Determining the Status of Ratoon Stunting Disease (RSD) in the Jamaican Sugar Industry

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ABSTRACT

Ratoon Stunting Disease (RSD) caused by the organism *Leifsonia xyli* subsp. *xyli* (previously named *Clavibacter xyli* subsp. *xyli*) is of major concern in many sugar producing countries of the world, as its impact on production can be very severe. Surveys were conducted on farms in the five ecological areas to determine the level of intensity and distribution of the disease in the Jamaican sugarcane industry. Sugarcane stalks 10-12 months old, were randomly selected and tested for the presence of the RSD pathogen, using the Tissue Blot Immunoassay Technique (TBIT). The incidence of the disease, expressed as a percentage of stalks infected, ranged from 0 – 14.0% on the estates, with Duckenfield being the highest (14.0%) followed by Worthy Park (10.0%) and Frome (3.8%). The relatively high incidence at Duckenfield was associated with the variety D14146 (46.7%). The other varieties showing high levels of occurrence islandwide were BJ82156 (13.3%), B41227 (6.6%), BJ7627 (5.8%), and BJ7465 4.1%. The incidence of the disease amongst the non-estate farms ranged from 0 – 5.9%. The results indicated that, though fairly widespread in distribution, the presence of RSD across the industry was relatively low since the mean incidence did not exceed 10%.

INTRODUCTION

Ratoon stunting disease (RSD) has been recognized as a factor limiting sugarcane production in most sugarcane producing countries. Since the disease was first discovered in Queensland, Australia in 1944-45 Steindl (1961), its impact has warranted serious concern as loss in yield can reach over 60%, especially in ratoon crops, Scarlett (1980), depending on the susceptibility of the variety and the weather conditions.

RSD is caused by the bacterium *Leifsonia* (*Clavibacter*) *xyli* subsp. *xyli* which infects the xylem vessels of the sugarcane plant. So, a discoloration of the internal tissue near the lower part of the node of the stalk, when split, is sometimes noticeable. A series of experiments conducted in 1952, had shown that a fairly accurate indication of the disease was the presence of these reddened vascular bundles (Steindl, 1961). Iglesia (2003) reported that a gummy substance, including the bacterium, blocked many of the vessels of the plant vascular system. The absence of specific external symptoms has made RSD very difficult to identify in the field, but stunting with reduced growth and shortened slender internodes are the general external expressions, which can be caused by a wide range of other factors. The presence of the pathogen however can be positively determined by laboratory analysis of which the tissue blot immunoassay technique (TBIT) is one that was recently developed.

Ratoon stunting disease has now attained widespread geographical distribution. In a report from

Barbados, Paulraj (2001), widespread occurrence of RSD was found in commercial fields. The percentage positive fields found in 86% of the plantations sampled, ranged from 25% to 80%. In Louisiana, the disease incidence across all cultivars was 22% (Damann 1990). French Guyana reported RSD in samples of variety B64277, (Feldmann *et al*., 1997). In 1992, the survey conducted in Guyana by a U.S. pathologist indicated RSD was not present. A survey done in Trinidad in 1990 indicated that although present, the population of RSD was low (SAC Handbook**,** 2000). Belize Sugar Industries Ltd (BSI) reported the presence of the disease in approximately 1.3% of stalks sampled (Daugrois, 2006). The varieties found infected were JA6419 (Cuban variety), C8751 and BBZ8505, with C8751 the most heavily infected variety giving an infection rate (percentage of positive fields) of 4.5%. In a survey conducted in Martinique as reported by Truffaut (1996), out of 104 samples taken, 23% was diagnosed with the disease. In Guadeloupe 4.3% of samples were positive to RSD. However, because of a healthy seedcane nursery scheme (**in vitro** multiplied plants), the disease was not found during a two year survey (Daugrois, (1998 and 2001). The Cuban sugar industry has mentioned RSD since 1953 and according to a report by Iglesia *et al*. (2003), the level of infection was 81% with an estimated yield impact of 19%. Field studies in South Africa showed yield reduction of 20%-40 % (Bailey and McFarlane, 1998).

Since the early 1950s, Jamaica has been listed among the countries with ratoon stunting disease. Preliminary work undertaken at Long Pond on Co421, suggested the presence of RSD in Jamaica (Smith, 1955; Anon., 1955). Consistently high correlation between intensity of symptoms and effect of the disease in Co421 was recorded, Smith and Manser (1958), and a depression of yield by 29% in the 1st ratoon crop was noted (Anon., 1956).

