

Effect of Aerated Steam on the Red-Rot Pathogen in Sugarcane Stalks

Shaw-ming Yang

Research plant pathologist, U.S. Sugarcane Field Laboratory, Agricultural Research, Science and Education Administration, U.S. Department of Agriculture, Houma, LA 70361; present address, U.S. Department of Agriculture, Southwestern Great Plains Research Center, Bushland, TX 79012.

A contribution from AR, SEA, USDA, in cooperation with the Louisiana Agricultural Experiment Station.

The author thanks O. M. Cifuentes for assistance in steam-treating the sugarcane.

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

Accepted for publication 8 January 1979.

ABSTRACT

YANG, S. M. 1979. Effect of aerated steam on the red-rot pathogen in sugarcane stalks. *Phytopathology* 69:702-705.

Autoclaved sugarcane leaf-pieces were used to study the viability of *Colletotrichum falcatum* in bud tissues of sugarcane cultivars Co 290 and CP 65-357 treated with aerated steam and untreated. Aerated-steam treatment (AST) at 51 C for 4 and 5 hr killed *C. falcatum* in 61 and 75% of naturally infected buds, respectively, and AST at 52 C for 4 and 5 hr killed *C. falcatum* in 88 and 91%, respectively. Aerated-steam treatment at 52 C

for 4 and 5 hr, killed the pathogen in naturally infected leaf sheaths and leaf midribs, stalk borer-tunneled internodes, and inoculated internodes. No more than one bud per stalk was found to have viable *C. falcatum* after AST of stalks at 52 C for 4 hr. Aerated-steam treatment at 52 C for 4 hr did not affect the germination of buds of CP 65-357, but significantly reduced the germination of buds of Co 290.

Additional key words: *Physalospora tucumanensis*.

Red rot of sugarcane (*Saccharum* interspecific hybrids), which is caused by *Physalospora tucumanensis* Speg. (conidial state = *Colletotrichum falcatum* Went), is a major disease of sugarcane and has caused the failure of important commercial cultivars in many areas of the world, including Australia, India, Taiwan, Louisiana (USA), and Hawaii (USA) (1).

The red-rot pathogen is present in or on seed cane at planting; it occurs in the growth cracks, in an incipient infection in buds and leaf scars, and in tunnels bored in the stalks by larvae of the sugarcane borer (*Diatraea saccharalis* F.) (1). The most important means of transmission of the pathogen from one crop to the next in Louisiana is in seed cane that is taken from infected stalks (1). Elimination of the pathogen in seed cane before planting, therefore, would be expected to control red rot.

Cifuentes et al (3) reported that aerated-steam treatment (AST) at 51 C for 4 hr controls ratoon stunting disease of sugarcane, but they did not determine whether AST would eliminate the red-rot pathogen in the seed cane.

This paper describes a technique for detection of *C. falcatum* in seed canes, and reports the results of 2 yr of study on the effect of AST on the survival of *C. falcatum* in naturally infected buds, leaf scars, leaf sheaths, leaf midribs, borer tunnels, and inoculated internodes.

MATERIALS AND METHODS

A technique was developed to detect viable *C. falcatum* in bud, leaf scar, and other tissue samples of sugarcane. Each bud or leaf scar sample was placed on an autoclaved sugarcane leaf-piece (2.5 × 1.5 cm, autoclaved at 121 C for 4 hr) on sterile, moistened filter paper in a petri dish. There usually were eight leaf pieces per petri dish; some of the leaf pieces were left without a tissue sample to

This article is in the public domain and not copyrightable. It may be freely reprinted with customary crediting of the source. The American Phytopathological Society, 1979.

serve as control. After 4–5 days at room temperature (20–27 C), the sample was removed, and the leaf piece was examined microscopically at $\times 80$. The formation of setae with amerspore masses on the leaf piece (Fig. 1) indicated the presence of viable *C. falcatum* in the sample. Samples from stalks included the bud and four pieces of leaf scar from each of the 8 to 10 nodes of the mature part of the stalk. When other tissues were sampled, namely, leaf sheath, leaf midrib, sugarcane-borer-damaged internode, or inoculated internode, each sample consisted of eight subsamples placed on two to four leaf pieces; if one or more subsamples indicated the presence of *C. falcatum*, the entire sample was scored as positive.

Oatmeal agar (OMA) was also used for isolation of *C. falcatum* in buds (1,6) and leaves (1) of sugarcane; the dehydrated oatmeal agar (Difco) was suspended in cold water, heated to boiling with constant stirring and autoclaved (121 C, 15 min).

