

IDENTIFICATION OF SOURCES OF RESISTANCE TO SUGARCANE RED ROT

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Abstract

RED ROT, caused by *Colletotrichum falcatum* Went, adversely affects sugarcane stand establishment in Louisiana by rotting planted stalks. Since cultivar resistance is the most effective control method, a study was conducted to identify sources of resistance to red rot and evaluate variability within *Saccharum* species and *Erianthus*. *Saccharum spontaneum*, *S. robustum*, *S. sinense*, *S. officinarum*, *S. barberi*, and *Erianthus* clonal accessions were evaluated for resistance alongside susceptible commercial cultivars in three experiments. Harvested stalks were inoculated with *C. falcatum*. Following a 6-week incubation, each stalk was split and rated for red rot symptoms including number of nodes passed (NP), number of nodes rotted (NR), internode rot severity (IRS), and a rot index (RI) that takes into account both the extent of node damage and internode rot. Significant differences were detected among species and accessions within species for all traits. Among the *Saccharum* species, *S. barberi*, *S. robustum* and *S. spontaneum* exhibited the lowest red rot symptom severity means, while *S. officinarum* and *S. sinense* had the highest severity means. A high level of variation was observed within *S. spontaneum* with RI means ranging from two to 29. Three of 31 *S. spontaneum* accessions (10%) had an NR mean less than 5, and 26% had a mean less than 10. *S. barberi* exhibited a high level of resistance, with RI means ranging from one to 20. Four of 14 *S. barberi* accessions (29%) had an RI mean of less than 5 (highly resistant), and six (43%) had a mean of 10 or less (moderately resistant). The least amount of variation was seen among the *Erianthus* accessions, with all showing little, if any, red rot symptoms. The *Erianthus* genus possesses high resistance and should be used as parental material in breeding programs where enhanced red rot resistance is needed. Other more easily utilised sources of resistance to red rot within the *Saccharum* germplasm are selected *S. spontaneum* and *S. barberi* accessions.

Introduction

Red rot, caused by *Colletotrichum falcatum* Went, damages sugarcane (inter-specific hybrids of *Saccharum* L.) by rotting standing cane and planted stalks (Singh and Lal, 2000). In Louisiana, red rot adversely affects stand establishment by rotting planted seedcane (Abbott, 1938). Red rot is difficult to control through hot-water and fungicide treatments (Singh *et al.*, 2008). Therefore, the disease is controlled with host plant resistance. A heritability study conducted in Louisiana indicated that resistance can be increased by careful cross-based selection (Yin *et al.*, 1994). However, an additional finding of this study was that the frequency of resistance was low within both breeding and selection populations. The results suggested that the introgression of genes from related wild germplasm might be needed to enhance red rot resistance in the parent population.

In India, a high frequency of resistance was demonstrated among *S. spontaneum* clones (Satyavir *et al.*, 1994; Srinivasan and Alexander, 1971). The objectives of this study were to identify sources of red rot resistance in *Saccharum* species and *Erianthus* that might be utilised in future breeding efforts to develop resistant cultivars and evaluate variability in resistance within this material.

Materials and methods

Resistance screening experiments were conducted in 2004, 2005, and 2008. A total of 90 *Saccharum* and nine *Erianthus* clonal accessions were evaluated for resistance to sugarcane red rot. The accessions evaluated are part of a collection maintained at the USDA-ARS Sugarcane Research Unit, Houma, LA. *Saccharum* accessions tested represented five species, as well as a limited number of commercial sugarcane cultivars (Table 1). Variable numbers of accessions were evaluated each year. Accessions in *Erianthus* included four *E. arundinaceus*, two *E. bengalense*, and one each of *E. procerus* and *E. ravennae*.

Data were assessed yearly to determine if there were differences between species and among clones within each species during any given year. In addition, a set of clones from each of the *Saccharum* species was included in a multi-year analysis to determine genotype, environment, and genotype x environment interactions. Fourteen *S. barberi*, 11 *S. sinense*, seven *S. officinarum*, seven *S. robustum*, and 31 *S. spontaneum* clones were analysed in 2004 and 2008 along with the susceptible commercial cultivar LCP85-384. The screening population varied among experiments, and not all clones were included in the multi-year analysis due to the lack of some materials for additional testing and the receipt of new imports into the collection as the study progressed.

