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STUDIES ON ROOT ROT AND STALK ROT OF MAIZE IN NORTH QUEENSLAND CAUSED BY MARASMIUS SACCHARI WAKKER VAR. HAWAIIENSIS COBB AND MARASMIUS GRAMINUM (LIB.) BERK. VAR. BREVISPORA DENNIS

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SUMMARY

The occurrence is reported in North Queensland of two new root and stalk rots of maize caused by *Marasmius sacchari* var. *hawaiiensis* and *Marasmius graminum* var. *brevispora*. The incidence of these diseases is aggravated by hot, dry growing conditions. The former pathogen is more common than the latter and has a wider host range. Plant-house screening of an extensive collection of maize lines has shown that none is totally resistant to *M. sacchari* var. *hawaiiensis*, but field observations indicate that the hybrid QK37, which has been widely grown on the Atherton Tableland, possesses a reasonable level of field resistance. Soil treatment with benomyl effectively reduced the incidence of *M. sacchari* var. *hawaiiensis* in a pot trial but not in field trials. In field trials the application of a complete fertilizer has, on occasions, reduced disease incidence.

I. INTRODUCTION

Fungal root and stalk rots of maize caused by *Diplodia macrospora* Earle, *D. maydis* (Berk.) Sacc., *Gibberella fujikuroi* (Saw.) Wr., *Gibberella zeae* (Schw.) Petch, *Macrophomina phaseoli* (Maubl.) Ashby, *Physalospora zeicola* Ell. & Ev. and *Pythium aphanidermatum* (Edson) Fitzp. have previously been recorded in Queensland (Simmonds 1966).

In 1961, a season in which rainfall in North Queensland maize areas was below average, a hitherto undescribed stalk rot was found on maize on the Atherton Tableland. Losses as high as 32% infected stalks were recorded during this season in some badly affected fields. Since then observations have shown that the disease, although present each year, has caused most damage during dry seasons.

In 1967, another new stalk rot was recorded on maize in the same area. This disease was present in minor proportions only in that season and has recurred annually to the same limited extent.

This paper describes these two new stalk rots and discusses the isolation, proof of pathogenicity and identity of the causal fungi. The relative importance and distribution of the two diseases are also dealt with. In the case of the major pathogen the host range, soil moisture relationships and possibilities of control either by cultural means or by fungicides are reported.

II. THE PATHOGENS

(a) Symptoms

(1) *Marasmius sacchari* var. *hawaiiensis*.—Symptoms may appear at any stage of growth from the seedling onwards. Infected seedlings generally wilt and succumb rather rapidly to a conspicuous white rot of the roots and crown region (Figure 1). On older plants (Figure 2) early symptoms consist of a mild chlorosis of all foliage and a faint striped mottle of apical leaves together with a slight upward rolling of their laminae. Stunting, firing of lower leaves, rolling, bleaching and eventual wilting of apical leaves are the most noticeable subsequent symptoms. The lower leaf sheaths, particularly those at and below soil level, are cemented to the stalk by a mantle of white mycelium (Figure 3) and, if infected plants are uprooted, mycelial strands can be seen on the rotted root system and in the adhering soil. When the leaf sheaths are stripped from such plants, a characteristic necrotic streaking can be seen on the stalk. This is accompanied by a breakdown of the parenchyma and vascular tissue internally from the discoloured areas in the sclerenchyma. This characteristic necrosis may extend upward through six or more internodes. When the rot is well advanced the internal tissues in the base of the stalk are usually completely decomposed and white mycelium occupies the cavity. The rotting roots and stalk emit a characteristic mushroom type odour.

