Detection of smut mycelia in apical meristems of sugarcane buds[1]

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ABSTRACT

Two tests were conducted to detect the fungal mycelia of *Ustilago scitaminea* Syd. in apical meristems of sugarcane (*Saccharum officinarum* L.) buds. In the first test six varieties were selected from infected sugarcane fields, and in the second test three varieties obtained from a nursery field were artificially inoculated with the fungus. Sugarcane plants artificially inoculated with *U. scitaminea* were used as checks in the first test. Growing points were removed from the plant cane and stained for 4 to 18 h by using Sinha's technique. Microscopic observations indicated the absence and presence of smut mycelia in the apical meristem buds of healthy and infected sugarcane varieties. The staining method of growing point nodal buds can be useful for indicating fungicide efficacy in controlling sugarcane smut, and for sugarcane seed certification programs.

RESUMEN

Detección del micelio del carbón en meristemos apicales de yemas de caña de azúcar

Se realizaron dos ensayos para detectar el micelio de *Ustilago scitaminea* Syd. en meristemos apicales de yemas de caña de azúcar (*Saccharum officinarum* L.). En el primer ensayo se usaron seis variedades seleccionadas de cañaverales infectados y en el segundo se inocularon artificialmente con el hongo de tres variedades procedentes de un semilllero. En el primer esayo se usaron como testigos plantas inoculadas artificialmente con *U. scitaminea*. Se arrancaron los puntos de crecimiento de las yemas y se tiñeron por 4 a 18 horas usando la ténica de Sinha. Las observaciones microscópicas mostraron la presencia y ausencia del micelio del carbón en el meristemo apical de las yemas de las variedades de caña de azúcar infectadas y senas, respectivamente. El método de teñido de la yema puede ser útil para determinar la eficacia de los fungicidas que controlan el carbón de la caña de azúcar y en programas de certificación de semilla.

INTRODUCTION

The objective of this study was to detect postharvest smut mycelia in apical meristems of sugarcane nodal buds. Since sugarcane (*Saccharum officinarum* L.) is vegetatively propagated, seed pieces are used in germplasm exchange between improvement programs internationally. A rapid method to detect the smut (*Ustilago scitaminea* Syd.) in sugar-

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cane cuttings is necessary for quarantine programs to prevent movement of the smut pathogen by germplasm exchange. *Ustilago scitaminea* infects sugarcane plants chiefly through nodal buds (1, 8). The fungus maintains an association with the meristematic tissues inside bud scales, and subsequently new tissues with smut mycelium become differentiated into microsori (whip) containing spores (8). A rapid staining technique for detecting smut hyphae in the growing point of sugarcane buds was reported in 1982 by Sinha et al. (6). Farías (personal communication[3]) used the above technique in screening fungicides in sugarcane artifically inoculated with *U. scitaminea.*

MATERIALS AND METHODS

Two tests were conducted in the plant pathology laboratory at the College of Agricultural Sciences at the University of Puerto Rico, Mayagüez Campus. The presence of smut mycelia in apical meristems of sugarcane buds was determined with the staining technique reported by Sinha et al. (6). Growing points (GP) of nodal buds were stained with a cotton blue (0.1%) and sodium hydroxide (6%) aqueous solution within 18 h at room temperature; then GP were fixed with lactophenol in microslides for microscopic observations.

Test 1

Two sets of 8-month-old cane in second ratoon crop PR 66-2281, PR 67-1070, PR 69-2247, PR 980, PR 69-2110, and B 59-233 were selected from natural infection in sugarcane fields in southern Puerto Rico. One set, used as checks, were artifically inoculated with *U. scitaminea* by immersion in a spore suspension (5), then planted under glasshouse conditions. Check varieties were harvested when 8 months old. Eight buds per variety were removed from stalks, and their growing points were stained for 18 h before being fixed for microscopic examination.

Test 2

Two sets of three sugarcane varieties, PR 67-1070, PR 67-1355, and B 59-233, were selected from a nursery field at the Gurabo substation. One set was previously inoculated with the fungus before planting. Another set, noninoculated, was used as check. Eight young buds per variety were selected from 8-month-old plants grown in the glasshouse. Growing points were removed and stained for 5 h, then fixed in microslides for observation under a light microscope.

