CIBTech Journal of Microbiology ISSN: 2319-3867
An Open Access, Online International Journal Available at http://www.cibtech.org/cjm.htm
2020 Vol.9 pp.25-33/Sealy
Research Article

SMUT SPORE (*USTILAGO SCITAMINEA*) GERMINATION ON THE SURFACE OF SUGARCANE BUDS (*SACCHARUM*. SPP). (i) THE PRIMARY INFECTIOUS PROCESS

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ABSTRACT
Scanning Electron Microscopy was used to investigate the smut infection process of on the surface of sugar cane buds. Smut spores (*Ustilago scitaminea)* are self-inhibited when spores are clustered on the bud surface. Once germination has occurred the promycelium might penetrate the host directly, presumably dikaryotising in the host, or dikaryotise on the bud surface by the following, (a) fusion of promycelial strands from different spores to form one strand which penetrates the host, (b) fusion of promycelia to form appresorium and (c) promycelia bud to produce sporidia which, on fusion, leads to infection hyphae. Host factors such as bud hairs and their associated secreted glands might play a role in disease resistance.

Keywords: *Promycelia, Appresorium, Dikaryotise, Infection Hyphae, Promyclium*

INTRODUCTION
Plants and pathogens evolved together and during this coevolutionary process pathogens developed systems which enabled them to parasitize plants, whereas plants developed sophisticated mechanisms to defend themselves. These mechanisms can be divided into two major categories (a) preformed resistance factors and (b) induced resistance factors, both of which can be further subdivided into structural and chemical factors. As the name suggests preform refers to factors existing prior to the arrival of the pathogen while induce refers to factors developing after the arrival of pathogen.
There are two barriers of infection (Dean, 1982), characterized as a pre-infection barrier and a post-infection barrier (Lloyd and Pillay, 1980). They suggested that the pre-infection barrier is associated with the bud scales which provide both chemical and physical resistance to the entry of promycelium. The post infection barrier occurs after the fungus enters the host and is probably more chemical than physical (Lloyd, and Pillay, 1980).
Besides the cuticle's function as a barrier, cuticular materials are capable of stimulating or inhibiting the growth of pathogens (Royle, 1976). The hydrophobic nature of the cuticular surface can prevent water films from accumulating on plant surfaces and this might alter the rate of infection (Blakeman and Atkinson, 1976; Blakeman and Sztejnberg, 1973; Martin *et al.,* 1957). For example, waxes present on surfaces, such as leaves and buds may not readily retain drops of spore suspensions. Leaf waxes show both inhibitory and promontory properties (Blakeman and Atkinson, 1976; Blakeman and Sztejnberg 1973; and Martin *et al.*, 1957).
Before infection occurs the spores must germinate on the bud surface (Bock, 1964; and Waller, 1969, 1970). The germination time is 6 hours (Bock, 1964; Sealy, 1988; Singh and Budhraja, 1964; Waller, 1969). The type of germination which ensues depends on the substratum and is of two types, hyphal or sporidial (Waller, 1969).
In this research I attempted to understand the behavior of germinating *Ustilago scitaminea* Sydow (smut) spores on the surface of sugarcane buds and factors leading to the production of infectious hyphae. Attention was focused on the presence of hairs on the bud surface and their possible involvement in the

prevention of infection. Cultivars were obtained from the West Indies Sugar Cane Breeding Station in Barbados and the research was done at the University of The West Indies.

MATERIALS AND METHODS

One bud nodal setts were excised from seven month old ratoon canes of resistant, moderately resistant and susceptible cultivars. 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) chlorox (commercial solution of 1.05% available chlorine) and rinsed with distilled water. Nodal setts were placed in containers lined with damped paper towels and then a drop of spore suspension (1000000 spores per ml) was placed on the buds. The containers were sealed and maintained at 25C for 6 hours. The buds were excised, frozen on dry ice and placed in a desiccator containing KOH pellets (16) for three (3) weeks. The dried buds were then 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 15-20mA using an Edwards Sputter Coater S150B. The specimens were viewed with a Philips 505 Scanning Electron Microscope. At least 10 buds per cultivar of each of the three types of cultivars were examined.