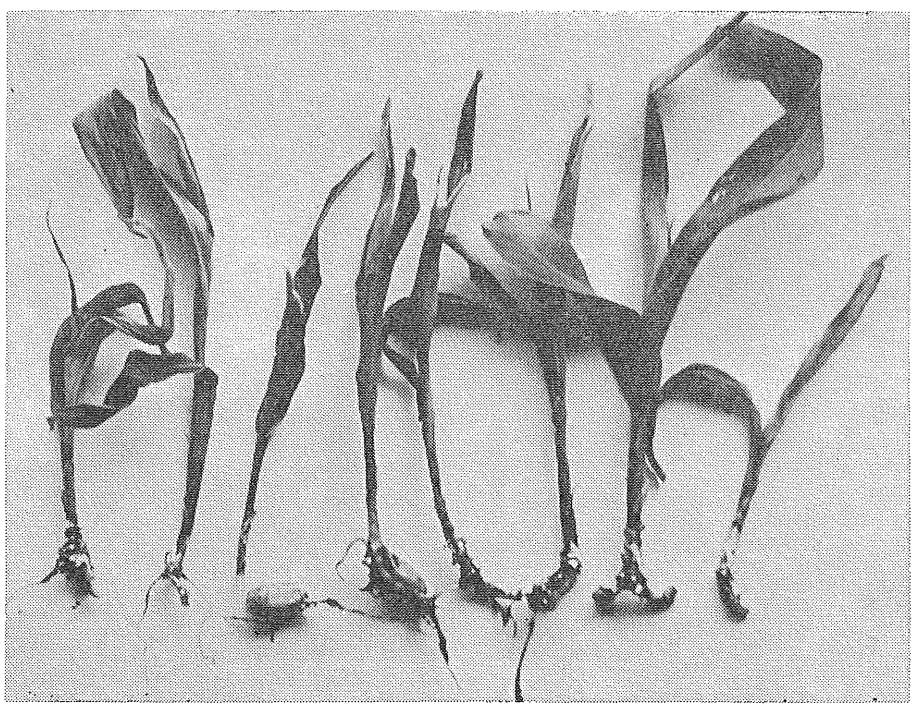

Figure 1.—Seedling blight (*Marasmius sacchari* var. *hawaiiensis*).

Figure 2.—Maize plant killed by root and stalk rot (*Marasmius sacchari* var. *hawaiiensis*).

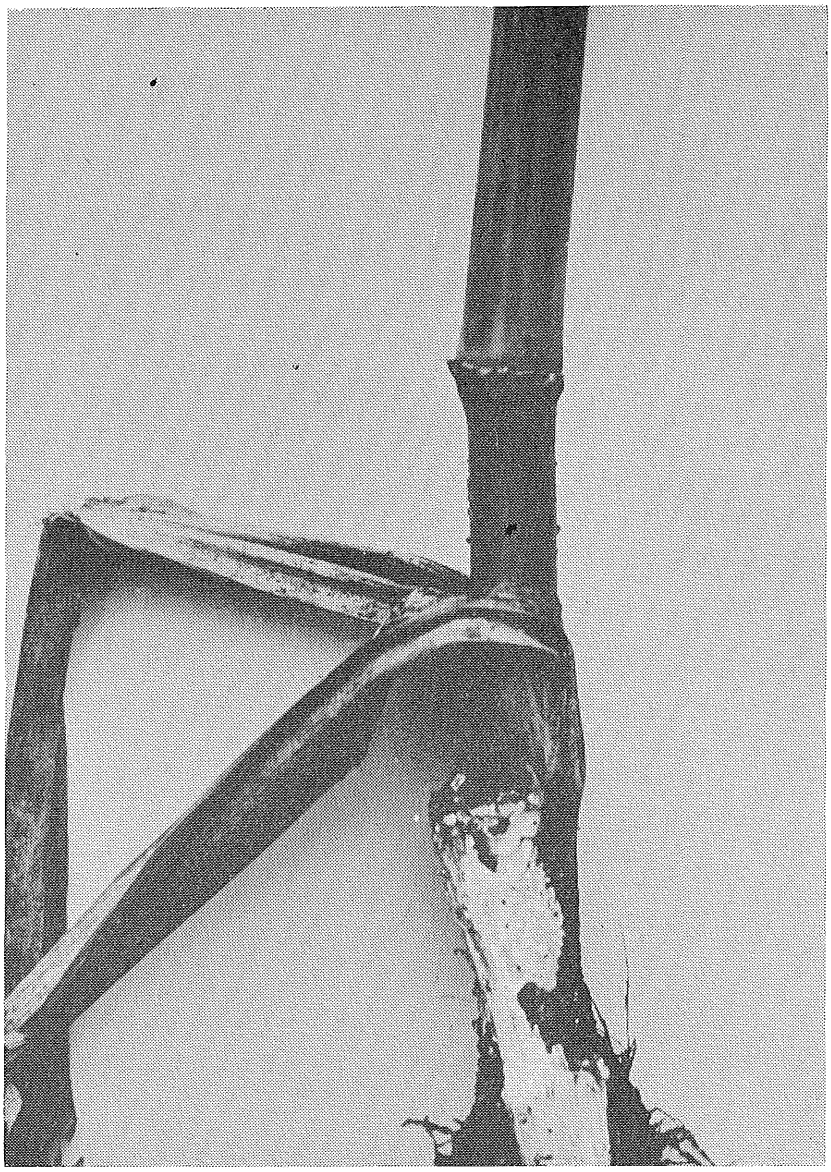

Figure 3.—Dead stalk showing white fungous mantle (*Marasmius sacchari* var. *hawaiiensis*).