[3]Graciela M. Farías, Famailla Regional Exp. Stn., Tucumán, Argentina. S. A. (Personal communication).

RESULTS AND DISCUSSION

Presence of smut mycelium was generally evident in excised lateral GP of sugarcane varieties with smut whip symptoms and in latent infection (tables 1, 2). The fungal mycelium was stained dark blue when GP were placed in the stain solution overnight (18 h) (fig. 1B, 2B), but a 5-h staining period was enough to detect the smut mycelia (fig. 2A). Sinha et al. (6) and Farías[3] reported a 3 1/2-to 4-h period for staining of the smut hyphae.

Test 1

Microscopic observations indicated the presence of smut mycelium in almost all buds for PR 980 and B 59-233 (table 1). However, PR 66-2281, PR 67-1070, and PR 69-2247 did not show any fungus hyphae in nodal buds. The presence of mycelium was evident only in one or two artificially infected buds of control checks. Sugarcane plants of PR 980, selected from natural infection fields near Aguirre, showed smut whip symptoms from side shoots (lalas) of stalks (fig. 3). PR 980 has been reported as smut susceptible in Guyana, Jamaica (2), and Zimbabwe (4). However, no infection was observed in 5 buds removed from this variety. Echávez-Badel (*unpublished*) observed that PR 980 had a moderate level of smut resistance in plant cane; however, the smut intensity increased 50% when this variety was ratooned.

TABLE 1.—*Detection of smut mlycelia in apical meristem of lateral buds of 6 sugarcane varieties with natural infection of* Ustilago sciteminea

		Smut mycelia[1]							
		Number of nodal buds							
Variety	Inoculation[2]	1	2	3	4	5	6	7	8
PR 66-2281	no	–	–	–	–	–	–	–	–
	yes	–	–	–	–	–	+	–	–
PR 67-1070	no	–	–	–	–	–	–	–	–
	yes	–	–	+	–	–	–	–	–
PR 69-2247	no	–	–	–	–	–	–	–	–
	yes	–	–	–	–	–	+	–	+
PR 69-2110	no	+	–	–	–	+	–	–	–
	yes	–	+	+	–	–	–	–	–
PR 980	no	–	–	+	–	+	–	+	–
	yes	+	–	–	–	+	+	+	–
B 59-233	no	+	+	+	+	+	–	+	+
	yes	+	+	+	+	+	+	+	+

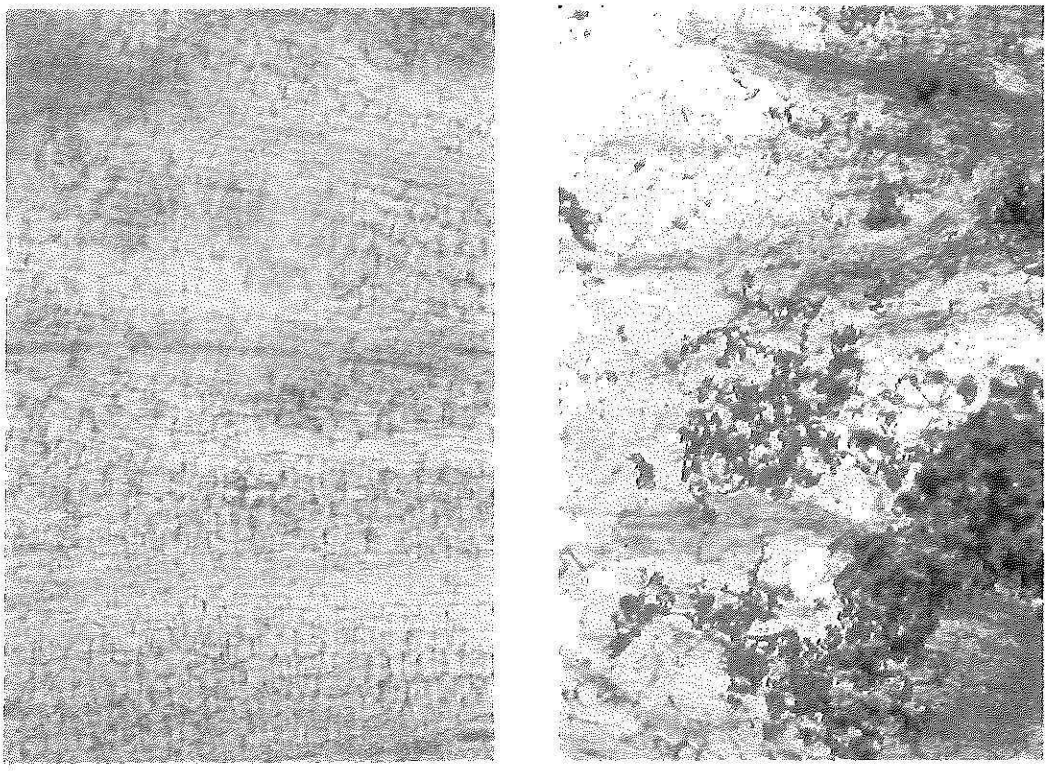
[1] + = Presence of smut mycelia; – = absence of smut mycelia.

