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Research Article

SMUT SPORE (*USTILAGO SCITAMINEA*. SYDOW) GERMINATION ON THE SURFACE OF SUGARCANE BUDS (*SACCHARUM* SP.), ITS CORRELATION WITH DISEASE RESISTANCE RATINGS, AND A TOOL FOR ASSESSING DISEASE RESISTANCE

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ABSTRACT
Scanning Electron Microscopy (SEM) was used to assess smut spore germination on the surface of sugarcane buds. There is a good correlation (r=0.8760) between the field resistance ratings of the 10 cultivars and the Mean Percentage Spore Germination (MPSG) on the surface of sugarcane buds. This good correlation implies that this method is useful in assessing the resistance ratings of cultivars.

INTRODUCTION
The major route of entry of the smut fungus (Ustilago scitaminea Sydow) is through the bud (Fawcett, 1944; Bock, 1964; Waller, 1970). Young buds are very susceptible to infection with resistance increasing with bud age (Bock, 1964; Byther and Steiner, 1974). Before infection occurs the spores must germinate on the bud surface. The germination time is about 6 hours (Bock, 1964; Waller, 1969; Trione, 1980; Sealy, In Press.). Spore germination occurs when the required number of spores, free water (moisture) and temperature are available for a specific time interval. After germination the fungus enters the host within 6-36 hours (Alexander and Ramakrishnan, 1980). This occurs through infection hyphae, the fusion of promycelial strands from different spores to form one strand which then penetrates the host or the fusion of promycelia to form an appresorium which then produces an infection strand (Waller, 1969; Alexander and Ramakrishnan, 1980, Sealy, 2020).
The morphology of the bud supplies physical resistance to the entry of the infection hyphae. Waller (1970) observed that the bud is most susceptible to penetration. Singh and Budhraja (1964) also suggested that bud scales are barriers against infection probably by delaying or preventing infection hyphae reaching the meristematic cells. They observed that the application of viable spores over a bud, after removing the scales, followed by incubation and planting of the inoculated seed-piece resulted in 100% infection in susceptible cultivars of sugarcane. Waller (1970) found that there is a correlation between a number of bud characters and resistance to smut, namely, the presence of a flange, bud grove, type of germination, bud size, time to burst and the growth rate. Fawcett (1944) observed that infection occurs at the base of the bud beneath the outer scale. Muthusamy (1974) studied the variation in susceptibility to smut in relation to bud sprouting, bud size, bud shape, position of the germ pore and the incidence of the stalk borer (Chilo indicus). A strong positive correlation was observed between the smut incidence and bud sprouting in standing canes. He observed that the sprouting of buds in the two varieties was due to the attack of the stalk borer. However, the high percentage of sprouting and the presence of the stalk borer infection did not always predispose cultivars to smut.

According to Muthusamy (1974) the position of the germ pore was subapical in most resistant varieties and apical in susceptible ones. Correlations were also found between smut incidence and bud size. Cultivars with large buds were more likely to sprout and to be infected by smut. Dormant buds are more resistant to smut than germinating buds (Waller, 1970). Waller (1970) suggested that the increased susceptibility of germinated buds is associated with swelling of the bud and an increase in the area within the bud. He thought that the swelling would permit easier access of spores between bud scales, and the increase in the area would allow for greater probability of penetration by the infection hyphae. Infection

hyphae on reaching the meristematic region of the bud undergo a period of latency. The mycelium min the host then spreads intercellularly, feeding through haustoria (Waller, 1970; Singh and Agnihotri, 1978; Alexander and Ramakrishnan, 1980).

Lloyd and Pillay (1980) found that the fungus in the cane stalk was largely associated with peripheral vascular bundles especially xylem tissue. Other workers have reported its presence in the parenchyma adjacent to vascular bundles (Alexander and Ramakrishnan, 1980), and even in the phloem (Peros and Chagvarchef, 1984). Bock (1964) noted that an infected bud produces a shoot and the growth of the mycelium of the pathogen keeps pace with the meristematic region.

The assessment of host resistance is by inoculation, both artificial and natural. The various methods of inoculation summarized after Lee-Lovick (1978) and others are:

(a) Spores are applied at the ends of cuttings
(b) Spores are dusted on buds at planting
(c) Spores mixed in soil before planting
(d) Soaking cuttings in a spore suspension (Bachchav et al, 1979; Whittle, 1982)
(e) Spores applied to young buds on standing canes
(f) Spores introduced into wounds at the base of shoots or around buds or roots
(g) Application of spores to cane flowers and the resultant infected seeds grown
(h) Inoculation of uprights (Benda and Koike, 1985)
(i) Dip minus bud scale inoculation (Rampersad and Brathwaite, 1985)

The most commonly used technique for the inoculation of buds is an aqueous suspension of spores. Various modifications in time of dipping and spore concentrations are used (Byther and Steiner, 1974; Bachchav *et al.*, 1979; Dean, 1982; Whittle, 1982). For natural inoculation in the field, it is widely accepted that rationed canes give a truer picture of the resistance of the various cultivars to smut (Walker, D.I.T, Personal communication).

This study investigated smut spore germination on the surface of sugarcane buds since it seemed possible that one line of the plant's defense against smut might operate at the level of the infection court, namely, the bud surface, and a tool for assessing cultivar resistance.

