 33 and 75%; CP 76-331, 22 and 55%; CP 70-321, 18 and 54%; CP 74-356, 16 and 55%; and CP 72-370, 3 and 34%. Disease incidence in CP 76-331 stalk samples was 2% in the eastern area and 36% in the western area, reflecting the asymmetric distribution found in the release plots in 1984 and 1985. Disease incidence across all cultivars was 22% of 3,607 stalks and 59% of 184 fields. Incidence of RSD for all cultivars increased approximately 10% annually after heat treatment of seed through the fifth year. The 22% incidence in standing cane across all cultivars, plus a 10% increase presumably attributable to mechanical spread by the harvester before replanting, suggested that one-third of the seed planted in Louisiana in 1986 may have had RSD.

Ratoon stunting disease (RSD) of sugarcane is cited as being the most important sugarcane disease in the world (13). This is attributable in part to its cryptic and chronic nature, a classic example of an endemic disease sensu Vanderplank (15). The disease is caused by the xylem-limited, fastidious bacterium *Clavibacter xyli* subsp. *xyli* Davis et al (8,9). The disease is spread by planting infected sugarcane stalks or by harvesting with a mechanical harvester or other implements that introduce the bacterium into the plant's xylem vessels. No biological vector other than man has been detected. Yield loss attributable to RSD in Louisiana ranges from 5 to 32% over the 3-yr crop cycle, depending on cultivar and growing conditions (10).

The primary approach to the control of RSD is to plant disease-free seed and adopt sanitary cultural practices that prevent the introduction of the causal organism by machinery or other means. Heat treatment has been used to remove the RSD bacterium from seed. Because a low percentage of infected stalks escape being cured and healthy stalks are reinfected by mechanical transmission, an ongoing program of heat treatment is required. The cost-benefit ratio for heat treatment was found to be $1 to $11.50 (12). Nevertheless, the control practice is not widely used in Louisiana (6). Another approach to propagate disease-free seed is to develop meristem-tip cultures that are free of disease. The regenerated plantlets are transplanted to the field. Stalks derived from the plantlets free from RSD are sold for vegetative propagation.

A major problem in managing RSD in Louisiana is the selection of clean sources of vegetative seed. The disease symptoms are obscure and internal, and growers are not able to determine the disease status of their prospective seed plots. Development of a diagnostic technique, alkaline-induced metaxylem autofluorescence (AIMA), has allowed detection of the cryptic disease (5). The AIMA assay was used in the present study to assess the geographic distribution and frequency of RSD in 26 of 38 secondary station plots from which cv. CP 76-331 was released to growers in the fall of 1984 and in 184 commercial fields of sugarcane in 1986. Abstracts of this work have been published (4,7).

MATERIALS AND METHODS

Distribution of RSD among secondary station plots. Fourteen plots of sugarcane (*Saccharum* interspecific hybrid) cv. CP 76-331 located along the Mississippi River and Bayou LaFourche (eastern area) were sampled on 13 August 1984 as plant cane and 16 September 1985 as first ratoon. Plots along Bayou Teche (western area) were sampled 5 September 1984 and 13 September 1985. The eastern and western cane-growing areas are separated by the Atchafalya Basin. Plots are located on commercial farms throughout the sugarcane belt and maintained by the farm labor and equipment. Sampling of individual plots started from a corner nearest the access road and continued on a diagonal across the field to the other corner until either 10 (1984) or 20 (1985) basal two-node cuttings of sugarcane stalks were collected. An attempt was made to span the entire field. The samples from each plot were processed in the laboratory the next day.

In 1984, the 10 samples from each field were recut to excise a section of stalk internode 8–10 cm in length. The section

Approved for publication by the director of the Louisiana Agricultural Experiment Station as manuscript 88-38-2626.

Accepted for publication 6 November 1990 (submitted for electronic processing).

was centrifuged in a 50-ml conical bottom centrifuge tube at 1,000 *g* for 3 min to extract xylem contents. The internode was removed from the centrifuge tube and a cross section (1–3 mm thick) was cut freehand with a knife or single-edged razor blade. The cross section was placed on a glass slide and 0.1 M Tris, pH 10, was applied to the cut surface. An aliquot of the xylem extract was removed from the tube with a disposable pipet and dropped on the same glass slide as the cross section. The drop was covered with a No. 1 coverslip. All samples were observed for the prescence of the characteristic coryneform *C. x. xyli* in darkfield microscope images of the xylem extract. The extracts were observed at ×500 with a Leitz Ortholux II microscope with a Phaco 1 ×32 long-distance objective in conjunction with phase ring II on the condenser. In 1985, only the AIMA was performed.

The AIMA assay was done as previously described (5), with a Wild dissecting scope fitted with a Leitz epifluorescence attachment (Wild-Leitz USA Inc, Rockleigh, NJ). A positive diagnosis for RSD was rendered when the secondary walls of metaxylem vessel elements fluoresced bright red.

