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.14-19/Sealy
Research Article

INOCULATION OF STANDING CANES OF DIFFERENT SUGARCANE CULTIVARS WITH SPORE SUSPENSIONS OF THE SMUT FUNGUS (*USTILAGO SCITAMINEA* SYDOW)

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ABSTRACT
Standing canes of nine sugar cane cultivars in Barbados were inoculated with spore suspension concentrations of 10^5, 10^4, and 10^3, spores ml^{-1} with water as the control. A 25 ml polyethylene vial (Taab. Labs. Ltd., Reading, U.K), with moist cotton wool placed in the bottom, was sealed with masking tape over each bud, to create a humid environment. Colour coding was necessary to identify the different spore suspension concentrations on the sugarcane cultivars. The vials were removed and the percentage of the buds which were germinated scored at bi-weekly intervals. The results were consistent with the resistance ratings of the most resistant cultivar (B80689). This method is suggested as a tool for assessing disease resistant ratings of sugarcane cultivars.

INTRODUCTION
Ustilago scitaminea Sydow is basidiomycete of the order Ustilaginales or smut fungi and is found throughout the world. The taxonomy of smuts is based largely on teliospore morphology with spore size, colour, ornamentation and shape being especially important (Lee-Lovick, 1978). Dean (1982) stated that in sugarcane there were two barriers of resistance against the smut fungus (Ustilago scitaminea Sydow). Lloyd and Pillay (1980) characterized these two barriers namely, a pre-infection barrier and a post infection barrier. They suggested that the pre-infection barrier is associated with the bud scales which provide both physical and chemical resistance to the entry of the mycelium. The post-infection barrier occurs after the fungus enters the host and is probably more chemical than physical. The major route of entry the fungus is through the bud (Fawcett, 1944; Bock, 1964; Waller, 1970). Young are very susceptible to infection with resistance increasing with age (Bock, 1964; Byther and Steiner, 1974). Chona (1943) and McMartin (1948) (both cited in Bock, 1964) recognized two phases of infection in growing cane in the field: (a) primary infection and (b) secondary infection. Primary infection was defined arbitrarily as the infection of nodal buds on standing cane stalks. This occurs when the required number of spores, free water (moisture) and temperature are available for a specific time interval. Such conditions allow for the germination of spores and entry of the fungus. Secondary infections occur on one or several of the numerous secondary shoots which develop from the primary shoot which itself results from the growth of a nodal bud.

Before infection occurs the spores must germinate on the substratum. The type of germination which then ensues depends on the substratum and is of two types-hyphal or sporidal (Waller, 1969). Germination on young leaves buds or inert media is hyphal while mature leaves and cut canes surfaces support sporidial develop (Waller, 1969). Within the host plant, smut hyphae are diploid (Trione, 1980). Alexander and Ramakrishnan (1977) found that the dikaryotic condition was achieved prior to entry of the bud by infection hyphae. They also found that the fungus entered the bud 6-36 hours following imbibitions of water by spores and that it did so by circumvention rather than penetration of the bud scales. Singh and Agnihotri (1978) stated that the fungus can be isolated from the infected apical and lateral bud meristems of canes showing the initial symptoms of disease.

The major symptom of the disease is the production of a smut whip. This is a black, whip-like structure which grows from the central core of the meristematic apex to a maximum length of 90 cm. immediately before whip production there is an increase in the activity of the apical meristem and the rapid

accumulation of the mycelium at its periphery (Waller, 1969). The growth pattern of the apical meristem changes and it becomes intercalary in function, acting as the basal meristem of the smut whip. Tissue differentiating above results in continued growth of the cane. Trione (1980) observed that the fungus grew rapidly in the developing whip relative to other sugarcane tissue.

In the modified apex, the vegetative hyphae change physiologically and cytologically into a reproductive phase that yields a large number of spores. Trione (1980) found that the vegetative hyphae in the sori located in the surface layers of the whip were mono-nucleated and irregular in shape and length. Hyphae in the sori on the outer portion of the whip were different from those in other parts of the whip.

