Stain Technique for Detection of Smut Hyphae in Nodal Buds of Sugarcane

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ABSTRACT

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A rapid staining technique was developed to demonstrate hyphae of *Ustilago scitaminea* in the growing point of nodal buds of sugarcane (*Saccharum* spp.) within 4 hr. Hyphae were present in all the growing points of buds from diseased canes but not in buds from healthy canes. This technique can conveniently be used by quarantine stations and seed certifying agencies for screening sugarcane seed material.

Sudden outbreaks of smut disease of sugarcane (*Saccharum* spp.) caused by *Ustilago scitaminea* Syd. have been recorded in countries or areas where smut was previously absent or unknown (1,3,4,6). The spread of the disease from one region to another is often through inadvertent movement of infected seed material, inasmuch as infected dormant nodal buds and healthy buds cannot be distinguished. Quarantine stations and seed certifying agencies examining buds alone cannot detect the disease with accuracy in a limited time because sugarcane is a perishable material. The only dependable method for the detection of smut at present is to plant the buds in a glasshouse and wait for the appearance of smut whips, which may take several months.

According to Bock (2), the smut fungus penetrates through the lower portion of the bud below the scales, becomes established in the meristematic region of the dormant bud, and undergoes a period of latency. This report describes a reliable and rapid technique for detecting smut hyphae in the growing point of nodal buds of sugarcane.

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MATERIALS AND METHODS

Laboratory study. Twenty-five healthy and the same number of diseased canes with apical whips were taken from an 8-mo-old crop of sugarcane cv. Co 1158. Healthy canes were obtained from a disease-free crop. All the canes were detrashed and the smut whips removed. Nodal dormant or sprouted buds of each cane were serially scooped out, and remnants of rind and dried bud scales were removed. The trimmed bud was held between thumb and forefinger, keeping the proximal (basal) end upward. Several transverse slices were removed with a sharp razor until folds of the bud scales were visible. The growing point, situated exactly in the center of the exposed portion of the bud, was removed by applying a slight pressure to the bud held in the same position with the thumb and forefinger. This allowed the growing point to be slipped out and then picked up with forceps without injury.

The growing points were put separately in watch glasses containing distilled water. A 0.1% aqueous solution of trypan blue stain and a 6% solution of sodium hydroxide were mixed in equal amounts. One milliliter of stain solution was placed in a small vial; the growing point was transferred to it with a camel's hair brush and allowed to stand for 3.5 hr. Subsequently, the growing points were removed to distilled water, thoroughly washed, transferred to 80% ethanol for 2 min for dehydration, and then placed in a vial containing 1 ml of lactophenol. The vials were then heated to boiling for 2 min to remove excess stain and clear the tissue. Finally, each growing point was placed on a microslide and mounted in lactophenol. The coverslip was gently pressed to keep the conical growing point in one plane. Presence or absence of the fungus in the growing point was observed under a light microscope at ×256.

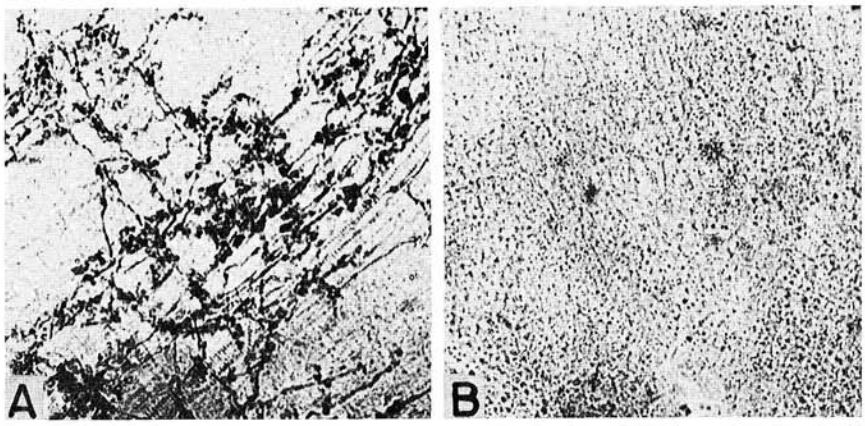

Fig. 1. Growing points of nodal buds of sugarcane: **(A)** Hyphae of *Ustilago scitaminea* in the growing point of smut-infected bud. **(B)** Growing point of healthy bud.

Microplot study. In a study to confirm the laboratory findings, 150 four-bud setts (seed material) of Co 1158 were cut from healthy and smutted canes. One terminal bud of each sett was scooped out and the growing point examined for the presence of the smut fungus by the staining method described above. Concomitantly, setts from healthy and diseased plants, each with three viable buds, were planted in separate microplots (6.3×7.0 m^2). Subsequent to sprouting, all but 100 plants per plot were removed. These plants were observed every week for 7 mo. Plants or clumps were removed upon the first appearance of a smut whip.

RESULTS

Microscopic examination of the growing points removed from buds of diseased canes both in the laboratory study and the microplot study all showed the presence of a network of branched fungal hyphae that was stained dark blue (Fig. 1A). In several buds, hyphae extended from the bottom to the tip of the growing point; in some other buds, the hyphae were confined to the basal portion only. On the other hand, none of the growing points from buds of healthy canes showed any trace of fungal hyphae (Fig. 1B).

In the microplot in which diseased setts were planted, smut whips appeared in all the 100 plants at different stages of their growth. Conversely, all plants raised from healthy setts remained free from smut whips through the end of the crop season.

DISCUSSION

The consistent presence of a network of fungal hyphae in all growing points of buds taken from diseased canes and the absence of hyphal structures in the growing points of buds from healthy canes indicated that the hyphae observed were those of the smut pathogen. An earlier observation by Bock (2) indicated that during growth of an infected shoot, the pathogen colonizes each primordium that subsequently develops into a nodal bud. Production of smut whips in plants raised from diseased setts substantiates the above conclusion. Waller (5) concluded that any shoot primordium produced from an infected meristem would be diseased and that plants that become infected during their initial growth stages produce a succession of smut whips for a considerable period during crop growth. Circumstantially, it could be surmised that the fungal hyphae present in the growing points of diseased buds are those of the smut fungus.

Inasmuch as the whole detection procedure can be completed within a 4-hr period and smut infection can be detected in buds earlier than the symptom expression on planting, this technique can be a useful tool, especially for quarantine stations and seed certifying agencies.

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