Depending on the extent of infection prior to tasselling, diseased plants may be barren or the ear reduced in size and the grain pinched.

(2) *Marasmius graminum* var. *brevispora.*—The extensive internal and external necrosis characteristic of *M. sacchari* var. *hawaiiensis* is not produced by *M. graminum* var. *brevispora,* tissue breakdown being confined to those areas actually invaded by the fungus. Typically, therefore, the roots, mesocotyl and

basal internodes are discoloured and rotted and the lower leaf sheaths are cemented to the stalk by a white fungous mantle. Leaf sheaths higher up the stalk are eventually infected as the disease progresses. Dead leaf sheaths are straw-coloured and when they are stripped from the stalk a fine white mycelium can be found on their inner surfaces. Sporophores of the causal fungus have been found on such material.

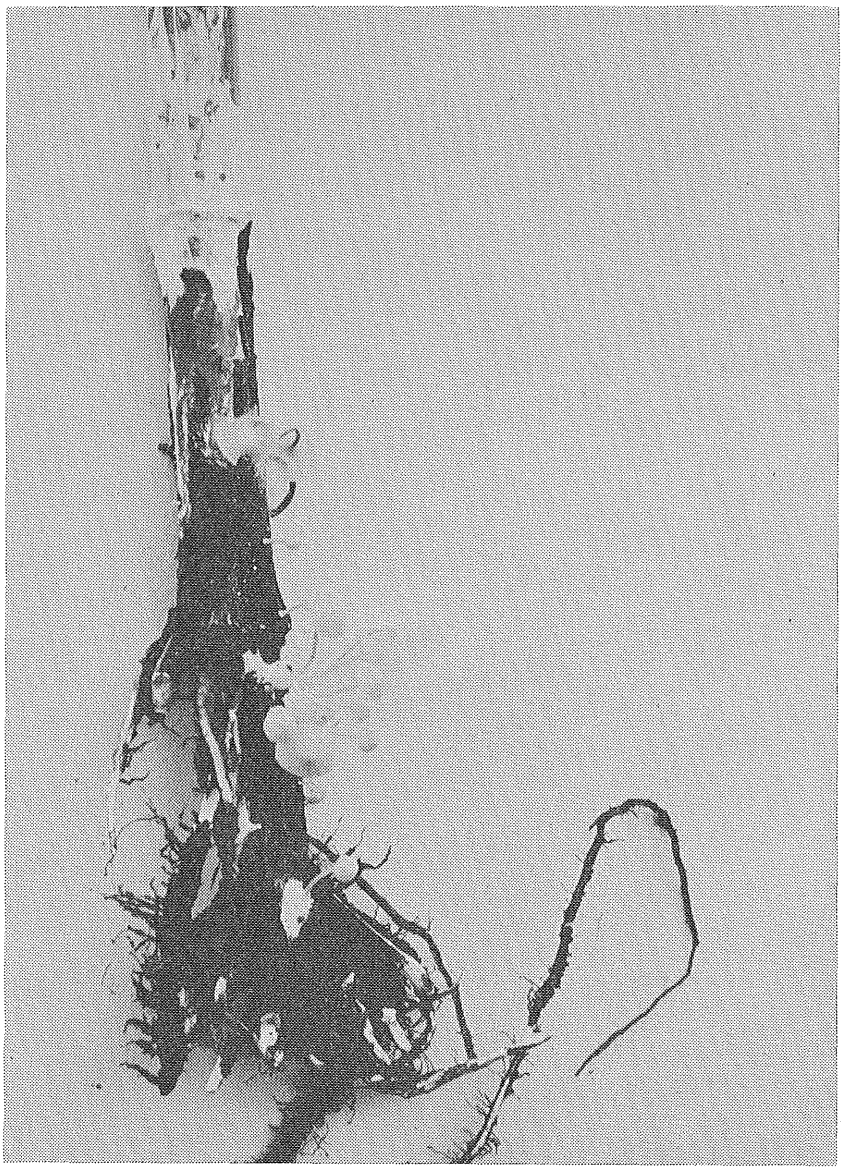

Figure 4.—Dead stalk showing white fungous mantle and sporophores (*Marasmius sacchari* var. *hawaiiensis*).