The early stages of whip emergence are variable and depend on the rate at which young leaves surrounding the whip unfurl and the rate of whip growth. Bock (1964) showed that temperature affects the rate of whip production and that 30^0C was the optimum. Smut whips grow for about 12 weeks and during this time the diseased canes increase in height by a maximum of 2.0 meters. The faster growth of the diseased canes often results in the old whips being above the canopy, therefore facilitating spore dispersal. A smut whip can produce about 10^9 spores per day and a total of 10^{11} spores during its growing period (Waller, 1969). In dry conditions spores are rapidly shed from emerging whips have been exposed for over two days. In wet conditions spore dispersal is hindered and most of the spores form a hard cake on the whip. Van der Plank (1963) found that several successive generations of a parasite can occur during the growth of the crop and this leads to the multiplication of the disease. Waller (1969) observed that the smut incidence increased from 0 to 100% over a 22 month period and there is a latent period of six months in the field. The proportion of smutted plants increases markedly with the successive ratoon crops-secondary crops from existing root stocks (Antoine, 1961).

Both rain fed and surface irrigation conditions increase disease incidence (Bock, 1964; Waller, 1969). The deposition of spores in the field is variable (Waller, 1969). In crops where the canopy is dense few spores reach the soil beneath. The down- wind side of the crop shows the highest deposition of the spores. Freshly deposited spores showed a variable of 80% (Waller, 1969). Spores are deposited on all the exposed surfaces of the cane. Those deposited on the upper surface are thought to be washed into leaf axils by rain and the majority becomes lodged around the nodal bud of the cane.

Infected plants are generally stunted with thin stalks with narrow, stiff leaves often at an acute angle. In exceptional cases smut galls are seen on young leaves with an off white membranous covering which on rupturing exposes smut spores (Singh and Agnihotri, 1978). Various structural abnormalities have also been observed in the flowers of diseased plants (Singh and Agnihotri, 1978).

To date there seems to be no effective control measure. However, the disease incidence can be minimized by planting resistant varieties, planting healthy setts, removing whips as they appear and ploughing out diseased rations (Bock, 1964; Waller, 1969; Singh and Agnihotri, 1978). This experiment investigated the resistance of sugar cane cultivars using different spore suspension concentrations in the inoculation of standing canes.

MATERIALS AND METHODS

Inoculation of Standing Canes

Spores were harvested from recently produced whips on field grown sugarcane and stored in a desiccator at 25^0C until required. Spores were suspended in sterile distilled water containing Tween 80 (0.1%v/v) to prepare spore suspensions. All experiments utilized the most apical bud on the stalk from which the leaf sheath could be removed in its entirety, and this is referred to hereafter as the standard bud. Nine month old sugarcane ratoon plants of the following cultivars were inoculated: B80689, B71383, B74541, B73382, B62163, B73385, B63118, B72177, and B7316. For each cultivar, 30 stalks were inoculated in each case, each stalk being on a different plant. Using a micropipette, 10ul of spore suspension of 10^5, 10^4, 10^3 spores ml^{-1} and, in the case of controls, distilled water was applied to the surface of the standard bud. A 25ml polyethylene vial (Taab. Labs. Ltd, Reading, U.K), with moist cotton wool placed in the bottom, was sealed with masking tape over each bud, to create a humid environment. After two days, the

vials were removed and the percentage of germinated buds scored at two weekly intervals. Buds were scored as germinated when there is a parting of the bud scales and the shoot development could be observed. Colour-coding with coloured tape allowed treated stalks to be easily identified.