The methods of detecting viable *C. falcatum*, sugarcane leaf-piece and OMA, were compared by using buds halved longitudinally, one-half of each bud for each method. In two tests with cultivar Co 290, there were a total of 156 buds from 20 stalks. The bud-halves for OMA were soaked in 0.74% NaOCl solution for 10 min, in sterile water for 5 min, dried, and then placed on OMA, four per plate. The other bud-halves were soaked in sterile water for 5 min, dried, and placed on autoclaved leaf-pieces. After 4–5 days, each bud-half on autoclaved leaf-pieces was examined directly under the microscope to determine the number of buds infected with *C. falcatum*; the number of infected buds determined directly was compared with the number of bud-halves scored positive by the OMA and leaf-piece methods.

Sugarcane was treated with aerated steam in a hot-air treatment unit in the Department of Plant Pathology, Louisiana State University, Baton Rouge (4). Steam-heated air was injected into the

chamber from the bottom. Samples were placed on a shelf, with five shelves in a unit. Thermocouples were placed inside and outside of cane stalks, and at various locations throughout the cane within the chamber. Temperature in the cane stalks and chamber reached equilibrium (51 or 52 C) within 1 hr and was maintained (± 1 C) for 1–5 hr. In order to prevent the treated samples from becoming contaminated with *C. falcatum* during transportation, each treated sample was kept in a polyethylene bag after treatment. Disposable plastic gloves were used to place the treated sample in the polyethylene bag. New gloves were used for each sample. The untreated samples also were kept in polyethylene bags.

Stalks (seed canes) of sugarcane cultivars Co 290 and CP 65-357, and leaves of CP 65-357 with midrib lesions, were collected in the field 1 day before treatment. Cultivar Co 290 is highly susceptible to *C. falcatum* (1) and CP 65-357 is moderately susceptible (2). In the 1976 test, for each cultivar 30 stalks stripped of leaf sheaths and leaf blades, 30 leaves, and 30 inoculated two-node cuttings were treated at 51 and 52 C for 2–5 hr. Ten leaves and 10 cuttings of each cultivar were removed from the treatment chamber after 2, 3, and 4 hr of treatment, and 15 stalks were removed after 4 and 5 hr. In the 1977 test, 15 stalks of each cultivar, 15 stalks of CP 65-357 tunneled by the sugarcane borer, and 15 stalks of CP 65-357 inoculated with *C. falcatum* in the field were treated at 52 C for each 4 and 5 hr. The stalks used in 1977 were not stripped. Each treatment was repeated once. Untreated samples were used as controls for each treatment.

Two-node cuttings were inoculated in the laboratory and growing stalks were inoculated in the field 8–12 wk before heat treatment. Mixed spore suspensions of isolates of *C. falcatum* were injected into holes drilled into the internode. Alternate internodes were inoculated on stalks of growing canes. Before inoculation, the sur-