(b) Isolation, Pathogenicity and Identification

(1) *Isolation and pathogenicity.*—Isolations from diseased material were made on to potato dextrose agar (P.D.A.) plus antibiotics (streptomycin sulphate, chloromycetin and chlortetracycline—50 mg of each per litre). Fungal mats were grown when required on potato dextrose broth. Inoculum for infection studies was grown on steamed and autoclaved wheat grain. For soil inoculations in pots a mix containing equal volumes of peat moss, fine sand and loamy soil was used. After sterilization by dry heat a complete fertilizer was incorporated at a rate of 2½ oz/10 gal, together with lime at 6 oz/10 gal to raise the pH to a reasonable level and a pinch of magnesium sulphate. Grain inoculum 21 days old was incorporated into the top 4 in. of the potting mix at the rate of 2½-3 oz in each 2 gal plastic planter. Maize seed of the hybrid GH128 (unless otherwise specified) was sown immediately after. When wound inoculation of the stalk was employed either wheat grain inoculum or a sterile water suspension of a mycelial macerate prepared in a blender was introduced into punctures made with a small cork-borer in the bases of the stalks.

Both fungi were recovered from diseased plants by isolating directly from the white fungous mantles between leaf sheaths and stalks or from visible mycelium in the rotted internal tissues of stalks or roots. Isolations were also made from sporophores.

For both species the cultures on P.D.A. were white and typical of basidiomycetes. *M. graminum* var. *brevispora* was less vigorous and aerial mycelium was absent or scant. Pigmentation of the agar substrate was lacking. *M. sacchari* var. *hawaiiensis* varied both in the amount of aerial growth and in pigment production but most fresh isolates grew a copious stranded aerial mycelium and some gave rise to a light brown pigmentation.

Cultures of both species isolated from lesions or from sporophores were found to be pathogenic by either the stem wounding (Figure 5) or soil inoculation technique. Typical stem rot and root rot symptoms were produced and the causal organisms were reisolated.

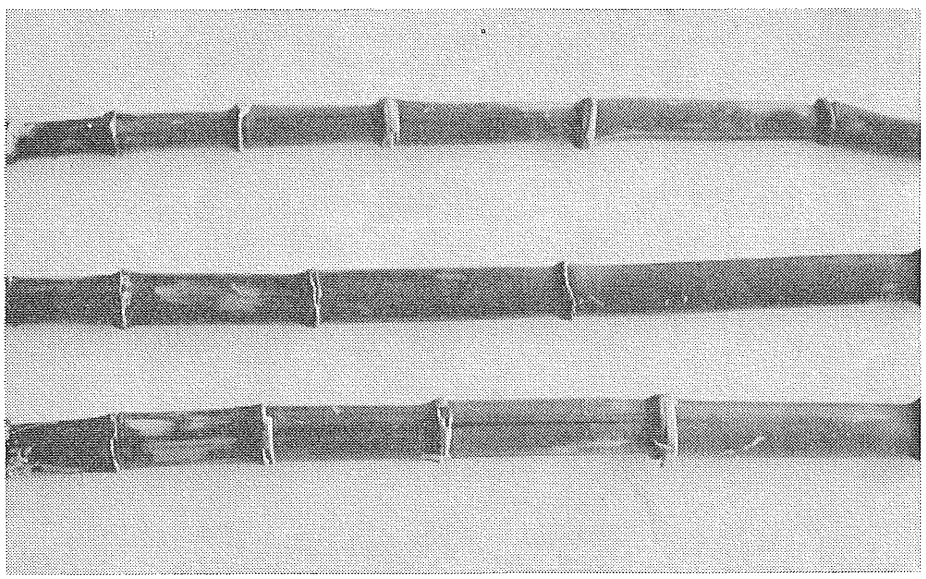

Figure 5.—Infected stalks showing external necroses (*Marasmius sacchari* var. *hawaiiensis*).

In soil inoculation, which might be expected to more closely parallel natural infection in the field, stem lesions were found in many instances to originate in the crown region from infected leaf sheaths. In other cases, either the seed or the mesocotyl was originally infected and the rot penetrated into the crown from these sites. Root infection (Figure 6) was either direct through root contact with the inoculum or originated from a progressive invasion of seminal or adventitious roots from mesocotyl or crown lesions.