Table 1—Number of *Saccharum* and *Erianthus* accessions screened for resistance to red rot in 2004, 2005, and 2008, and across all three years (Total number). A multi-year analysis was conducted on a subset of *Saccharum* clones in 2004 and 2008.

Accession type	Total number	2004	2005	2008	2004 and 2008
Commercial hybrid	6	1	6	2	1
<i>Erianthus</i> spp.	9	1	9	9	0
<i>S. barberi</i>	17	15	15	15	14
<i>S. officinarum</i>	9	9	3	6	7
<i>S. sinense</i>	11	11	11	11	11
<i>S. robustum</i>	11	9	0	7	7
<i>S. spontaneum</i>	36	32	26	33	31

The accessions were planted in February 2004, 2005, and 2008 from nodal cuttings and grown in 37.8 L containers in a soil-less potting medium. In December of each year, four undamaged stalks of each accession were cut, and the leaves were removed. Stalks were then dipped in a 0.26% solution of sodium hypochlorite for 10 minutes to surface disinfect and they were inoculated with *C. falcatum*. A 3.2-mm-diameter hole was drilled into, but not through, the centre internode of all four stalks of each accession, and 100 μ L of a conidial suspension of *C. falcatum* (3.8×10^6 spores/mL) was introduced. Stalks were wrapped inside a perforated sheet of polyethylene and kept moist for 6 weeks at room temperature. Following incubation, all stalks were split longitudinally and assessed for disease severity. Each stalk was rated for the following symptoms: i) number of nodes passed (NP) by internode rot symptoms in each direction from the inoculation point, ii) number of nodes exhibiting rot symptoms (NR), and iii) internode rot severity (IRS) in each internode, including the one inoculated and three in either direction. IRS was visually rated 1–6 based on the amount of rot observed in each internode, where 1 \leq 10%, 2 = 11 to 25%, 3 = 26 to 50%, 4 = 51 to 75%, 5 = 76 to 90%, and 6 > 90% of the internode tissue exhibited red discoloration. A single IRS rating was given to each stalk by averaging the seven internodes

assessed. A rot index (RI) was calculated based on a combination of traits. It was calculated as $RI = (NP + NR) \times \text{Log}_{10} (IRS+1)$.

The effect of species and accession on red rot susceptibility was determined by analysis of variance using the PROC MIXED procedure in SAS (SAS Institute, 2001) with replication and years as random variables. Differences between species and accession least square means were compared using the pdiff option (Saxton, 1988) at the 0.05 probability level.

Results and discussion

A species effect was found for all four traits analysed in all three single-year analyses. In 2004, differences were found among clones within all *Saccharum* species for all traits analysed. The single *Erianthus* accession included in the 2004 analysis was more resistant than the average of other species for all traits. On average, *S. robustum* and *S. barberi* were the most resistant of the *Saccharum* species. In 2005, less significance was seen among the accessions within species. For every trait, all nine *Erianthus* clones showed near complete resistance to red rot. This resulted in a lack of significance among accessions within this genus, and the *Erianthus* genus, as a whole, was more resistant than all the *Saccharum* species. Despite the relatively large number of *S. sinense* clones (11) included in 2005, the only traits showing significant differences among the clones were NR and RI. A lack of significance was observed among the three *S. officinarum* clones for NP, NR, and RI in 2005, but this could be an artifact of the low sample size and does not necessarily indicate a lack of overall variation within the species. Differences were detected for all traits among clones within *S. barberi* and *S. spontaneum*. In 2008, *Erianthus* again showed complete resistance, while all other species showed differences among accessions.

This study did not attempt to evaluate variability in resistance within commercial cultivars. A previous study (Yin *et al.*, 1994) found a high frequency of susceptibility in the current cultivar population.

When data from all three years were combined, differences were found among species for NP, NR, IRS and RI. The nine *Erianthus* clones showed near complete resistance and were more resistant than the other species for all four traits. The species were ranked in order of average resistance for each of the four measured traits, and these rankings were summed to give an overall ranking of resistance. The order of overall ranking was as follows: *Erianthus* > *S. robustum* > *S. barberi* > *S. spontaneum* > *S. sinense* and *S. officinarum* > commercial sugarcane (hybrid clones). While the number of clones per species in the combined 3-year data set was unbalanced, it gave a general idea of relative resistance among the species.