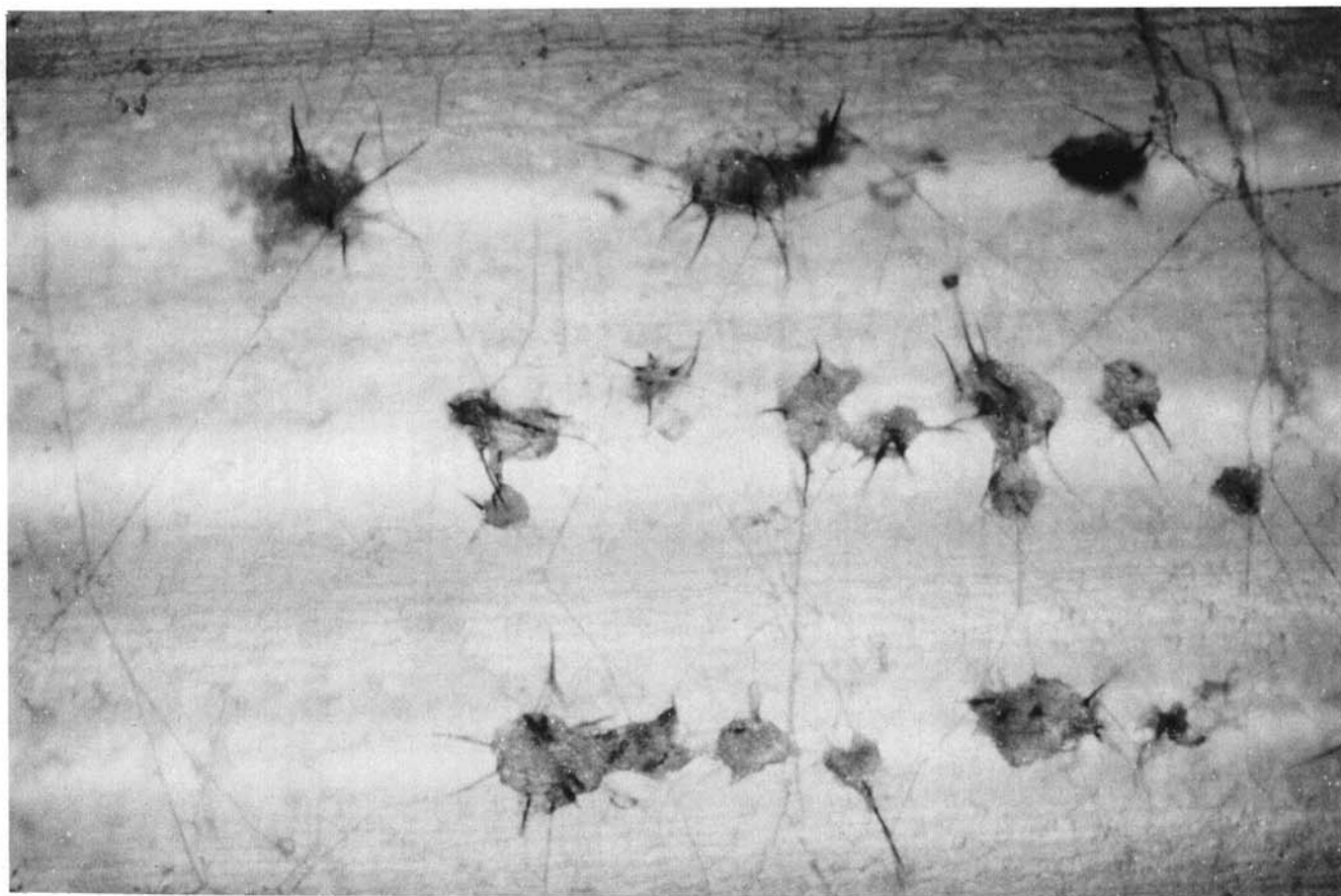


Fig. 1. Photomicrograph showing setae with amerspore masses of *Colletotrichum falcatum* yielded from a sugarcane bud incubated on an autoclaved sugarcane leaf piece. Growth of other fungi from the same bud on the leaf piece did not mask the identity of *C. falcatum*, the causal agent of red rot of sugarcane.

face of two-node cuttings was disinfected with tap water and 1% NaOCl solution. The inoculated cuttings were incubated in polyethylene bags on laboratory benches.

For studying the effect of AST on germination of sugarcane, one-node cuttings were prepared from five stalks treated at 51 and 52 C for 4 and 5 hr and five untreated stalks. In 1976, the cuttings were placed between layers of newspaper that had been moistened with 0.5% Lysol solution in wooden flats and incubated at 36 C for 30 days. In the 1977 tests, the one-node cuttings were planted in sterilized vermiculite in flats and incubated on greenhouse benches (20–36 C) for 30 days. Water was added as needed. In the second 1977 test, five treated and five untreated stalks of CP 65-357 also were planted in the greenhouse.

RESULTS

Detection of *Colletotrichum falcatum* in buds of untreated sugarcane stalks on autoclaved leaf-pieces and on oatmeal agar (OMA). The leaf-piece method detected *C. falcatum* in more buds than OMA. A direct microscopic examination showed that the fungus was present in 94 of 156 buds compared by the half-bud method. Of the 94 buds, the autoclaved leaf-piece method and OMA detected 81 and 65% infection, respectively. The difference in percentage of infection detected with the media was significant ($P = 0.05$, LSD test).

In this experiment, as in all subsequent tests, autoclaved leaf-pieces left as controls showed no *C. falcatum*.

Species of *Curvularia*, *Mucor*, and *Trichoderma* were found in most of OMA plates. They covered the plates and made it difficult to detect colonies of *C. falcatum*. Some fungi also were found on autoclaved leaf-pieces, but these did not mask *C. falcatum* (Fig. 1).

Percentage of buds infected with viable *Colletotrichum falcatum* per untreated stalk. *Colletotrichum falcatum* was present in all of 60 untreated stalks of Co 290, but not in all of the 60 stalks of CP 65-357. It was not found in every bud of tested stalks. The highest percentage of buds infected with *C. falcatum* was 72% per stalk. The average percentage of buds infected with viable *C. falcatum* per stalk was 52% for Co 290, and 30% for CP 65-357. The difference was significant ($P = 0.01$, LSD test).