RESULTS

Figures 1-7 show the events that did occur over a 6 hour period on a sugarcane bud surface once the required amount of spores, free water (moisture) and temperature were available. In Figure 1, all the spores are clustered together and are ungerminated. Although this Scanning Electron Micrograph (SEM) relates to a resistant cultivar (B71383), clustered spores on moderately resistant and susceptible cultivars showed little or no germination. The clustering of spores on the bud surface therefore leads to self-inhibition of spore germination. Figures 2 and 3 show germinated spores and an appressorium on the bud surface. Germ tubes (promycelia) fuse to form the appressorium (Figure 3) which are attached to the bud surface. These presumably form a dikaryotic phase (Singh and Budhraja, 1964) which produces infection hyphae and spreads throughout the host (Alexander and Ramakrishnan, 1980, Singh and Agnihotri, 1978; Waller, 1970). Fusion was described between sporidia, promycelium and hyphae resulting in dikaryotisation but these were not on the bud surface (Alexander, and Srinivasan, 1966). On the other hand, such a phenomenon is possible as is depicted by Figure 4 where there are germinated spores on the bud surface budding to produce sporidia and infection hyphae. An apressorium is also present (Lee and Bostock, 2006.) and this occurred on the very susceptible cultivar, B7316. Figure 5 also shows the development of sporidia from the promycelium of a germinating spore. In this case the substratum is the bud surface of a moderately resistant cultivar, B73385.

Figure 6 shows a phenomenon that is known for *Ustilago maydis* (Fisher and Holton, 1957) but not mentioned in the literature for *Ustilago scitaminea*. Promycelia from different spores that become elongated and septate (notches on hyphal strand) fuse at a particular point (dikaryotise) and then penetrate the bud surface. This occurred on the very susceptible cultivar, B49119. The fusion of promycelia from different spores as well as appressoria formation supports the concept of sexuality in the fungus (Alexander, and Srinivasan, 1966). Promycelium produces long, septated hyphae which act as infection threads. This could be supported by the Scanning Electron Micrograph finding noted in (Figure 7) where there is direct penetration of the bud surface (B60267, moderately resistant) by the promycelium of the germinating spore. Dikaryotisation presumably occurs within the host.

DISCUSSION

The morphology of the bud supplies physical resistance to the entry of infection hyphae (Waller, 1970). The sugarcane bud is most susceptible to fungal penetration by the smut pathogen and there is a correlation between a number of bud characters and resistance to smut, namely, the presence of a flange, the presence of a bud grove, type of germination, bud size, time to burst and growth rate (Waller,

1970).These particular items were not observed in this study as germination was the only aspect studied. However, bud groves might be involved in the clustering of spores and therefore preventing germination of smut spores (Figure 1). Bud scales are suggested to be barriers against infection and probably delay or preventing the infection hyphae reaching the meristemic cells (Fawcett, 1944). This was not confirmed by this study because of the surface nature of the investigation.

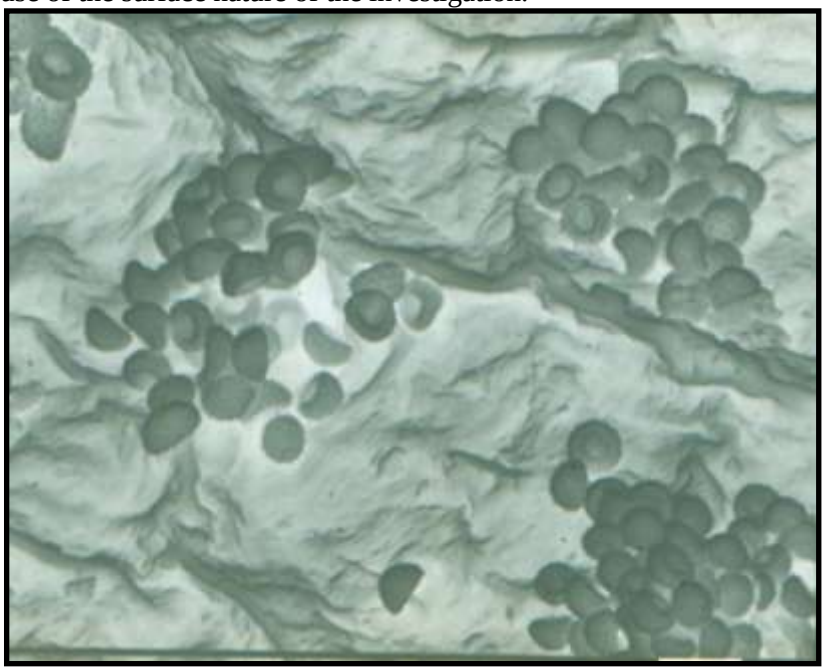

Figure 1: Ungerminated spores on the bud surface of the cultivar B71383 (resistant) The clustering of spores cause self inhibition of spore germination (Mag. X 2,500)