RESULTS AND DISCUSSION

Results

Table 1: Field Resistance Ratings to smut disease for sugarcane cultivars used in this study (Walker, D.I.T., Personal Communication). The most resistant cultivar is rated one, and 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

DISCUSSION

When standard buds on standing canes were inoculated with smut spore suspensions of various spore concentrations, there was generally a higher proportion of germinated buds as the inoculums level increased (Figures 1 and 2). The inoculated plants of all cultivars showed a higher number of germinated buds than the controls confirming that such increase was due to the smut fungus. Cultivar B80689, considered to be the most resistant cultivar (Table 1), showed no bud germination even after 70 days. The other cultivars fall broadly into two categories, those where inoculation at different spore loads gave similar bud germination (Figure 2. B72177, B73382, B73385) and those where more marked differences between germination at different inoculums levels were evident (Figure 1, B71383, B63118, B62163, B74541, B7316). The resistant cultivars B73183 and B63118 and the moderately resistant B62163 showed the greatest difference in the number of buds germinating at different spore loads.

This suggests that spore density plays an important role in disease resistance of these cultivars. The moderately resistant cultivars B72177, B73382, and B73385 showed similar bud germination and different spore density levels suggesting that resistance break down at the lowest spore density. On the other hand the susceptible cultivar B7316 showed differences in bud germination with increased spore load as was more typical of the resistant cultivars. Since 10ul of spore suspension was applied in each case this means those spore densities 10^5, 10^4, and 10^3 spores ml^{-1} represent approximately 1000, 100, and 10 spores on the bud respectively! These results are less clear-cut than those reported by Byther and Steiner (1974) who got virtually no whips on resistant cultivars and whips on susceptible cultivars. In

most cultivars a low level of germination was evident in the controls, possibly due to the high humidity of the chamber. Tillering from apical buds on standing canes may be a sign of infection (Byther and Steiner, 1974). This was the basis for using bud germination as a sign of infection on the stalk. Unfortunately, it was not possible to continue monitoring this experiment right to whip production since this was carried out on a commercial holding and harvest time intervened. In fact, there was some discrepancy in the events of sprouting. When the experiment was terminated, shoots with up to 20 cm long whips had developed on inoculated canes of the susceptible cultivar B7316, whereas for other cultivars there was virtually no further bud growth beyond the initial germination. This difference is important especially since B7316 considered the most susceptible in the study, is not readily distinguishable from other more resistant cultivars. Clearly, further use of this method must incorporate measurements of bud growth and ultimately whip formation. Alternatively, staining of germinated buds would confirm the presence of the fungus and further validate this approach.