Elimination of *Colletotrichum falcatum* in the tunnels of sugar-

cane borer larvae and in inoculated internodes by aerated-steam treatment. When sugarcane borer-tunneled internodes were tested on autoclaved leaf-pieces, *C. falcatum* was detected in 44% (28 of 64) of them. *Colletotrichum falcatum* was isolated from less than 3% (2 of 67) of tunneled internodes of stalks treated with aerated steam at 52 C for 4 hr and from none (zero of 66) of those treated at 52 C for 5 hr. The tunneled internodes for each treatment were obtained from 20 stalks in two tests.

Colletotrichum falcatum was recovered from 100% (40 of 40) of the inoculated two-node cuttings and from 84% (36 of 43) of the inoculated internodes of growing stalks. The AST at 51 or 52 C for 2 hr eliminated *C. falcatum* from the cuttings, and the AST at 52 C for 4 hr eliminated it from the internodes of unstripped stalks. Shorter treatments were not tried with inoculated stalks.

Reduction of the percentage of buds with viable *Colletotrichum falcatum* by aerated-steam treatment at 51 and 52 C for 4 and 5 hr. The percentage of buds infected with viable *C. falcatum* was significantly reduced by AST, according to Waller and Duncan's Bayesian multiple comparison test (7) (Table 1). The reduction increased with the increases in treatment temperature and time. The reductions were similar whether the stalks were stripped (1976 test) or unstripped (1977 test).

In both cultivars, *C. falcatum* was detected in significantly fewer leaf scars on stalks given AST than on untreated stalks. For Co 290, the percentage of nodes with leaf scars yielding *C. falcatum* was 30% for untreated stalks; 11 and 8% for stalks treated at 51 C for 4 and 5 hr, respectively; and 5 and 3% for those treated at 52 C for 4 and 5 hr, respectively. The comparable figures for CP 65-357 were 21%, 8, and 6%, and 3 and 2%, respectively.

Elimination of *Colletotrichum falcatum* in the leaf sheaths and midribs by aerated-steam treatment. *Colletotrichum falcatum* was detected in 82% of untreated leaf sheaths and in 95% of untreated leaves with leaf-midrib lesions. The red-rot pathogen was not detected in the leaf sheaths after treatment at 52 C for 4 hr. The detection of *C. falcatum* from leaves with midrib lesions was reduced 85, 98, and 98%, respectively, by treatment at 51 C for 2, 3, and 4 hr; and reduced 88, 100, and 100%, respectively, by treatment at 52 C for 2, 3, and 4 hr.

Effect of aerated-steam treatment on seed cane germination of the

TABLE 1. Effect of aerated-steam treatment of whole sugarcane stalks on infection with *Colletotrichum falcatum* in buds and on germination of one-node cuttings prepared from the treated stalks

Year ^a	Cultivar	Treatment		Infection with <i>C. falcatum</i> in buds		Germination of one-node cuttings	
		Temperature (C)	Time (hr)	Buds from 20 stalks tested (no.)	Infection (%)	Cuttings from 5 stalks tested (no.)	Germination (%)
1976	Co 290	51	4	172	25	78	89
		51	5	162	14	80	75
		52	4	160	7	79	69
		52	5	167	5	74	54
		Room ^b		165	60	74	92
	CP 65-357	51	4	175	17	79	92
		51	5	171	12	76	89
		52	4	164	8	74	85
		52	5	162	6	73	55
		Room		179	46	81	95
WD-B ^c (k = 100) LSD					12.0		15.6
1977	Co 290	52	4	164	5	68	65
		52	5	168	3	73	42
		Room		161	52	74	90
	CP 65-357	52	4	163	4	85	90
		52	5	162	3	75	71
		Room		165	35	83	91
WD-B (k = 100) LSD					14.4		12.5

^aStalks were stripped of leaves before treatment in 1976, but not in 1977.

^bRoom temperature was about 24 ± 3 C.

^cWD-B LSD indicates Waller-Duncan Bayesian LSD (7).

two cultivars. In one-node cuttings, the two cultivars differed in tolerance of the heat treatment (Table 1). The germination of buds of Co 290 was significantly reduced by AST at 51 C for 5 hr, and 52 C for 4 hr, but the germination of CP 65-357 was significantly reduced only by AST at 52 C for 5 hr. The effect of AST on germination of cuttings of sugarcane borer-damaged and artificially inoculated stalks (data not shown) was similar to its effect on germination of cuttings for the uninoculated control. Tests have not been run with canes of varying degree of hardness. In these experiments, field-grown canes were used. No attempt has been made to harden or adapt the cane to increase resistance to heat.