[2] yes = Pieces of seed cane were immersed in 5 x 10^6 smut spores per ml of water.

TABLE 2.—Detection of smut mycelia in apical meristem of buds of 3 sugarcane varieties artificially inoculated with *Ustilago scitaminea* by immersion in a spore suspension

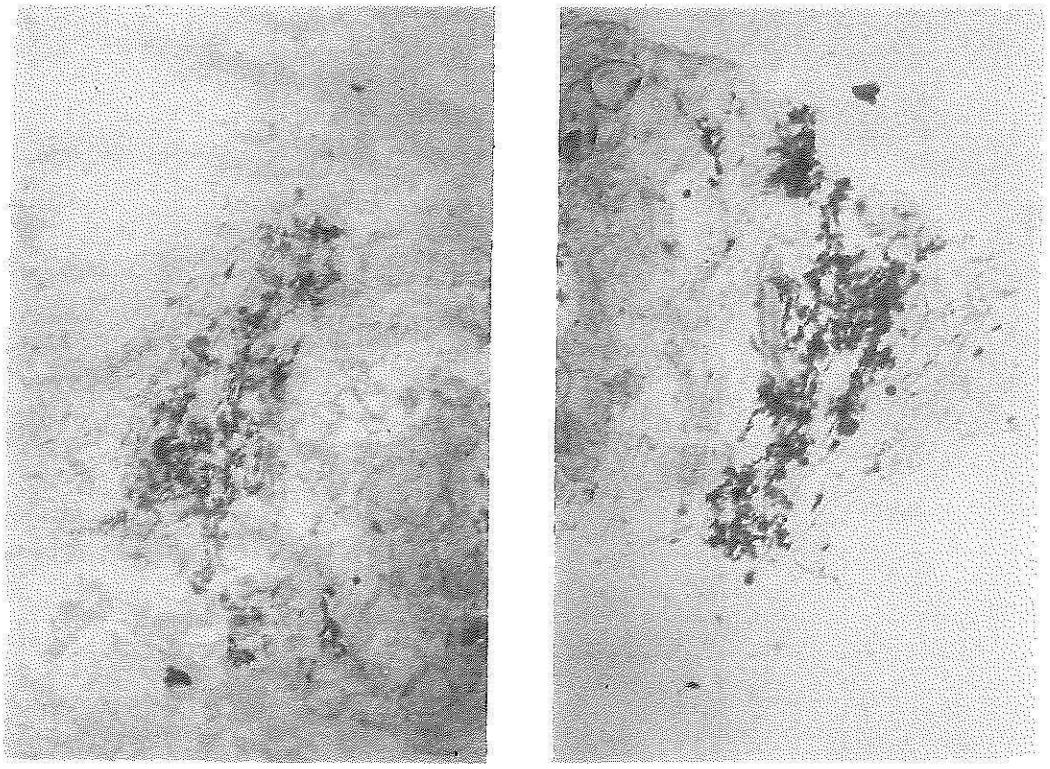
		Smut mycelia[1]							
		Number of nodal bud							
Varieties	Inoculation	1	2	3	4	5	6	7	8
PR 67-1070	yes	–	–	–	+	–	–	–	–
	no[2]	–	–	–	–	–	–	–	–
PR 67-1355	yes	–	–	–	–	–	–	+	–
	no	–	–	–	–	–	–	–	–
B 59-233	yes	+	+	+	+	+	+	+	+
	no	–	–	–	–	–	+	–	+

[1] + = Presence of smut mycelia; – = absence of smut mycelia.
[2] Pieces of seed cane were immerced in distilled water.



