Resistance of Sugarcane Relatives Injected with *Ustilago scitaminea*

D. M. BURNER, Research Geneticist, M. P. GRISHAM, Research Plant Pathologist, and B. L. LEGENDRE, Research Agronomist, U.S. Department of Agriculture, Agricultural Research Service, Sugarcane Research Unit, Houma, LA 70361-0470

ABSTRACT

Burner, D. M., Grisham, M. P., and Legendre, B. L. 1993. Resistance of sugarcane relatives injected with *Ustilago scitaminea*. Plant Dis. 77:1221-1223.

We evaluated the resistance of 102 clones of sugarcane (*Saccharum* spp.) relatives to *Ustilago scitaminea*, causal agent of sugarcane smut, in two greenhouse experiments. Relatives included *Erianthus* spp. section *Ripidium*, *S. barberi*/*S. sinense*, *S. officinarum*, *S. robustum*, *S. spontaneum*, and *Saccharum* interspecific hybrids (cultivars). Clones of *Erianthus* spp. section *Ripidium* were the most resistant and clones of *S. officinarum* and *S. robustum* were the most susceptible of the six taxonomic groups included in the first experiment. Clones in the second experiment, predominantly *S. spontaneum* from recent germ plasm collection expeditions, were assigned to one of three groups depending on their geographic origin. Clones from India, Indonesia, and the Philippines had means of 1, 37, and 51% infection, respectively. Exotic, smut-resistant clones will be crossed with elite clones to enhance smut resistance in Louisiana sugarcane germ plasm.

Sugarcane cultivars are vegetatively propagated, interspecific hybrids derived primarily from a few clones (genotypes) of four *Saccharum* species—*S. officinarum* L., *S. spontaneum* L., *S. robustum* Brandes & Jesw. ex Grassl, and *S. sinense* Roxb. (2). Realizing the need to preserve diverse germ plasm for future development of commercial cultivars, researchers have made several collections of wild sugarcane relatives (4). Plants are maintained in clonal repositories in India and the United States. In 1964, the Agricultural Research Service of the U.S. Department of Agriculture established a breeding program at the U.S. Sugarcane Field Laboratory in Houma, LA, with the objective of incorporating diverse germ plasm into new cultivars (12).

Sugarcane smut, caused by *Ustilago scitaminea* Syd. & P. Syd., has been reported from all sugarcane-producing areas of the world except Australia and Fiji (14). The first report of the disease in the Western Hemisphere was from Argentina in 1940. The distribution remained limited to central South America until the 1970s. Smut was reported in Hawaii in 1971 (5) and in Guyana in 1974 (17). During the next decade, the disease continued to expand in distribution through northern South America, Central America, the Caribbean, and the continental United States (9). It was first reported in Louisiana in 1981 (18). Smut has threatened sugarcane industries in countries where large acreages are planted with susceptible cultivars (9).

The most effective way to control sugarcane smut is to use resistant cultivars (3,9,14). Strong genetic control of resistance (6,25,27) suggests that rapid progress could be made in developing resistant cultivars through breeding and selection. In Louisiana, smut-resistant progeny are routinely selected from crosses where parents were not selected for smut resistance (15), although resistant progeny are most reliably obtained from crosses between resistant parents (6). Advanced selections in the breeding program at Houma frequently exhibit resistance to sugarcane mosaic virus (SCMV) because of strict parental selection, although many are eliminated for smut susceptibility.

Smut resistance in sugarcane is influenced by nodal bud morphology (26), chemical inhibitors present in bud scales (19), and host physiology (11,26). To assess smut reaction, researchers typically use a dip inoculation assay in which nodal buds are immersed briefly in a suspension of teliospores, planted in a greenhouse, and evaluated in the greenhouse or field (1,6,9,15,27). Chen and Lo (7) used a pinprick method to inject teliospores into buds of a clone of *Miscanthus* Anderss., which generally produces insignificant stalks with no root primordia at stalk nodes (10). Teliospore injection circumvents the protection afforded by intact bud scales and provides an estimate of the physiological resistance to fungal development in the plant. Injection inoculation may induce greater smut infection than dip inoculation, and cultivars can respond differently to the two methods of inoculation (11,26).

Because varietal reaction to smut can be influenced by smut race or isolate, the environment, the experimental design and procedure, and interactions among these factors (6,9), empirical evaluation of smut resistance under local conditions appears to be necessary. *S. spontaneum* and *S. sinense* tend to have higher frequencies of resistant (23) or resistant plus moderately resistant (1) clones than do *S. barberi* Jesw., *S. officinarum*, and *S. robustum*. However, Chona (8) considered *S. officinarum* to be immune or highly resistant to smut compared to *S. spontaneum* and *S. barberi*. Results of these studies were based on dip inoculation. The smut reactions of *Erianthus* spp. and *Saccharum* spp. have not been compared. The objective of this study was to identify sources of resistance to *U. scitaminea* among a clonal collection of diverse sugarcane relatives for future use in the breeding program.