RSD incidence in commercial fields. County agents of the Louisiana Cooperative Extension Service from nine of the 16 sugarcane-growing parishes selected several growers from their parishes to sample potential seed sources. Sampling was done during the last week of August 1986 by collecting 20 basal two-node cuttings from either a diagonal or a V-shaped path across the entire field. Cuttings were placed in a plastic bag along with an identification card with the following information: parish, date of collection, farm/collector, crop year, field designation, field size, and years after heat treatment if treated. Samples were delivered to the lab, refrigerated, and processed as quickly as possible. The AIMA assay was performed as previously described (5), and the frequency of diseased stalks per 20-stalk sample was determined. Frequency data along with the information from identification cards were collated with a SAS software program (SAS Institute Inc., Cary, NC) on a personal computer. Frequencies of disease by cultivar, location, and years after heat treatment were determined.

RESULTS

Distribution of RSD among secondary station plots. In 1984, RSD in release plots of CP 76-331 appeared to be distributed in a geographically asymmetric manner. None was detected in the 14 plots in the eastern region of the sugarcane belt (i.e., Mississippi River and Bayou LaFourche area), but nine of the 12 plots in the western area along Bayou Teche had RSD (15% of the stalks). Diagnosis of the disease by autofluorescence was confirmed by observation of bacteria in the xylem fluid. In 1985, RSD was detected in four of 14 plots (3% of the stalks) in the eastern area and 11 of 12 plots (33% of the stalks) in the western area (Table 1).

The system of vegetative increase and distribution of sugarcane cv. CP 76-331

Table 1. Ratoon stunting disease in stalk samples from Louisiana release plots of sugarcane cultivar CP 76-331 in 1984 (plant cane) and 1985 (stubble cane crop)

Location	RSD incidence[a] 1984	1985
East—Mississippi River		
New Hope	0/10	0/20
Evan Hall	0/10	0/20
Graugnard Farms	0/10	0/20
Glendale	0/10	0/20
East—Bayou Lafourche		
Lula	0/10	0/20
Westfield	0/10	0/20
Glennwood	0/10	0/20
Little Texas	0/10	0/20
Cedar Grove	0/10	3/20
Thibodeaux Bros.	0/10	0/20
Leighton	0/10	2/20
Raceland	0/10	2/20
Georgia	0/10	0/20
McLeod	0/10	2/20
Total	0/140	9/280
Percentage	0	3
West—Bayou Teche		
Calumet Farms	1/10	4/20
Sterling	1/10	11/20
Sterling	3/10	14/20
Breaux Bros.	1/10	6/20
Oaklawn	0/10	2/20
Allain	2/10	8/20
Longman Parks	0/10	6/20
Great Southern	5/10	12/20
Ronald Hebert	0/10	0/20
Enterprise	1/10	11/20
Caroline	1/10	4/20
Triple V Farms	3/10	1/20
Total	18/120	79/240
Percentage	15	33

[a]Number of stalks that were positive for RSD per number sampled. The alkaline-induced metaxylem autofluorescence assay was used for RSD disagnosis in 1984 and confirmed by microscopic detection of the causal bacterium. Only the autofluorescence assay was used in 1985.

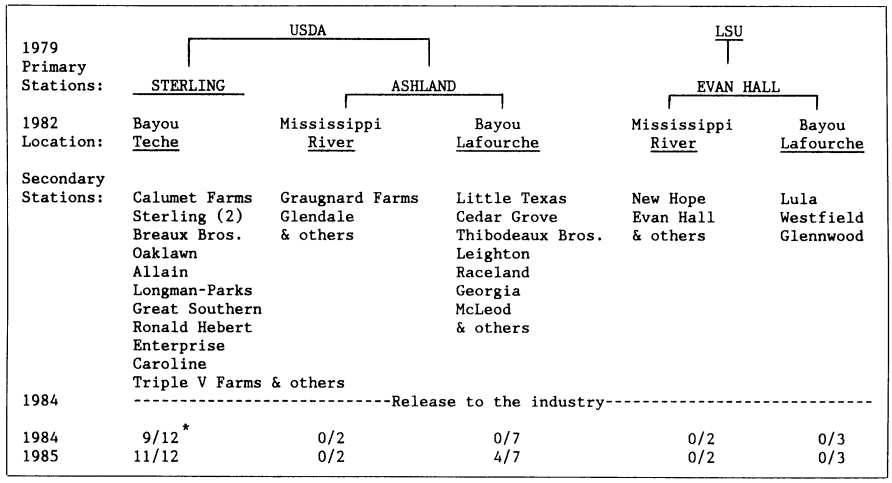

Fig. 1. Frequency of secondary station plots with ratoon stunting disease superimposed on the pathway of distribution and vegetative increase of healthy and diseased sugarcane cv. CP 76-331 during its final stages of selection and release to Louisiana growers. * = Number of secondary station plots that had at least one stalk sample positive per 10 or 20 stalks per field as determined by the alkaline-induced metaxylem autofluorescence method.