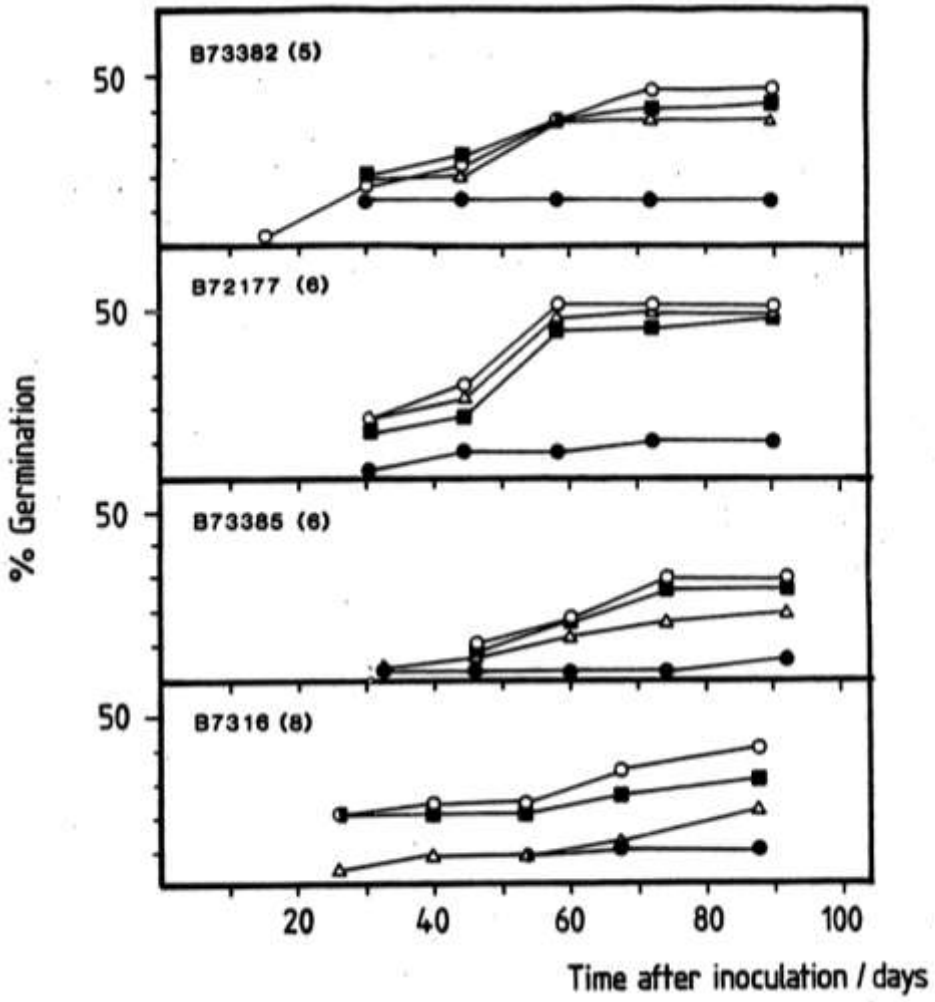

Figure 1: Effect of spore inoculums concentration on the germination of buds on 'standing cane' of different cultivars. Field resistance ratings are given in brackets after the cultivar numbers.

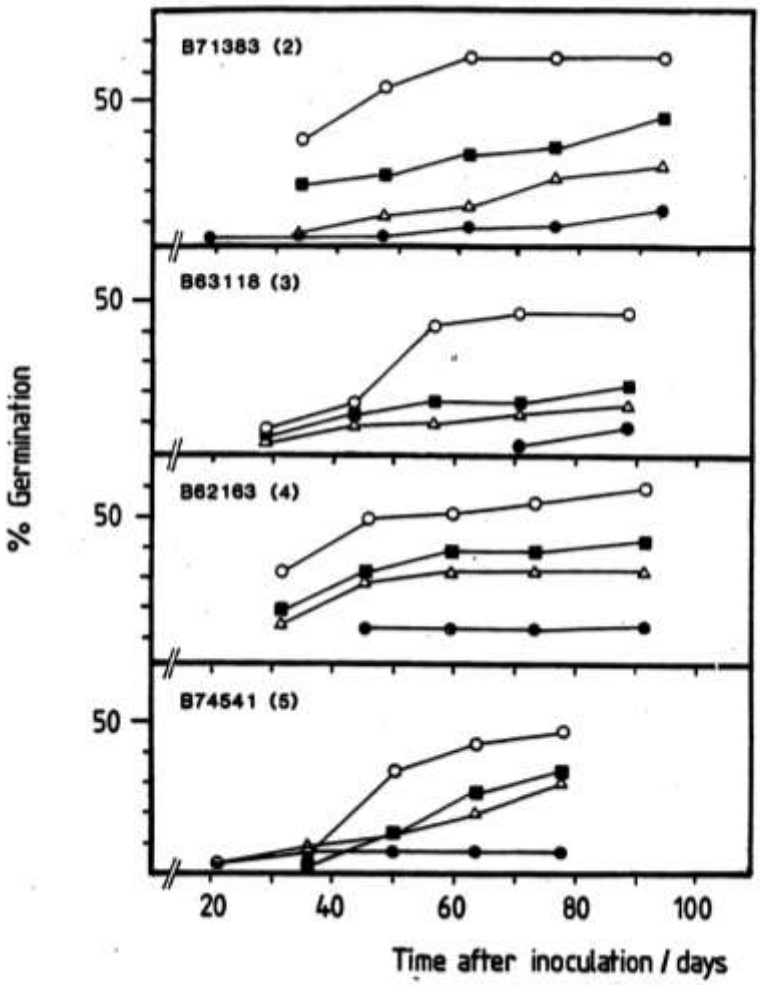

Figure 2: Effect of spore inoculum concentration on the germination of buds on 'standing cane' of different cultivars. Field resistance ratings are given in brackets after the cultivar numbers.