A subset of each of the *Saccharum* species and the commercial sugarcane cultivar LCP85-384 were included in a 2004 and 2008 multi-year analysis to determine significant genotype, year, and genotype by year interactions. Significant differences ($\alpha=0.05$) were found between species for all traits except IRS (Table 2). Year and species by year effects were significant for NP, NR, IRS, and RI ($\alpha=0.05$). The species were analysed individually to determine the effect of year, accession and accession by year on each of the four red rot criteria (Table 2). Significant effects were found for accession for all four traits; however, year was not always significant. For NP, no significant year effect was seen within *S. officinarum*, *S. sinense*, or the commercial cultivar, and for NR and IRS, year was insignificant for *S. barberi*, *S. spontaneum*, and the single commercial clone tested. RI showed no year effect for *S. barberi*, and LCP85-384. All accession by year effects were significant with the exception of NP for *S. robustum*. The ratio of variety F-values to interaction F-values was determined to assess the relative importance of variety by year interaction (Graybosch *et al.*, 1996). For all traits and species analysed, the variety F-values were greater than the interaction, with the exception of NP, IRS, and RI for *S. sinense*. There were no common *Erianthus* clones in 2004 and 2008, so they were not included in the multi-year analysis. However, eight of the nine clones analysed across the three years showed complete or near complete resistance to red rot in all traits analysed.

Table 2—Summary of red rot scores for accessions included in multi-year analysis. Species, number of nodes passed (NP), nodes rotted (NR), internode rot severity (IRS), and rot index^a (RI) are shown along with accession (A) and year (Y) mean separation and accession by year interaction (A*Y) analyses.

Species	NP				NR				IRS				RI			
	mean	A	Y	A*Y	mean	A	Y	A*Y	mean	A	Y	AY	mean	A	Y	A*Y
Commercial	7.8a	–	–	**	3.7ab	NA	NS	NA	53a	–	NS	–	20 a	–	NS	–
<i>S. officinarum</i>	6.3a	**	NS	**	3.3bc	**	**	**	43a	**	**	**	16 ab	**	**	**
<i>S. sinense</i>	4.7cd	*	NS	**	3.4b	**	**	**	41a	**	*	**	13 cd	**	**	**
<i>S. barberi</i>	4.3d	**	*	NS	2.6c	**	NS	**	36a	**	NS	**	11 d	**	NS	**
<i>S. robustum</i>	5.1bc	**	**	**	4.2a	**	**	**	38a	**	**	**	15 abc	**	**	**
<i>S. spontaneum</i>	5.5b	**	**	**	3.0bc	**	NS	**	39a	**	NS	**	14 bc	**	**	**

*indicates significance at the $\alpha=0.05$ level** indicates significance at the $\alpha=0.01$ level. NS=nonsignificant. ^aRI was calculated as $RI = (NP + NR) \times \text{Log}_{10} (IRS+1)$.

A previous study conducted by Yin *et al.* (1994) showed that RI had the highest heritability of the four traits measured. This trait aims to combine different susceptibility traits of the disease to obtain an overall assessment of damage to both the stalk and the buds. RI scores less than five were highly resistant (showing minimal stalk and bud rot), while those less than 10 were moderately resistant. Variation was observed for RI within each of the *Saccharum* species (Figure 1). The highest level of variation was observed within *S. spontaneum* with RI means ranging from 2 to 29. Three of 31 accessions (10%) had an RI mean less than 5, and eight (26%) had a mean less than 10. The percentages of *S. spontaneum* clones with resistant and moderately resistant ratings in the Indian studies were 37 and 65% (Srinivasan and Alexander, 1971) and 35 and 71% (Satyavir *et al.*, 1994), respectively. While less variation was observed among the *S. barberi*, a higher percentage of resistance was found within this species than was found within the others. Rot index ranged from one to 20 within *S. barberi*. Four of 14 accessions (29%) had a RI mean of less than 5, and six (43%) had a mean of 10 or less. The resistant and moderately resistant rating frequencies found in India for this species were 33 and 48% (Srinivasan and Alexander, 1971) and 6 and 22 % (Satyavir *et al.*, 1994), respectively.