MATERIALS AND METHODS

Clones of sugarcane and near relatives were tested for susceptibility to *U. scitaminea* in two greenhouse experiments. In the first experiment, 50 clones of interspecific hybrids (cultivars) of sugarcane and five groups (taxa) of near relatives were challenged with *U. scitaminea*. Cultivars were CP 65-357, CP 70-321, CP 72-356, CP 72-370, CP 73-351, CP 76-331, CP 79-318, and NCo 310. Clones of *Erianthus* Michx. sect. *Ripidium* Henrard were SES 288, *E. arundinaceus* (Retz.) Jesw.; Cane 2886, *E. bengalense* (Retz.) Bharadw.; US 63-6, *E. brevibarbis* Michx.; US 57-11-2, *E. longisetosus* Anderss.; Mardon, species unknown; Kalimpong, *E. procerus* (Roxb.) Raiz.; SES 372, *E. ravennae* (L.) P. Beauv.; and US 57-60-2, *E. rufipilus* (Stevd.) Griseb. *S. barberi* (clones Kalari, Matki Mango, and Semari) and *S. sinense* (clones China, Katha, and Zwinga) were grouped together because of their close phylogenetic relationship (10,22). *S. officinarum* clones were Badila, Creoula, IN 84-71, NG 28-2, NG 28-211, NG 51-146, NG 57-67, NG 77-62, NG 77-117, and Sylva. *S. robustum* clones were IJ 76-293, IJ 76-314, IJ 76-522, Molokai 5099, NG 77-21, NG 77-38, NG 77-77, NG 77-147, NG 77-160, and NG 77-199. *S. spontaneum* clones

Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture and does not imply its approval to the exclusion of other products that may also be suitable.

Accepted for publication 8 August 1993.

were Local collection (an escaped clone collected at Houma), Mandalay, SES 184B, SES 189, SH 249, Tainan 2n=96, Tainan 2n=112, and US 56-15-8.

The second experiment evaluated 52 clones collected in three geographic regions during recent germ plasm expeditions. All but three of these clones—IND 81-53 (*Vetiveria* sp.), IND 81-47 (*E. bengalense*), and IS 76-182 (*Miscanthus* sp.)—were *S. spontaneum*. Seven clones were from India (IND 81-47, IND 81-53, IND 81-80, IND 81-142, IND 81-144, IND 81-161, and IND 81-166). Twenty-seven clones were from Indonesia (IN 84-9, IN 84-10, IN 84-11, IN 84-12, IN 84-12-F1, IN 84-12-F2, IN 84-12-F3, IN 84-12-F8, IN 84-12-F10, IN 84-12-F13, IN 84-12-F14, IN 84-12-F16, IN 84-13, IN 84-16, IN 84-18, IN 84-21, IN 84-22, IN 84-27, IN 84-39, IN 84-42, IN 84-66, IN 84-69, IN 84-70, IN 84-88, IN 84-89, IN 84-91, and IS 76-182). Eight of the Indonesian clones (IN 84-12-F1 to IN 84-12-F16 in the preceding list) were selfed progeny of IN 84-12 (J. C. Comstock, *personal communication*). Eighteen clones were from the Philippines (PAL-84-1, PAL-84-4, PCA-SUR-84-5, PCA-NOR-84-7, PCAV-84-5, PCAV-84-6, PCAV-84-7, PCAV-84-11, PCAV-84-12, PCAV-84-20, PLAG-84-8, PPGN-84-2, PPGN-84-5, PPGN-84-8, PQ-84-3, PQ-84-4, PTAR-84-1, and PTAR-84-2).

When possible, lateral buds were used to propagate clones. Clones that did not form nodes with viable buds were propagated from tillers or rhizomes. Germinating buds or cuttings were randomly transplanted to compartments (5.2 × 5.2 × 7.6 cm) of Styrofoam trays containing steam-sterilized vermiculite. Each of the five replicates of each clone included one to six plants (not all transplants survived).

The injection inoculation procedure (13) was used with modifications. Plants were inoculated 60 days after transplanting with 0.25 ml of a suspension of *U. scitaminea* containing 5×10^6 teliospores per milliliter. The inoculum was injected into the leaf whorl just above the shoot meristem. Teliospores used for inoculation had been collected from whips (long, unbranched sori) of naturally infected stalks of CP 73-351. Plants were examined for smut symptoms weekly for 3 mo. Production of a whip was the infection criterion (11). Any plant with a whip was noted, removed, and destroyed.