A B

Fig. 1.—Growing points of PR 67-1070 (left) and B 59-233 (right) varieties. A) Growing point of healthy bud. B) Smut mycelia of infected bud when growing point was stained 18 h period.



A B

Fig. 2.—Growing points of nodal buds of B 59-2.33 sugarcane variety. A) Mycelia of *Ustilago scitaminea* when growing point was stained 5-h period. B) Infected bud when growing point was stained 18-h period.

Test 2

Mycelium was observed in all buds removed from B 59-233 sugarcane plants artifically inoculated (table 2). Echávez-Badel (*unpublished*) found 89% smut infection in B 59-233 sugarcane plants previously inoculated by immersion in a smut spore suspension. Infection is highest in buds from 8- to 12-month-old canes in susceptible varieties (7). PR 67-1070 and PR 67-1355 generally did not show the fungus mycelium in GP of nodal buds. Both varieties were resistant to smut in Puerto Rico (3).

The bud staining technique could be used to detect smut mycelium in sugarcane apical meristems before appearance of the first whip symptoms. Furthermore, this staining method can be useful in testing fungicide efficacy for smut and for certifying seed-pieces for planting or exchange.

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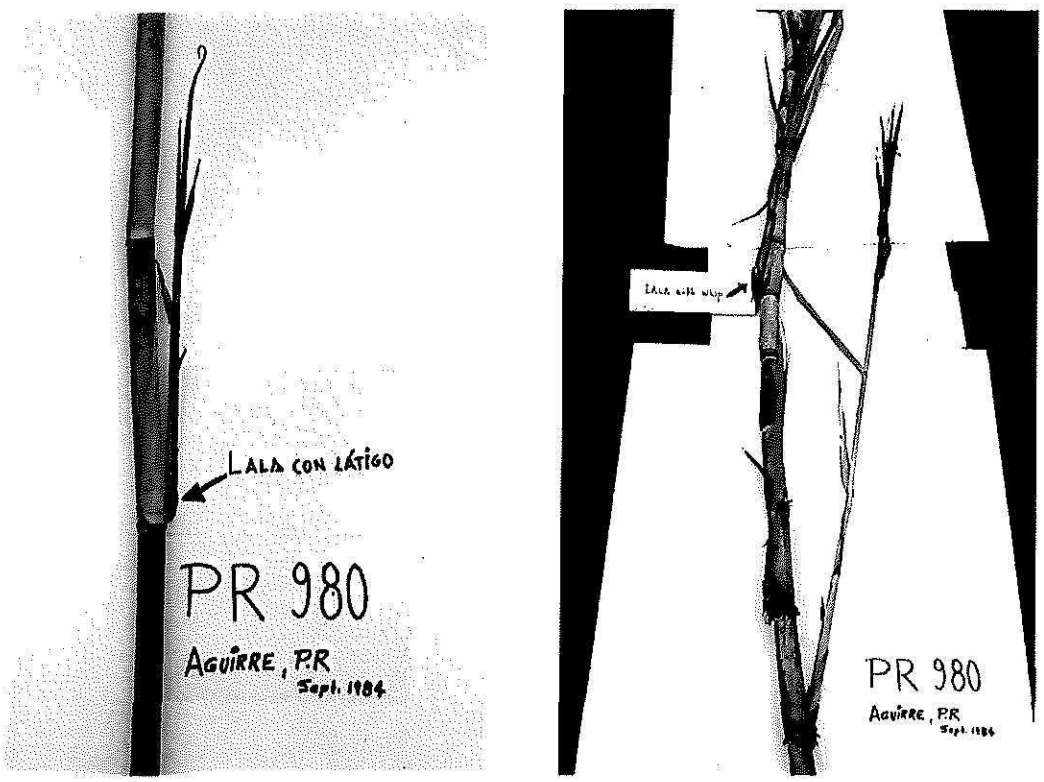

A B

Fig. 3.—Sugarcane variety PR 980 selected from infected sugarcane fields near Aguirre, P. R. Note smut whips from side shoots (lalas) of stalks.

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