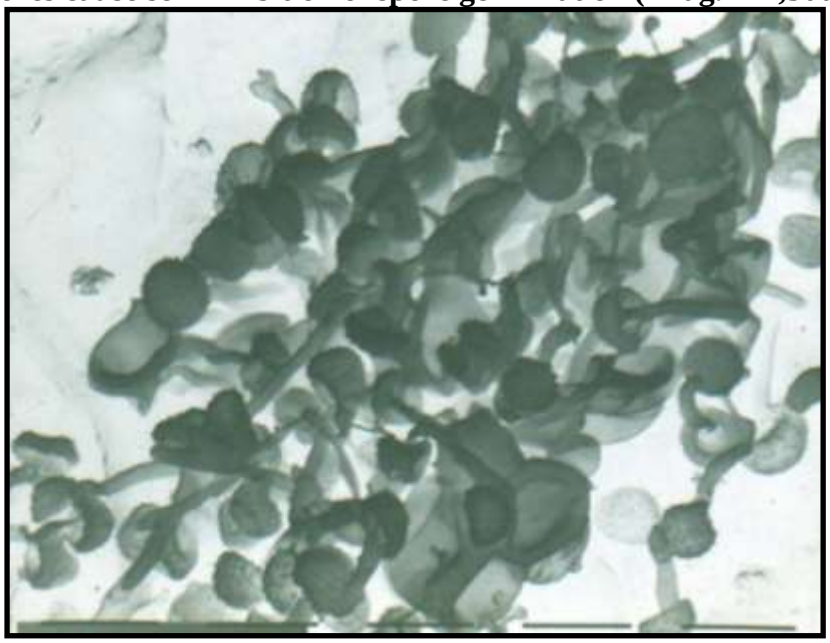

Figure 2: Germinated spores on the bud surface of the susceptible cultivar B7316 (Mag. X 1,500)

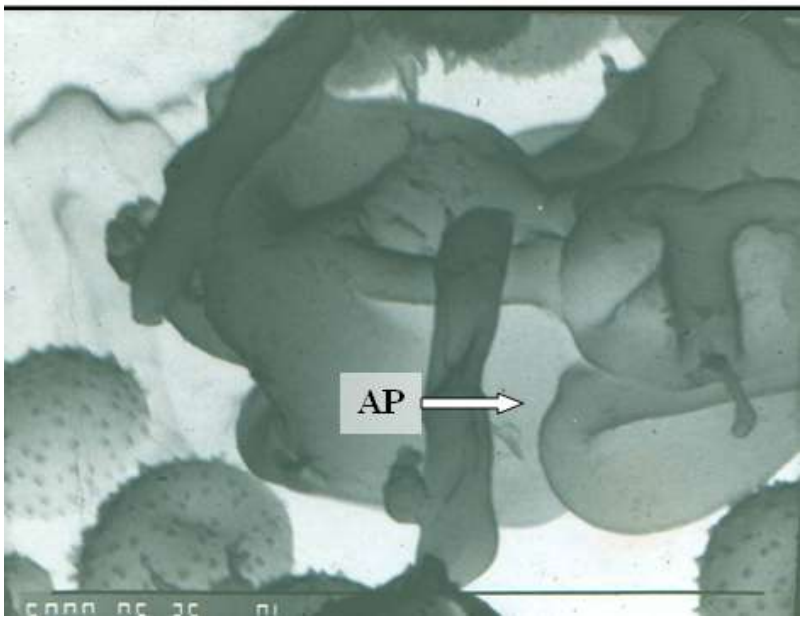

Figure 3: Germ tubes (promycelia) forming an appressorium (AP) on the bud surface of the susceptible cultivar B7316 (Mag. X 5,000).