When considering clones for disease resistance breeding, not only is the trait mean important, but also the variation within each species and the ease with which genes can be introgressed into commercial varieties. *Erianthus* clones showed the most consistent and most complete resistance to red rot. This suggests *Erianthus* could make an important contribution to an effort to enhance resistance in sugarcane. This is in agreement with a study conducted by Ram *et al.* (2001) who found that twelve out of 18 *Erianthus* clones tested showed resistance to red rot. However, it is not the only source of resistance genes, and certainly not the easiest to cross with elite sugarcane cultivars.

A wide range of variation was seen in *S. spontaneum*. While the overall average resistance of evaluated clones in this species was not as high as was demonstrated in India, there were still many clones that could be exploited for potential sources of resistance. *S. spontaneum* clones are more easily crossed with commercial sugarcane than *Erianthus* and produce more seed. Therefore, they are arguably the better candidates to utilise as more readily and rapidly exploitable sources of red rot resistance. Limited numbers of clones of the other *Saccharum* species were analysed, but the results indicate that, as a whole, *S. barberi* clones had a high level of resistance and could be easily exploited. *S. barberi* also had the highest ratio of variety to interaction F-values, indicating that, as a whole, this species is more stable across years and another good candidate for source genes for red rot resistance. The 3-year analysis results ranking suggested *S. robustum* might provide a source of resistance, but highly resistant clones were not detected in the species. Individual clones of *S.*

sinense, *S. robustum*, and *S. officinarum* might be identified that could serve as potential sources of resistance. However, individual clone resistance levels must be conclusively determined before using these *Saccharum* species in breeding due to variation among clones.

The significant interaction between year and accession for all species calls into question the ability to breed for resistance to red rot. However, this does not necessarily contradict successful breeding for the trait. As is observed with many screening studies, even with a significant interaction term, clones can be identified that have stable resistance across years. For example, in our multi-year analysis, we included 31 *S. spontaneum* accessions, and while statistical significance was found for the interaction between accessions and year, four clones were among the top 10 for resistance in both 2004 and 2008. This indicates that, with careful selection, enhanced resistance can be achieved through breeding. *S. spontaneum* clones identified as possessing red rot resistance in our study include but are not limited to: IND81-144, Guangxi 87-22, SES147B, and IN84-42. Of the *S. barberi* clones analysed, Dhalula and Ganapathy showed stable resistance. It does not appear necessary to heavily screen *Erianthus* clones for parental selection since most appear to possess near complete resistance to red rot. Furthermore, resistance to red rot in *Erianthus* makes it more attractive when considering the genus as a source of genes for other traits, such as low temperature tolerance. Transmitting an unfavourable trait, such as susceptibility to red rot, will not be a problem while introgressing the other traits of interest in this genus.