Plants were maintained throughout the experiment in a screened (30-mesh) greenhouse at temperatures between 24 and 35 C. Plants were treated weekly with diazinon for insect control and fertilized weekly with a commercial 15-30-15 fertilizer (Miracle-Gro).

Each experiment was arranged in a randomized complete block design with five replications. Most replications contained six plants per clone, but some had as few as one plant per clone. Data were recorded as simple percentage infection (presence or absence of a whip) of plants within each clone and replication. Percentages were transformed by $\sin^{-1} y^{1/2}$ and expressed in degrees (24), where y = infection percentage in decimal form. Zero percentages were equal to $\sin^{-1} (1/4n)^{1/2}$, where $n = 6$ plants for most plots. Because of missing data, percentage and transformed data did not always correspond exactly.

Transformed data were analyzed by the general linear models (GLM) procedure (21). Sources of variation for analysis of variance of experiment 1 were replication (4 df), taxon (5 df), clone within taxon (44 df), error (replication × taxon, 20 df), and residual (169 df). Sources of variation for analysis of variance of experiment 2 were replication (4 df), origin (2 df), clone within origin (49 df), error (replication × origin, 8 df), and residual (190 df). Simple means of clones averaged across replications (untransformed) and least squares means (transformed) were both computed. Multiple comparisons of least squares means were made with the t test option of the GLM procedure (21) with the error terms specified above. Conclusions were based on analysis of transformed data.

RESULTS

Mean percentage of infection differed among taxa in the first experiment (Table 1). *Erianthus* spp. had significantly less smut infection (10%) than the other taxa (26–47%). Infection varied considerably among clones within taxa. *Erianthus* spp. clones had 0% infection except for US 63-6, which had 63% infection. The range of infection was similar for clones within other taxa, except that no clones with 0% infection were found in *S. officinarum* or *S. robustum*. Means of *Saccharum* spp. cultivars, *S. spontaneum*, and *S. barberi*/*S. sinense* did not differ significantly from each other and were intermediate between those of *Erianthus* spp. and *S. officinarum* and *S. robustum*.

Among the cultivars tested, CP 72-356 and CP 72-370 had no infection. Cultivars CP 70-321, CP 79-318, and CP 76-331 had 7, 10, and 17% infection, respectively. Cultivars CP 65-357, CP 73-351, and NCo 310 had 34, 56, and 87% infection, respectively. Infection of *S. spontaneum* clones ranged from 0% (SES 184B, SH 249, and Tainan 2n=112) to 13% (Local collection and SES 189) to more than 50% (Tainan 2n=96 [51%], US 56-15-8 [63%], and Mandalay [73%]). Two clones in the *S. barberi*/*S. sinense* taxon (China and Katha) were heavily infected with smut (≥70%), while others had little (Semari [7%] and Matki Mango [13%]) or no (Kalari and Zwinga) infection. *S. officinarum* and *S. robustum* clones had 19% or more infection, except for Sylva (3%) and NG 77-77 (3%).

Smut reaction of clones in the second experiment differed according to region

Table 1. Differential reactions to *Ustilago scitaminea* among sugarcane (*Saccharum* spp.) relatives

Taxon	Number of clones	Infection: Untransformed: Simple mean (%)	Infection: Untransformed: Range[y] (%)	Infection: Transformed[z]
Erianthus spp.	8	10	0–63	0.40 a
Saccharum spp. cultivars	8	26	0–87	0.52 b
S. spontaneum	8	27	0–73	0.52 b
S. barberi/*S. sinense*	6	28	0–77	0.53 b
S. officinarum	10	43	3–78	0.70 c
S. robustum	10	47	3–75	0.76 c
Grand mean		32	0–87	0.59
Coefficient of variation (%)		65.6		37.0

[y] Range of clone means.
[z] $\sin^{-1} \sqrt{y}$, where y = infection percentage. Least squares means followed by the same letter do not differ significantly ($P < 0.05$) according to the t test.