The effect of AST on the germination of CP 65-357 may be less for whole stalks than for cuttings. Five stalks treated at 52 C for 4 hr, five treated for 5 hr, and five untreated stalks produced 34, 28, and 34 shoots, respectively.

DISCUSSION

The autoclaved leaf-piece technique has the following advantages over the OMA method for detection of *C. falcatum*: (i) Sensitivity—A higher percentage of buds infected with *C. falcatum* yielded the pathogen on leaf pieces than on OMA. Some of the apparently greater sensitivity of the leaf-piece technique may reflect a greater concentration of *C. falcatum* on the surface rather than within the tissue. The fungicidal effect of the surface disinfectant used with OMA might contribute to the apparently lower sensitivity of the OMA method. (ii) Specificity—Under the microscope, *C. falcatum* is easily distinguished from other fungi on the sugarcane leaf-piece. This facilitates its detection. (iii) Speed—A technician not trained in mycology easily can examine 50 leaf pieces in 1 hr (iv) Ease—Samples need not be surface sterilized.

Nonsporulating isolates of *C. falcatum*, which have been noted on OMA (5), have not been found on autoclaved leaf-pieces, but the search for them has been less extensive with the leaf-piece than with the OMA technique.

Although direct microscopic examination for *C. falcatum* would give an index of infection, it would not indicate whether the pathogen is viable. If the viability of the pathogen after heat treatment is to be determined, the technique must be able to demonstrate the growth of the pathogen.

The percentage of buds with viable *C. falcatum* per stripped stalk in the 1976 test was similar to that per unstripped stalk in the 1977 test. Also, the percentage of reduction in *C. falcatum* infections by AST was similar in unstripped and stripped stalks. Leaves and leaf sheaths attached to the stalks do not reduce the effectiveness of

AST.

Conditions strongly favoring set germination were used to determine the effect of AST on viability of buds. Under such conditions, *C. falcatum* does not always reduce set germination, even in a susceptible cultivar (1). This finding may be the reason that more than 90% of the cuttings from untreated stalks of cultivar Co 290 germinated even though *C. falcatum* was associated with more than 50% of them.

C. falcatum occurs in seed cane in borer tunnels, growth cracks, buds, and leaf scars, and the red-rot disease caused by this fungus is responsible for poor germination of sugarcane (1). Whether all infection sites are equally important for the poor germination of planted seed cane is unknown; under Louisiana conditions, red rot seems often associated with borer injury. AST at 52 C for 5 hr eliminated the pathogen from borer tunnels and inoculated internodes, and from 91% of buds, and 97% of leaf scars. Although the number of infected nodes per stalk required to reduce germination significantly in the field is unknown, the low frequency of infected nodes in treated seed cane, combined with the limited spread of red rot within the seed cane of the resistant varieties grown in Louisiana, suggests that AST at 52 C for 5 hr could effectively control the disease. This method warrants further research to determine if AST will control the disease under field conditions.

LITERATURE CITED

1. ABBOTT, E. V., and C. G. HUGHES. 1961. Red rot. Pages 262-287 in: J. P. Martin, E. V. Abbott, and C. G. Hughes, eds. Sugarcane Diseases of the World, Vol. 1. Elsevier, New York. 542 pp.
2. ANONYMOUS. 1973. Notice of release of sugarcane variety CP 65-357. Sugar Bull. 51:4.
3. CIFUENTES, O. M., R. J. STEIB, B. J. COCHRAN, and M. M. MAYEUX. 1976. The use of aerated steam for the control of ratoon stunting disease in Louisiana. Proc. Am. Soc. Sugar Cane Technol. 5(NS):127-129.
4. COCHRAN, B. J., M. M. MAYEUX, and R. J. STEIB. 1978. Aerated steam: A system for heat treating sugarcane to control ratoon stunting disease. La. State Univ., Agric. Exp. Stn. Bull. 709. 16 pp.
5. FONTENOT, D. B., M. J. GIAMALVA, and D. J. CHIASSON. 1972. Relative pathogenicity of isolates of *Colletotrichum falcatum* on sugarcane seed pieces. Proc. Am. Soc. Sugar Cane Technol. 2(NS):93-97.
6. SINGH, K. 1973. Hot air therapy against red rot of sugarcane. Plant Dis. Rep. 57:220-222.
7. WALLER, R. A., and D. B. DUNCAN. 1969. A Bayes rule for the symmetric multiple comparisons problem. J. Am. Statist. Assoc. 64:1484-1503.