Dr. Michael J. Davis, from the University of Florida, conducted an industry-wide survey in Jamaica in 1987 using the fluorescent antibody staining technique (FAST) but was unable to identify positives in the 61 fields sampled. However, another survey initiated in 2004 by SIRI, in conjunction with CIRAD, the French research agency in Guadeloupe, found L*xx* in 9 fields of the 64 sampled (Anon., 2004). Seven varieties showed positives, with D14146 in St. Thomas, showing the highest number of positives, whilst the highest number of infected fields occurred at Worthy Park (Falloon and Henry, 2004). The percent incidence of the disease recorded was 7%, as 26 out of a total of 384 samples (stalks) were infected.

In view of the potential loss from RSD and to obtain a more comprehensive picture of its presence in Jamaica, this study was undertaken to determine its intensity and distribution.

MATERIALS AND METHODS

The survey was a joint venture between SIRI, CIRAD and the Ministry of Agriculture and was conducted between late 2005 and early 2007 on 163 fields across the industry.

Cane Sampling:

At each 10-12 month old cane field, with a cycle of 6th ratoon and over, at a distance of 20 m inside the field along the perimeter, 15 sugarcane stalks were chosen at random. The stalks were cut as close to the ground as possible at the internode with a sharp machete. They were further cut to lengths of approximately 1 m, bundled, tied and labeled with tags bearing pertinent details of both field and variety. The collected samples were then taken to the SIRI laboratory where they were cut with a sharp knife 3 cm below a node. A cork borer of 1 cm in diameter was used to remove an inner core of cane. This core, containing xylem vessels, was then pressed for 15 seconds on a nitrocellulose membrane, with an absorbent paper below to absorb the excess cane juice. The blotted prints were then air-dried, covered with protective paper and placed in an envelope to be stored and analyzed at the Ministry of Agriculture's Bodles laboratory (St. Catherine, Jamaica) and random samples selected and sent to the CIRAD station in Guadeloupe for comparison. Both laboratories use the tissue blot immunoassay technique (TBIT) to detect the RSD pathogen.

Procedure

The *Leifsonia xyli* subsp. *xyli* detection process using Lxx antibody from goat was provided by CIRAD, Montpellier. The blotted membranes were immersed in 150 ml of 3% Bovine Serum Albumin (BSA) in Tris Buffered Saline (TBS buffer) for 30 mins. For the first antibody, the nitrocellulose membranes were incubated in goat L*xx* polyclonal antibody IgG diluted 1:5000 in 1% BSA in TBS for 1.5 hours at room temperature. The NC membranes were then washed with 3 rinses of 5 minutes each in TBS Tween (polysorbate detergent). For the second antibody, the rinsed membranes were then incubated in rabbit IgG alkaline phosphatase conjugate diluted 1:8000 in 1% BSA for 1 hour at room temperature. The membranes were again washed with 3 rinses of 5 minutes each in TBS Tween. Membranes were then immersed in alkaline phosphatase substrate for 5 to 20 minutes at room temperature. The reaction was stopped by dipping the membranes in distilled water. After drying, the prints were observed under the microscope where the colonized vascular bundles appeared blue.

RESULTS AND DISCUSSION

Six of the seven estates sampled, Appleton, Bernard Lodge, Long Pond, Frome, St. Thomas and Worthy Park, showed the presence of the pathogen. The seventh, Monymusk, showed zero infection in fields sampled. The highest incidence occurred in St. Thomas (14.0% of stalks sampled), followed by Worthy Park (10.0%) and then Frome with 3.8% (**Table 1**). The relatively high incidence in St. Thomas was associated with the previously determined susceptible variety D14146 which showed stalk infection of 46.7% (**Table 3**).

At Worthy Park, BJ82156 seemed to be favoured by the bacterium, as all the fields of this variety sampled showed the presence of the pathogen. Low pathogen intensity was also detected at Appleton, Bernard Lodge (inclusive of Innswood and Caymanas) and Long Pond. Negative results, i.e., the tissue-blot test did not detect the bacterium in the stalks sampled, were obtained at Monymusk and New Yarmouth.

Table 1: Ratoon Stunting Disease Survey, 2006, Summary – Estates

Estate	Number of fields Sampled	Number of fields Infected	Number of stalks Sampled	Number of stalks Infected	% Stalks Infected
Appleton	8	1	120	1	0.83
Bernard Lodge	28	3	402	4	1.00
Frome	20	8	290	11	3.79
Long Pond	13	2	189	3	1.59
Monymusk	14	0	204	0	0
New Yarmouth	4	0	60	0	0
St. Thomas	8	2	150	21	14.00
Worthy Park	10	6	150	15	10.00

Incidence of the pathogen amongst the non-estate farms was highest in Clarendon (5.9%), where 75% of the sampled fields were infected (**Table 2**). Most fields sampled were from St. Catherine, but the pathogen was detected in only 1.2% of stalks sampled. Infected fields on non-estate farms were 50% less than on the estates, which recorded an overall stalk infection of 2.1%. From a total of 2411 stalks sampled throughout the industry, 3.03% showed some degree of infection. These results have confirmed the presence of RSD at relatively low levels of intensity across the industry, except in two locations where the incidence was 10% and over.