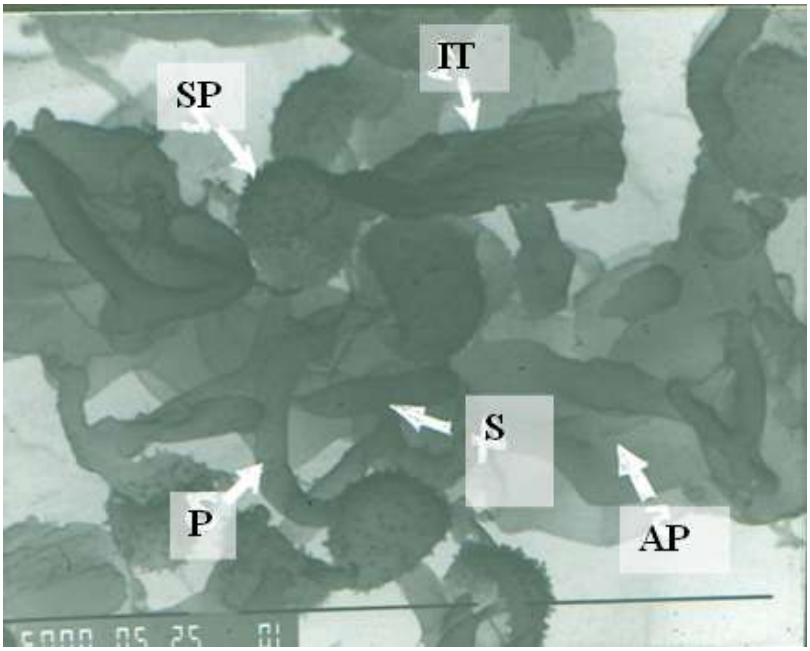

Figure 4: Germinated spores on the bud surface of the susceptible cultivar B7316, with promycelia budding to produce sporidia and infection hyphae. Appresorium was also seen. (Mag. X 3,500) Infection Thread (IT), A Smut Spore (SP). Promycelium(P), Sporidium (S) budding from promycelium and Apressorium (AP) on the bud surface.

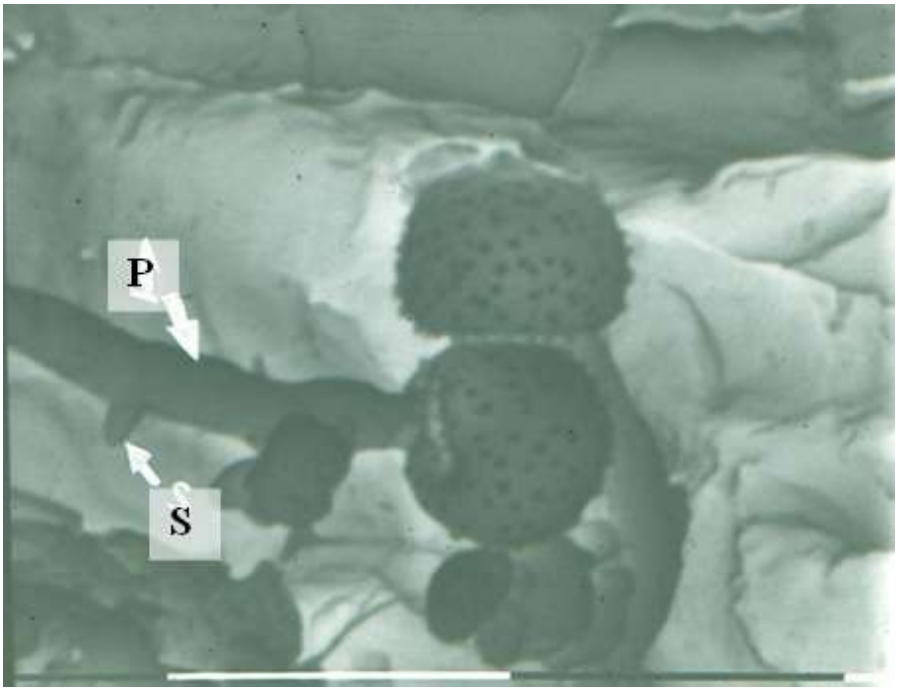

Figure 5: Spores germinating on the bud surface with promycelia (P) and developing sporidium (S) of the moderately resistant cultivar B 73385 (Mag. X5, 375).