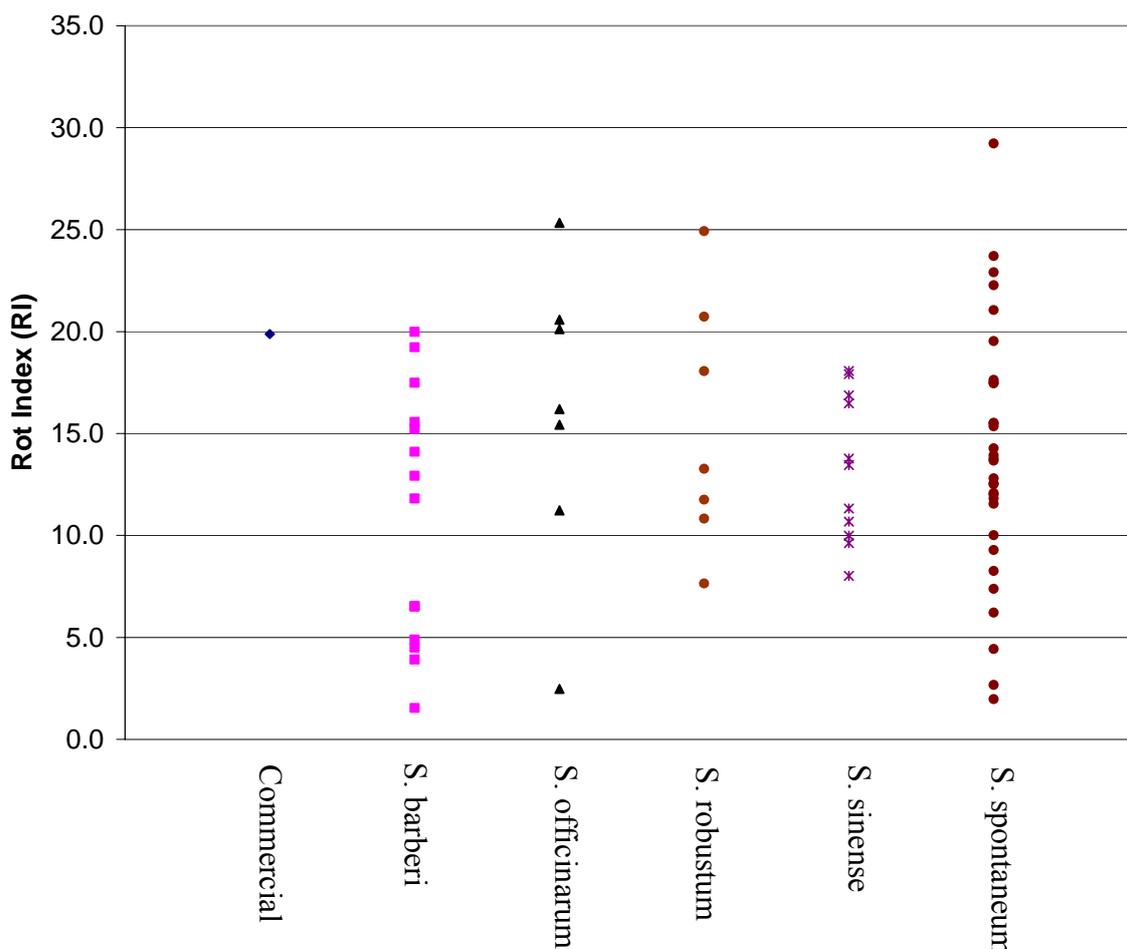


Fig. 1—Variation in rot index (RI) between and within five *Saccharum* species in comparison to the susceptible commercial sugarcane variety LCP 85-384. Sources of genetic resistance to sugarcane red rot can be found among the *Saccharum* species, particularly *S. spontaneum* and *S. barberi*.

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IDENTIFICATION DES SOURCES DE RÉSISTANCE À LA MORVE ROUGE DE LA CANNE À SUCRE

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MOTS CLÉS: *Saccharum*, *Colletotrichum falcatum*, *Glomerella*.

Résumé

LA MORVE ROUGE, causée par *Colletotrichum falcatum* Went, affecte de manière négative la plantation de la canne à sucre en Louisiane en provoquant une pourriture des boutures. Comme la résistance variétale est le moyen de lutte le plus efficace, une étude a été entreprise pour identifier les sources de résistance et évaluer leurs variabilités parmi les espèces de *Saccharum* et d'*Erianthus*. Les acquisitions clonales de *Saccharum spontaneum*, *S. robustum*, *S. sinense*, *S. officinarum*, *S. barberi*, et d'*Erianthus* ont été évaluées pour la résistance parallèlement aux cultivars commerciaux sensibles dans trois essais. Les tiges récoltées ont été inoculées au *C. falcatum*. Après six semaines d'incubation, chaque tige a été fendue et notée pour les symptômes de la morve rouge, en incluant le nombre de nœuds attaqués par la maladie (NP), le nombre de nœuds pourris (NR), la sévérité d'entrenœuds attaqués (IRS), et l'index de pourriture (RI), qui tient compte de l'étendue des dégâts causés au nœud et à l'entrenœud. Des différences significatives ont été détectées parmi les espèces et les acquisitions inter-espèces pour tous les caractères. Parmi les espèces de *Saccharum*, *S. barberi*, *S. robustum* et *S. spontaneum* ont manifesté les moyennes de symptômes les moins sévères, alors que *S. officinarum* et *S. sinense* avaient les moyennes de sévérité les plus élevées. Un niveau élevé de variabilité a été observé parmi l'espèce *S. spontaneum* avec des moyennes de RI s'étalant de 2 à 29. Trois des 31 acquisitions de *S. spontaneum* (10%) avaient une moyenne de NR de moins que 5, et 26% avaient une moyenne de moins que 10. *S. barberi* a manifesté un niveau de résistance élevé, avec des moyennes RI s'étendant de un à 20. Quatre des 14 acquisitions de *S. barberi* (29%) avaient des moyennes RI de moins que 5 (très résistantes), et six (43%) avaient des moyennes de 10 ou moins (modérément résistantes). La variabilité la plus faible a été observée parmi les acquisitions d'*Erianthus*, toutes démontrant peu ou pas de symptômes de morve rouge. Le genre *Erianthus* possède donc une résistance élevée et devrait être utilisé comme parent dans les programmes d'hybridation génétique là où la résistance est requise. L'autre source de résistance à la morve rouge, plus facilement accessible, est le germoplasme de l'espèce *Saccharum*, dont certaines acquisitions de *S. spontaneum* et *S. barberi*.