The time from inoculation until the first sign of infection has been used as an index of resistance for many diseases (Van der Plank, 1968). In the case of sugarcane smut disease, Peros and Baudin (1983) also found that for inoculated nodal setts this time delay before whip appearance correlated well with resistance ratings of cultivars. Sealy (1988) also observed a time delay from inoculation to bud germination. With the exception of the very resistant cultivar,B80689, which showed no germination, the others showed delayed times which are inconsistent with their resistance ratings (Sealy, 1988). Such inconsistencies are not unusual for sugarcane smut-Ustilago scitaminea sydow (Lee-Lovick, 1978).

As a procedure for determining the resistance of cultivars bud germination data from this method gives a clear indication of the high resistance of B80689, the most resistant cultivar

It is suggested that this method be used to assess sugarcane cultivars for smut-Ustilago scitaminea sydow-resistance since inoculated buds which germinate normally leads to whip production.

REFERENCES

Alexander, K. C and Ramakrishnan, K (1977). Studies of the smut disease (*Ustilago scitaminea* Syd.) of sugarcane: 4- Parasitism, Germination, dikaryotisation and Infection. *Proceedings of the International Society of Sugarcane Technologists*, **16** 469-472.

Antoine, R (1961). Smut. In: J.P Martin, E.V Abbott and G. Hughes (Editors). *Sugarcane Diseases of the World, Elsevier, Amsterdam* 327-354.

Bock, KR (1964). Studies on Sugarcane smut (Ustilago scitaminea) in Kenya. *Transactions of the British Mycological Society*, **44** 403-417.

Byther, R.S and Steiner, GW (1974). Comparison of inoculation techniques for smut Disease testing in Hawaii. *Proceedings of the International Society of Sugarcane Technologists Congress*, 15 280-288.

Dean, JL (1982). The Effect of Wounding and high pressure spray inoculation on the Smut reactions of Sugarcane clones, *Phytopathology*, **72**, 1023-1025.

Fawcett GL (1944). El 'carbon' de la cana de azucar. *Boletin de la Estacion Experimental Agricola de Tucuman* No. 47

Lee-Lovick G (1978). Smut of Sugarcane-*Ustilago scitaminea*. *Review of Plant Pathology*, **57,** 181-188.

Lloyd HL and Pillay M (1980). The development of an improved method for evaluating Sugarcane for resistance to smut. *Proceedings of the South African Sugarcane Technologists' Association*, **54** 168-172.

Peros JP and Baudin, P (1983). Etude de la variabilite' d' Ustilago scitaminea syd., agent du charbon de la canne a' sucre L' *Agronomie Tropicale* **38** 234-240.

Sealy, Earl A (1988). The Basis of Resistance of Sugarcane to the Smut Fungus (*Ustilago scitaminea* Sydow. *Research Thesis-University of the West Indies, Cave Hill, St. Michael, Barbados, W.I*

Singh K and Agnihotri VP (1978). Ustilago scitaminea Sydow, In: *J. Kranz, H. Schmutterer and W. Koch (Editors) Disease, Pests and Weeds in Tropical Crops*. 180-181; John Wiley and Sons, Chichester/New York/ Brisbane/Toronto.

Trione, EJ (1980). Teliospore formation by *Ustilago scitaminea* in Sugarcane. *Phytopathology*, **70** 513-516.

Van der Plank, J. E (1968). Disease Resistance in Plants. *Academic Press/ New York/ London*

Van der Plank, JE (1963). Disease Resistance in Plants. Academic Press, New York/London

Waller JM (1969). Sugarcane Smut, Ustilago scitaminea, in Kenya. I. Epidemiology. *Transactions of the British Mycological Society*, **52** 139-151.

Waller JM (1970). Sugarcane smut, *Ustilago scitaminea*, in Kenya II Infection and Resistance. *Transactions of the British Mycological Society*, **54** 405-414.