MATERIALS AND METHODS

One bud nodal setts were excised from 7 month old ratooned canes of the cultivars, B80689, B71383, B74541, B73382, B62163, B63118, B73385, H593775, B60267, and B7316. The standard bud, most apical bud on the stalk from which the leaf sheath could be removed in its entirety, was used in this experiment. The buds were surface sterilized by wiping with cotton swabs containing 20% (v/v) house hold bleach (commercial solution of 1.0% available chlorine, and rinsed with distilled water. Nodal setts and prewashed microscope slides were placed in plastic containers lined with damp paper towels and a drop of spore suspension (10^5spores ml^{-1}) was placed on buds and slides. The containers were sealed and maintained at 25^0C for 6 hours. The buds were excised, frozen on dry ice and placed in a dessicator containing KOH pellets (O'Brien and McCully, 1981) for 3 weeks. The dried buds were mounted on SEM stubs and Sputter coated with gold: palladium (60:40) alloy source for 3.5 minutes in an argon atmosphere and a current of 15-20 mA using an Edwards's sputter coater S150B.

The specimens were viewed with a Philips 505 Scanning electron Microscope (Botany Department, U.W.I, Mona, Jamaica) and scored for percentage germination based on a random field of approximately 100 spores. Controls, consisting of a drop of spore suspension on a prewashed microscope slide, were scored for percentage germination by light microscopy after 6 hours, using 15 (w/v0 aqueous aniline blue to stain germ tubes. Spores with germ tubes protruding were scored as having germinated (Manners, 1966). For each cultivar 6 buds were examined.

Results and Discussion

When smut spore suspensions were placed on the surface of sugarcane buds of the various cultivars the Mean Percentage Spore Germination (MPSG) varied between 30 and 655 (Figure 1). The field resistance ratings of the cultivars are given in Table 1.

Table 1: Field Resistance Ratings to Smut disease for sugarcane Cultivars used in this Study. (Walker, D.I.T, Personal Communication- the West Indies Central sugarcane Breeding Station). The most resistant cultivar is rated zero, the least resistant nine (Hutchinson, 1969).

SUGARCANE CULTIVAR	RESISTANCE RATING
B80689	1
B73183	2
B63118	3
B62163	4
B74541	5
B73382	6
B73385	6
B60267	6
B72177	6
H593775	7
UCW5465	7
B7316	8

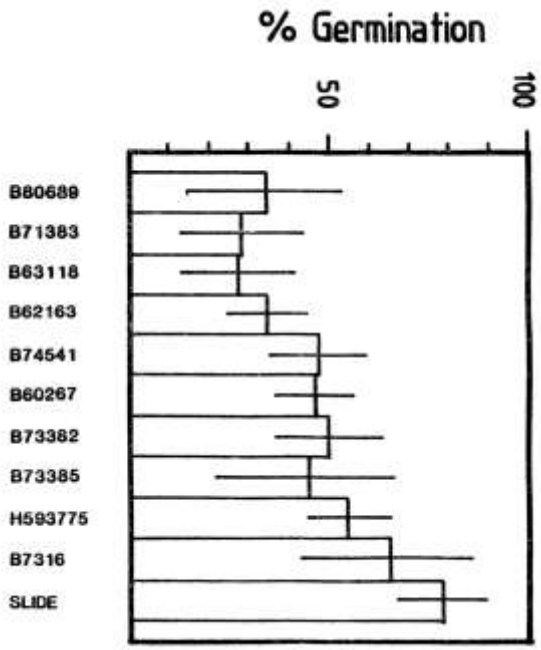

Figure 1: Germination of smut spores after 6 hours incubation on Sugarcane bud surfaces. Cultivars are ranked in order of decreasing resistance to smut disease. (Mean of six replicates +/- 95% confidence limits.

The MPSG on the bud surface increased as host resistance decreased and was consistently lower than that of spores germinating in water on a microscope slide. Resistant cultivars B80689, B71383, and B63118, and the moderately resistant B62163 inhibited smut spore germination the greatest with values for MPSG between 28 and 35%. Spore germination on moderately resistant cultivars like B74541, B60267, B73382, and B73385 was characterized by MPSG values around 50%. Susceptible cultivars B7316 and H593775 gave MPSG values closer to those of spores germinated in water. Put simply, there is clearly a trend of increasing inhibition of spore germination with increasing resistance to smut. In fact the correlation between smut resistance ratings of the cultivars and MPSG on the bud surface (r_s =0.876. n=10, p=0.0085) is very good.

The results show a good correlation between spore germination on the bud surface and field resistance to smut disease (Figure 1). While the disease resistance of plants is considered to be multifactoral (Misaghi, 1982), the close relationship I have demonstrated suggests the bud surface may be a major site for resistance for the plant. On the other hand, there is only a 305 difference in MPSG between germination on the most resistant and most susceptible cultivars and it is not clear that this could account for such contrasting resistance in the field. Whatever the fundamental implications of these results, they are important in that they validate this technique as a useful and simple method for screening new cultivars for smut resistance.

Based on the resistance ratings of the cultivars and their MPSG on the bud surface the following is suggested as a basis for screening cultivars:

Mean Percentage Spore Germination	**Classification**
Below 35%	Resistant
36-50%	Moderately Resistant
Over 50%	Susceptible

This method is faster than conventional field trials and while buds from 7 month old ratooned canes were used in these experiments it might be possible to use canes of a younger stage.

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