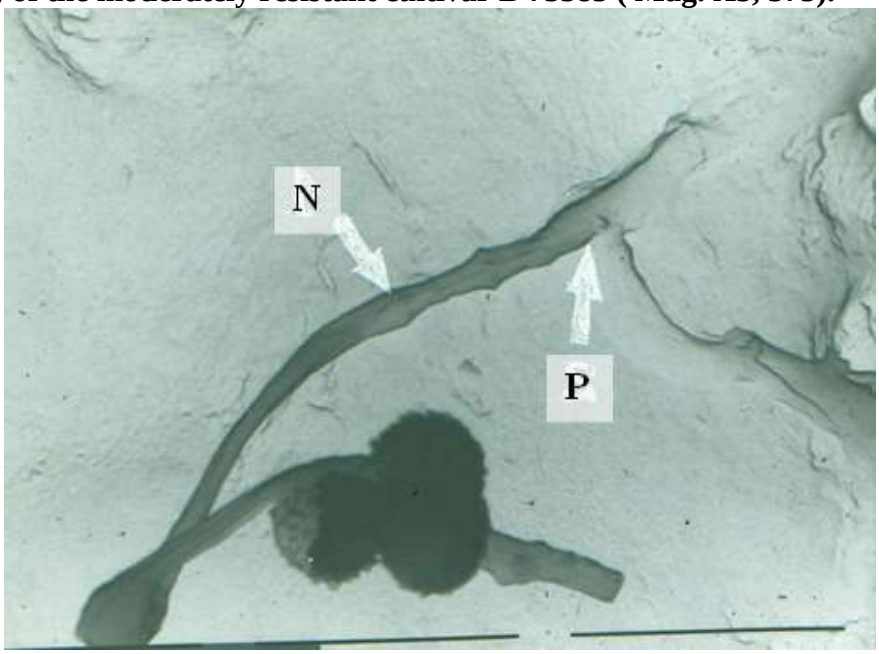

Figure 6: Fused spores germinating on the bud surface of the susceptible cultivar B49119 producing a promycelium which combines with another promycelium before penetrating the bud surface. (Mag. X 3000). Notches (N)- regions of septation; Fusion of promycelial strands (P) –dikaryotisation..

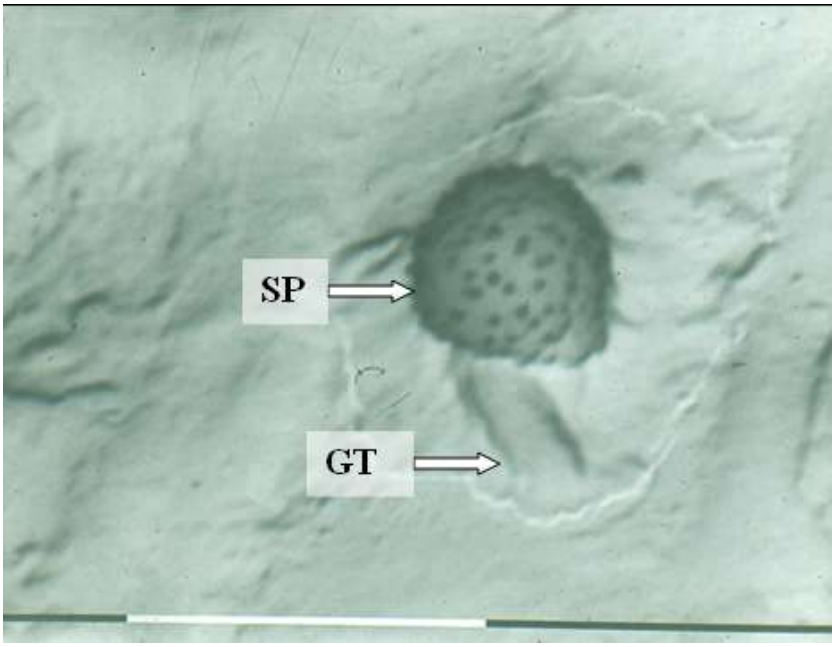

Figure 7: A germinated spore (SP) on the bud surface with the germ tube (GT)-promycelium, penetrating the bud surface of the moderately resistant cultivar B60267 (Mag. X 5,375).