Table 2. Differential reactions to *Ustilago scitaminea* of *Saccharum spontaneum* clones from three geographic regions

Origin	Number of clones	Infection: Untransformed: Simple mean (%)	Infection: Untransformed: Range[y] (%)	Infection: Transformed[z]
India	7	1	0–7	0.28 a
Indonesia	27	37	0–89	0.65 b
Philippines	18	51	0–83	0.81 c
Grand mean		37	0–89	0.65
Coefficient of variation (%)		65.4		32.6

[y] Range of clone means.
[z] $\sin^{-1} \sqrt{y}$, where y = infection percentage. Least squares means followed by the same letter do not differ significantly ($P < 0.05$) according to the t test.

of origin (Table 2). Means differed significantly ($P < 0.05$); clones from India were most resistant, followed by clones from Indonesia and the Philippines. Indian clones showed no smut symptoms, except for IND 81-142 (7% infection). Of the Indonesian clones, six were uninfected (IN 84-10, IN 84-12-F10, IN 84-12-F14, IN 84-22, IN 84-27, and IN 84-39), seven had 4–17% infection (IN 84-9, IN 84-13, IN 84-21, IN 84-42, IN 84-88, IN 84-89, and IN 84-91), and the remaining clones had infection levels ranging from 29 to 89%. Selfed progeny of the smut-susceptible clone IN 84-12 (63% infection) segregated into uninfected (see above) and susceptible (>63% infection) classes. Among clones from the Philippines, one (PCA-SUR-84-5) had no infected plants, and one (PQ-84-3) had 13% infection; the remaining plants from the Philippines had 30–83% infection.

DISCUSSION

Mean smut resistance of *Erianthus* spp. was greater than that of other taxa. This is the first documented report comparing smut reactions of *Erianthus* and *Saccharum* taxa. Our observations of the smut reactions of *S. officinarum* and *S. robustum* support the finding by Alexander et al (1) that these species tend to have comparatively low frequencies of smut-resistant clones. In contrast, Chona (8) reported that *S. officinarum* was immune or highly resistant to smut. This discrepancy may have been caused by differences in the method of inoculation and the clones tested.

Smut infection of cultivars was similar to that found in dip inoculation studies (M. P. Grisham and B. L. Legendre, *unpublished*). The cultivars CP 70-321 and CP 65-357, which have excellent and intermediate field resistance, respectively (M. P. Grisham and B. L. Legendre, *unpublished*), had means of 7% (moderately resistant) and 34% (susceptible) infection, respectively, with injection inoculation. Three clones (NG 57-67, NG 77-21, and IJ 76-293) that were reported as resistant (0% infection) in a dip inoculation study (1) were susceptible (32, 58, and 61% infection, respectively) in this study. Clones of *S. barberi* and *S. sinense* (except Semari) were also tested by Miller et al (20), who reported that they ranged in infection from 0 to 52%. Results in our study were generally consistent except for China, which had 70% infection with injection inoculation, compared to only 7.6% infection with dip inoculation (20). These data confirm that the inoculation procedure influenced the smut reaction of some clones (11,26).

In a previous study (16), we reported that clones of *Erianthus* spp., *S. spontaneum,* and *S. barberi*/*S. sinense* were more resistant to SCMV than those of other taxa. Also, the Indian clones were more resistant to SCMV than those from the Philippines and Indonesia. Because *Erianthus* spp. and Indian clones of *S. spontaneum* have demonstrated significant resistance to both smut and SCMV, this germ plasm warrants further study in breeding programs for disease resistance in sugarcane.

Injection inoculation was useful in identifying potential sources of smut resistance in plants that differ greatly in growth habit. Unlike mechanical resistance, physiological resistance to fungal development in the plant may be a genetic characteristic more easily preserved during breeding and selection for large-diameter, low-fiber stalks for commercial planting. However, Dean (11) theorized that physiological resistance is more likely to be lost through genetic change in the pathogen than is mechanical resistance. Further study is needed to determine whether dip and injection inoculation methods are mutually predictive of field resistance.

ACKNOWLEDGMENTS

We wish to thank Patricia Angelette, Southern Bibbins, and Druis Bourg (USDA-ARS, Houma, LA) for technical assistance and Wayne K. Langholff (USDA-ARS, Stoneville, MS) for statistical assistance. Clones tested in this study are available from the USDA-ARS Subtropical Horticulture Research Unit in Miami, FL.