Table 2: Ratoon Stunting Survey, 2006, Summary – Farmers' Holdings

Farmers' Location	Number of Fields Sampled	Number of Fields Infected	Number of Stalks Sampled	Number of Stalks Infected	% Stalks Infected
Clarendon	8	6	118	7	5.93
Frome	9	1	133	4	3.01
St. Elizabeth	5	0	73	0	0
St. Catherine	29	3	427	5	1.17
St. Thomas	7	1	95	2	2.11
			846	**18**	**2.12**

Fifteen varieties showed the presence of the pathogen.- D14146, BJ82156, B41227, BJ8252, BJ7314, BJ7627 and to a lesser extent, BJ7465, seemed to be the relatively susceptible varieties, with D14146 being the most susceptible (**Table 3**). The varieties showing no incidence of the RSD pathogen were B49119, BJ8226, BJ7262, BJ7555, BJ7230, J9501 and BJ8207. However, BJ8226 and BJ7262 (with stalk infection of 20.8% and 41.7% respectively) had shown the pathogen in the 2004 survey.

Table 3: Ratoon Stunting Disease Survey, 2006, Summary – Varieties

Variety	Number of Fields Sampled	Number of Fields Infected	Number of Stalks Sampled	Number of Stalks Infected	% Stalks Infected
BJ7504	39	4	589	5	0.85
BJ7015	22	1	313	1	0.32
BJ7627	8	3	120	7	5.83
B41227	3	1	45	3	6.67
B49119	2	0	30	0	0
BJ8252	1	1	15	1	6.67
BJ8226	5	0	69	0	0
BJ82119	4	2	58	2	3.45
BJ7452	11	1	163	1	0.61
BJ7262	3	0	45	0	0
BJ7465	20	6	292	12	4.11
BJ82102	4	1	60	1	1.67
BJ78100	15	3	211	5	2.37
BJ82156	5	5	75	10	13.33
BJ7555	1	0	15	0	0
UCW5465	6	1	88	1	1.14
BJ7451	3	1	45	1	2.22
BJ7230	1	0	15	0	0
J9501	1	0	15	0	0
BJ7314	2	1	30	2	6.67
BJ8207	2	0	45	0	0
D14146	2	2	45	21	46.67
UK	3	0	43	0	0
Total	**163**	**33**	**2411**	**73**	**3.03**

Table 4 shows the incidence of fields testing positive for RSD at different locations and six of the varieties, viz: **BJ7314, BJ7627, BJ7465, B41227, BJ82156 and D14146**, that showed fairly high incidence of the disease in Jamaica. The rate of infection (percentage of positive fields) ranged from 11 to 60% on the estates, whereas for the farmers the rate was 11% or over, with the highest rate (75%) recorded in Clarendon. Two varieties, BJ82156 and D14146, seemed to be favoured as the infection rate recorded for both was 100%. BJ82156 was associated with Worthy Park with the highest percent of positive fields (60%). Although D14146 was located only at St. Thomas Sugar Co, only 25% of total estate fields sampled were positive. Farmers' fields recorded 14% positives. The relatively widespread occurrence of RSD in Worthy Park and Clarendon could possibly be due to the use of mechanical harvesters in those areas.

Table 4: Percentage positive fields for RSD at different locations/ varieties in Jamaica

Location/Estates	**# of Fields Sampled**	**% of Positive Fields**
Appleton	8	13
B/Lodge	28	11
Frome	20	40
Long Pond	13	15
St. Thomas	8	25
Worthy Park	10	60
Location/Farmers		
Clarendon	8	75
Frome	9	11
St. Catherine	29	10
St. Thomas	7	14
Variety		
BJ7314	2	50
BJ7627	8	38
BJ7465	20	30
BJ41227	1	33
BJ82156	5	100
D14146	2	100

CONCLUSION

While the presence of RSD in Jamaica has been re-confirmed by this survey, the incidence of the disease was found to be relatively low, since the mean incidence did not exceed 10%. The fairly high rate of infection (percentage of positive fields) found at Worthy Park and Clarendon might be attributed to the extensive use of mechanical harvesters which is a known means of spreading the disease. The decontamination of tools used to harvest sugarcane should be given serious consideration. Heat treatment of newer varieties prior to distribution to growers and the establishment of clean seedcane nurseries both underway in the Sugar Industry Research Institute should aid in arresting the spread of RSD in Jamaica.

ACKNOWLEDGEMENT

The author wishes to extend sincere appreciation to Messrs. Malcolm Bennett-Easy and Errol Henry for their input in this paper. Sincere thanks are extended to Dr. Lisa Myers, Bodles Research Centre, Ministry of Agriculture,for the invaluable work done in the laboratory analysis of the samples, and to Mr. Trevor Falloon for approva lto present these results.

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