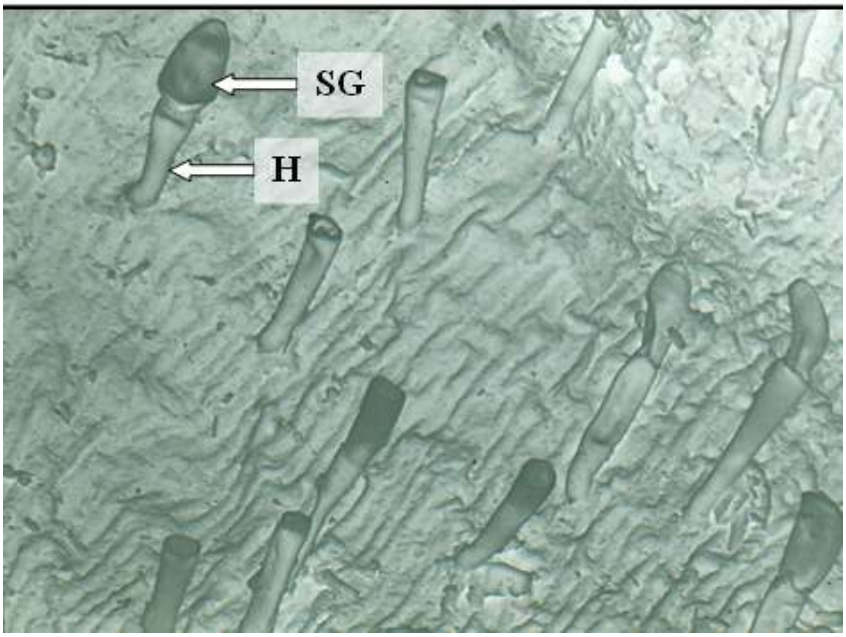

Figure 8: Hairs (H) on the bud surface of the resistant cultivar B71383. Some hairs have lost their secretary gland (SG) and some of the intact ones are deflated suggesting that their contents were released. (Mag.X 2,500).

The most important morphological characteristic in relation to resistance to sugar cane smut (*Ustilago scitaminea*) according to this study is the presence of hairs on the bud surface with secretary glands (Figure 8). Plant hairs may be involved in resistance of pathogens by host plants by the secretion of toxic substances (Hafix, 1952, Misaghi, 1982, Weinhold, and Hancock, 1980). There is no known relationship between the presence of bud hairs on the bud surface of sugar cane cultivars and resistance to smut. However, bud hairs may be important chemically, as secretary structures since there are secretary glands at their apex (Figure 8). To a lesser extent hairs could prevent the direct contact of spores with the bud surface. Whether the response to the presence of the pathogen by the hairs is spontaneous or constitutive in terms of chemical secretion is unknown. But an interesting observation (Figure 8) is the presence of a secretary structure in a socket at the top of the hair. This presumably falls to the bud surface releasing its contents and an upright structure remains showing a circular hollow (Figure 8). Cultivars which contain hairs on their bud surface also have numerous prickles on their stalk. Such cultivars as B80689 and B71383 are resistant to sugarcane smut. Although sugarcane buds contain waxes on their bud surface, and consists of cuticles, no work has been done to determine the contribution of these two parameters to the pre-infection process. However, waxes present on the bud surface may not readily retain drops of spore suspensions as was done in this experiment.

It is important to note that structural features alone might not protect plants from invasion by pathogens, but can provide a delay which may or may not have an effect on the outcome of the disease in some host pathogen systems. The delay they provide may allow the plant time for a more effective defense response (Conti *et al.*, 1985; Misaghi, 1982; and Royle, 1976).

The different ways of dikaryotising by the pathogen (*Ustilago scitaminea*) as noted in Figures, 2, 3, 4, 5, and 6- on the bud surface supports the concept that these might be evolutionary adaptations employed by the pathogen to overcome the host's resistance mechanism.This idea holds if one considers the direct penetration of the bud surface by the promycelium (Figure 7) as the most primitive of the infectious mechanisms.. None of the evolutionary adaptations associated with spore germination appears to be cultivar specific.

This study confirmed the fusion between promycelia, the formation of sporidia, dikaryotization (2), direct penetration of the bud surface (Bock, 1964; Waller, 1969, 1970) and the formation of appresorium on the bud surface. Apressorium formation is noted for other fungi as well as smut (Alexander and Srinivasan, 1966; Lee and Bostock, 2006). Apressoruim formation was also confirmed by this study. Both hyphal and sporidial germination were observed on the surface of the cultivars studied (Figures 2, 4, 5 and 7). This phenomenon was identified on different surfaces (Waller, 1969).

The primary infectious process of sugar cane smut (*Ustilago scitaminea*) starts with the germination of spores. Maximum spore germination occurs after 6 hours. Once germinated the promycelium penetrates the host directly, form sporidia, dikaryotise, and then penetrate the host, fuse two promycelia and then penetrate the host or form appressoria before penetration. Self inhibition of spore germination occurs when spores are in close proximity of each other on the bud surface.

ACKNOWLEDGEMENTS

The author would acknowledge Mr. D.I.T Walker, Director, West Indies Central Sugarcane Breeding Station, Groves, Barbados, for identifying the cultivars, and to, Mr. M Wood and Mr. G. Skeete of Edgecumbe Limited for their cooperation while collecting sugarcane cultivars. I would also like to sincerely thank, Professor G. Sidrak of the Department of Botany, University of The West Indies, Mona Campus, Jamaica, for allowing me to use the Scanning Electron Microscope in his department and to Mrs. Williams for her technical assistance while using the sputter coater and the electron microscope.

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