Table 2. Frequency of ratoon stunting disease by sugarcane cultivar and geographic region in 1986 in Louisiana[a]

Cultivar	Sample size[b]	Disease frequency (%) East[c]	West[c]	Total
CP 65-357	964	32.5	36.3	33.2
CP 74-383	384	23.6	59.0	32.8
CP 76-331	423	1.7	36.3	22.4
CP 70-321	1,092	22.0	10.6	18.3
CP 72-356	220	17.0	0.0	15.5
CP 72-370	495	2.2	10.0	3.4
CP 70-330	19	74.0	...	74.0
Total	3,607			
Mean		20.8	26.6	22.2

[a]Stools from which these samples were taken were destined for use as vegetative seed in the fall.
[b]Number of stalks of each cultivar sampled.
[c]Geographic region of the Louisiana sugarcane growing area separated by the Atchafalaya Basin.

during the final stages of testing and selection is outlined in Figure 1. The highest incidence of RSD occurred in secondary plots of CP 76-331, which had been supplied vegetative seed from the Sterling primary station.

Incidence of RSD in commercial fields. In 1986, 43 growers submitted samples from 184 fields of commercially grown sugarcane that were prospective sources of vegetative seed. The overall frequency of RSD across all cultivars was 22% of 3,607 stalks sampled (Table 2). The RSD incidence by cultivar appeared to be distributed among three classes when calculated on the percentage of stalks infected with RSD (Table 2) or on the frequency of fields infested with RSD (Table 3). High-incidence cultivars were CP 65-357 and CP 74-383; intermediate-incidence cultivars were CP 70-321, CP 72-356, and CP 76-331; and CP 72-370 was a low-incidence cultivar. Because there was only one field of CP 70-330 sampled, it was not classified.

The RSD frequency across all seven cultivars (1,635 stalks from 82 fields) as a function of time (years) after heat treatment for control of RSD increased about 10% per year and was about 37% after 5 yr (Fig. 2). The frequency of RSD in samples that growers indicated had not been heat-treated was 33% (1,259 stalks from 63 fields).

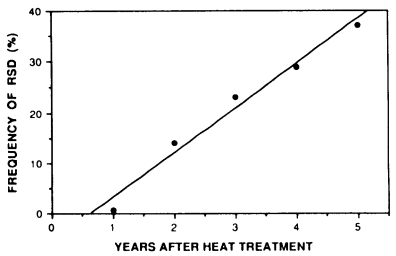

Fig. 2. Frequency of ratoon stunting disease in sugarcane cultivars grown in Louisiana in 1986 regressed over years after heat treatment of seed cane. Each point from the first through the fifth year after heat treatment is the mean incidence from 17, 30, 20, nine, and six fields, respectively ($Y = -5.62 + 8.78X$) ($R^2 = 0.98$).

DISCUSSION

The RSD was asymmetrically distributed in CP 76-331 for both 1984 and 1985, with a high incidence in the western and a low incidence in the eastern areas of Louisiana (Table 1). The plots were sampled in 1985 to confirm the distribution found the year before and to estimate the spread that occurred during the 1984 harvest in the release plots. Disease levels in the plots in 1985 may reflect the true incidence of disease in the seed the growers received in 1984. This assertion is based on the assumption that the unmeasured frequency of infection by the causal bacterium upward into healthy stalks cut for seed cane at the 1984 harvest was approximated by the frequency of infection of the healthy rooted stubble found in the plots in 1985.

Ratoon stunting disease was detected in 1985 in the eastern area in four plots along Bayou LaFourche where no RSD was detected in 1984. This could be attributed to the introduction and/or spread of RSD by the harvester. Also, the probability of detection was increased by doubling the sample size and may be illustrative of the limits of the sampling protocol used. The probability from a binomial distribution of finding one or more positive samples out of 10 or 20 when the incidence is 0.05, 0.10, or 0.15 is 40, 65, and 80% for 10 samples and 64, 88, and 96% for 20 samples per plot. Therefore, the probability is low for detecting a disease incidence of <0.05 with a 10- or a 20-stalk sample.

Approximately 1,773 t of stalks (0.9 kg per stalk = 1.97 million stalks) of sugarcane cv. CP 76-331 was released from 38 secondary station plots to the Louisiana growers in the fall of 1984. This represents between 197,000 (10 stalks per stool) and 394,000 (five stalks per stool) stools or individual plants of sugarcane. The 520 total samples taken in 1985 represent between 0.13 and 0.26% of the stools released.