LITERATURE CITED

1. Alexander, K. C., Madhusudhana Rao, M., Mohanraj, D., Prakasam, N., and Padmanaban, P. 1991. Sources of resistance to red rot and smut among different sugar cane species. Sugar Cane 1991(6):10-13.
2. Arceneaux, G. 1967. Cultivated sugarcanes of the world and their botanical derivatives. Proc. Int. Soc. Sugar Cane Technol. 12:844-854.
3. Benda, G. T. A. 1987. Breeding for disease resistance. Pages 161-179 in: Copersucar International Sugarcane Breeding Workshop. Copersucar, São Paulo, Brazil.
4. Berding, N., and Roach, B. T. 1987. Germplasm collection, maintenance, and use. Pages 143-210 in: Sugarcane Improvement Through Breeding. D. J. Heinz, ed. Elsevier Publishing, Amsterdam.
5. Byther, R. S., Steiner, G. W., Ladd, S. L., and Heinz, D. J. 1973. Smut disease of sugarcane in Hawaii. Plant Dis. Rep. 57:966-968.
6. Chao, C. P., Hoy, J. W., Saxton, A. M., and Martin, F. A. 1990. Heritability of resistance and repeatability of clone reactions to sugarcane smut in Louisiana. Phytopathology 80:622-626.
7. Chen, Y. H., and Lo, C. C. 1989. Disease resistance and sugar content in *Saccharum-Miscanthus* hybrids. Taiwan Sugar 36(3):9-12.
8. Chona, B. L. 1957. Sources of resistance to diseases in breeding varieties of sugarcane. Indian J. Genet. Plant Breed. 17:257-268.
9. Comstock, J. C., Ferreira, S. A., and Tew, T. L. 1983. Hawaii's approach to control of sugarcane smut. Plant Dis. 67:452-457.
10. Daniels, J., and Roach, B. T. 1987. Taxonomy and evolution. Pages 7-84 in: Sugarcane Improvement Through Breeding. D. J. Heinz, ed. Elsevier Publishing, Amsterdam.
11. Dean, J. L. 1982. The effect of wounding and high-pressure spray inoculation on the smut reactions of sugarcane clones. Phytopathology 72:1023-1025.
12. Dunckelman, P. H., and Breaux, R. D. 1972. Breeding sugarcane varieties for Louisiana with new germplasm. Proc. Int. Soc. Sugar Cane Technol. 14:233-239.
13. Ferreira, S. A., and Comstock, J. C. 1981. A new inoculation technique for sugarcane smut, *Ustilago scitaminea.* (Abstr.) Phytopathology 71:873.
14. Ferreira, S. A., and Comstock, J. C. 1989. Smut. Pages 211-229 in: Diseases of Sugarcane—Major Diseases. C. Ricaud, B. T. Egan, A. G. Gillaspie, Jr., and C. G. Hughes, eds. Elsevier Publishing, Amsterdam.
15. Grisham, M. P., and Breaux, R. D. 1988. Effectiveness of screening for smut resistance in the sugarcane selection program in Louisiana. J. Am. Soc. Sugar Cane Technol. 8:19-22.
16. Grisham, M. P., Burner, D. M., and Legendre, B. L. 1992. Resistance to the H strain of sugarcane mosaic virus among wild forms of sugarcane and relatives. Plant Dis. 76:360-362.
17. James, G. L. 1976. Sugarcane smut infection in Guyana and Martinique. Sugar J. 38(12):17.
18. Koike, H., Fontenot, D., Damann, K., and Schlub, R. 1981. Smut of sugarcane in Louisiana. Plant Dis. 65:1018.
19. Lloyd, H. L., and Pillay, M. 1980. The development of an improved method for evaluating sugarcane for resistance to smut. Proc. Annu. Congr. S. Afr. Sugar Technol. Assoc. 54:168-172.
20. Miller, J. D., Dean, J. L., and Tai, P. Y. P. 1982. Relation between taxonomic group and resistance to smut in Chinese and North Indian sugarcanes. Sugar J. 45(5):18-19.
21. SAS. 1988. SAS/STAT User's Guide, Release 6.03 Edition. SAS Institute, Inc., Cary, NC.
22. Sreenivasan, T. V., Ahloowalia, B. S., and Heinz, D. J. 1987. Cytogenetics. Pages 211-253 in: Sugarcane Improvement Through Breeding. D. J. Heinz, ed. Elsevier Publishing, Amsterdam.
23. Srinivasan, K. V., and Alexander, K. C. 1971. Sources of resistance to red rot and smut in the species of *Saccharum.* Sugarcane Pathol. Newsl. 6:6-7.
24. Steel, R. G. D., and Torrie, J. H. 1980. Principles and Procedures of Statistics. McGraw-Hill, New York.
25. Walker, D. I. T. 1980. Heritability of smut resistance. Sugarcane Breeders Newsl. 43:19-23.
26. Waller, J. M. 1970. Sugarcane smut (*Ustilago scitaminea*) in Kenya. II. Infection and resistance. Trans. Br. Mycol. Soc. 54:405-414.
27. Wu, K. K., Heinz, D. J., and Meyer, H. K. 1983. Heritability of sugarcane smut resistance and correlation between smut grade and yield components. Crop Sci. 23:54-56.