IDENTIFICACIÓN DE FUENTES DE RESISTENCIA A LA PUDRICIÓN ROJA DE LA CAÑA DE AZÚCAR

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PALABRAS CLAVES: *Saccharum*, *Colletotrichum falcatum*, *Glomerella*.

EL MUERMO rojo o pudrición roja, causada por *Colletotrichum falcatum* Went, afecta negativamente el establecimiento de la caña de azúcar en Luisiana debido a la pudrición de los tallos sembrados. Debido a que la resistencia de los cultivares es el método de control más eficaz, se realizó un estudio para identificar las fuentes de resistencia a la pudrición roja y evaluar la variabilidad dentro de cada especie de *Saccharum* y *Erianthus*. Clones de accesiones de *Saccharum spontaneum*, *S. robustum*, *S. sinense*, *S. officinarum*, *S. barberi* y *Erianthus* se evaluaron en su resistencia a la enfermedad junto a cultivares comerciales susceptibles, en tres experimentos. Tallos cortados fueron inoculados con *C. falcatum*. Después de una incubación de 6 semanas, cada tallo se dividió longitudinalmente y calificaron los síntomas de la pudrición roja, incluyendo el número de nudos atravesados (NP), número de nudos podridos (NR), la severidad de la podredumbre del entrenudo (IRS), y un índice de pudrición (RI) que tiene en cuenta tanto la magnitud del daño de los nudos como la pudrición de los entrenudos. Se detectaron diferencias significativas entre las especies y las adhesiones dentro de las especies para todas las variables. Entre las especies de *Saccharum*, *S. barberi*, *S. robustum* y *S. spontaneum* exhibieron la menor severidad media de incidencia de la pudrición roja, mientras que *S. officinarum* y *S. sinense* exhibieron la mayor severidad media de incidencia de la enfermedad. Un alto nivel de variación se observó en *S. spontaneum* con RI que tuvo un rango de variación de 2 a 29. Tres de 31 accesiones de *S. spontaneum* (10%) tuvieron una media de NR inferior a 5, y 26% tenían una media de NR menor de 10. *S. barberi* mostró un alto nivel de resistencia, con medias de RI que variaron del uno al 20. Cuatro de 14 accesiones de *S. barberi* (29%) tuvieron una media de RI inferior a 5 (muy resistente), y seis (43%) tuvieron una media de RI de 10 o menos (moderadamente resistente). La menor variación en los resultados se observó entre las accesiones de *Erianthus*, con todas mostrando muy poco o ningún síntoma de pudrición roja. El género *Erianthus* posee una alta resistencia y debe ser utilizado como material progenitor en los programas de mejoramiento que requieran mejorar la resistencia a la pudrición roja. Otras fuentes de mayor facilidad en su utilización como fuentes de resistencia a la pudrición roja en el germoplasma de *Saccharum*, se pueden buscar en las accesiones de *S. spontaneum* y *S. barberi*.