The 33% incidence of RSD in the 12 western plots of cv. CP 76-331 found in 1985 suggests that one-third of the seed of that cultivar planted in the western area in 1984 may have been diseased. Clearly, the indexing of plants from prospective seed sources for RSD incidence could be used to limit the distribution of diseased seed into commercial fields. The pattern of distribution of vegetative seed of this cultivar from the ninth year (1979) after its selection until its release (1984) suggests that the high incidence of RSD in the western area developed during the 3 yr of vegetative propagation at the Sterling primary station. In the fall of 1982, CP 76-331 from the Sterling primary station was distributed to 16 secondary stations in the western area along Bayou Teche (Fig. 1). The mean RSD incidence in the first ratoon crop across the 10 stations sampled other than Sterling was 22%. The mean incidence at the two plots at Sterling was 63%. Two other secondary stations, Enterprise and Great Southern, approached the high disease incidence found at Sterling.

The RSD incidence in cultivars from commercial fields differed with the growing area (Table 2). The asymmetric distribution in CP 76-331 (about 2% in the eastern area and about 36% in the western sugarcane area of Louisiana) in 1986 may be explained by the similar asymmetric distribution of RSD in the seed cane of this cultivar released to growers in 1984 (Table 1). The growers in the eastern area received relatively clean seed cane and maintained it, whereas growers in the western area received diseased material and maintained it. This result emphasizes the need to test for RSD and select disease-free stalks for propagation in order to control this disease.

Several sugarcane-producing countries have made estimates of RSD incidence. South Africa reported a decline in RSD incidence from 32 to 21% in infested commercial fields between 1977 and 1983, whereas seed cane field infestation levels dropped from 25 to 12% over the same time. The decline was presumably attributable to the utilization of a diagnostic service for detection of the causal bacterium. In three of the warmer regions of South Africa, 40–55% of the commercial fields were infested with RSD (1). In 1986, the incidence found in Lousiana seed cane (22.4% of the stalks from 59% of the fields) is similar to that reported from South Africa. In Australia, RSD incidence in commercial fields from 1983 to 1986 varied between 18 and 37% (14). In Taiwan, RSD occurred in up to 73% of the sampled stools of NCo 310 (3). As a result of a heat-treatment program for RSD, the incidence dropped to 14% (2).

In our survey, the first progeny of heat-treated cane had a very low incidence of RSD, as would be expected for an effective control. The frequency of disease each year thereafter increased by about 10%. By 5 yr after heat treatment,

Table 3. Frequency of sugarcane fields with ratoon stunting disease and percentage of acreage by cultivar in Louisiana in 1986[a]

Cultivar	Louisiana acreage (%)	Fields sampled		Sample fields infested (%)
		Sample (%)	No.	
CP 65-357	24	27	49	71[b]
CP 74-383	9	11	20	75
CP 76-331	2	12	22	55
CP 70-321	41	30	56	54
CP 72-356	9	6	11	55
CP 72-370	9	14	25	36
CP 70-330	4	0	1	100
Total	98	100	184	
Mean				59

[a]Samples were taken from stools destined for use as vegetative seed in the fall.
[b]A field was considered infested with ratoon stunting disease if one or more of the approximately 20 stalks sampled per field was autofluorescence positive.

there would have been four harvests. Each harvest would be capable of introducing or spreading RSD in a field. The incidence of RSD in the samples that growers indicated had not been heat treated was 33%. Thus, although heat treatment was initially effective, reintroduction of disease by mechanical harvesters brought it back to ambient levels within 5 yr. This reinforces the need for implementing an ongoing program of heat treatment and/or planting of clean seed cane for effective control of RSD.

In conclusion, RSD incidence in Louisiana was estimated by the alkaline-induced metaxylem autofluorescence assay (5). Growers were informed of the estimates of RSD incidence in their prospective seed cane sources. Information concerning the disease levels in commercial sugarcane cultivars was obtained and appeared similar to those found in other sugarcane-producing areas of the world. These levels may be indicative of the inherent infectability/susceptibility of the various cultivars. In infectivity titration experiments, the frequency of infection was correlated with a cultivar's sensitivity to yield loss from RSD (11). The 22% incidence of RSD in standing cane plus an average 10% annual increase presumably attributable to mechanical spread by the harvester before replanting, was evidence that one-third of the seed cane commercially planted in Louisiana in 1986 had RSD.

ACKNOWLEDGMENTS

We thank Windell Jackson, Charley Richard, and Herman Waguespack, Jr., of the American Sugar Cane League for their congenial help in sampling the secondary station plots. We also thank the county agents and growers who participated in collecting the samples and Jeff Hoy, Lori Grelen, and Roy Navarre for their technical assistance. We thank David Pope, University of Georgia, for the SAS collations of survey data and Vernon Wright of the LSU Department of Experimental Statistics for discussions of sampling.

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