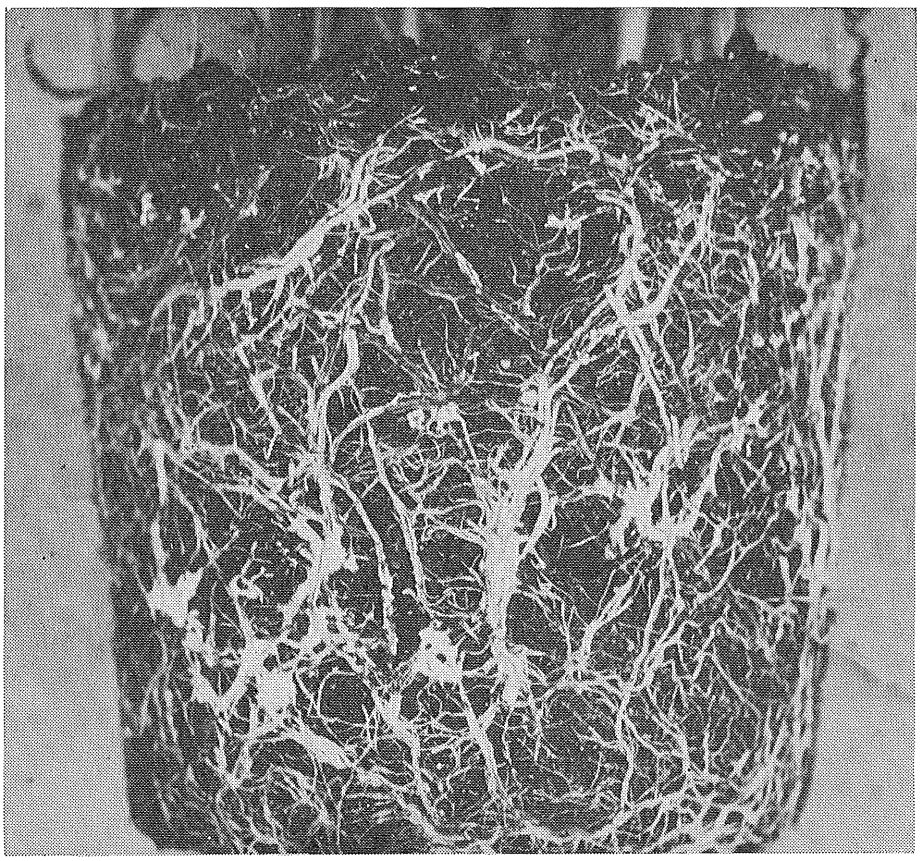

Figure 6.—*Marasmius sacchari* var. *hawaiiensis* infecting maize roots in inoculated potting mix.

(2) *Identification of M. sacchari* var. *hawaiiensis*.—Early field surveys and pure culture experiments failed to produce sporophores. Fruiting bodies were first found in 1965 on dead stalks in infected maize fields on the Atherton Tableland. Their production at that time and since coincided with a long spell of showery, overcast weather. They have also been produced in the laboratory by enclosing the basal stalk and roots of dead plants in plastic bags.

A short description of the sporophore follows:—Pileus up to 26 mm, white, campanulate at first then expanding; stipe up to 26 mm, white, bulbous and villose at point of attachment to substrate, central, sometimes excentric; gills simple or bifurcate, adnate; spores hyaline, clavate, papillate at point of attachment, 10-15 × 3.5-4.5 microns. Spore print white.

The minimum and maximum temperatures as determined by growth on P.D.A. plates in a multi-temperature incubator were 12°C and 40°C, while the optimum fell within the limits 30-32°C.

The fungus was identified as *Marasmius sacchari* Wakker var. *hawaiiensis* Cobb. This identification was subsequently confirmed by Mr. D. Pegler of the Royal Botanic Gardens, Kew.

(3) *Identification of M. graminum* var. *brevispora*.—Sporophores of this fungus are quite commonly found on infected stalks and stubble in the field and are sometimes produced on stalks infected by artificial inoculation. The main characters of the fruiting structures are:—Pileus up to 4-5 mm diam., convex with a shallow umbilicus and a dark central papilla, striate-sulcate to the umbilicus, orange; stipe slender, brown and smooth, up to 25 mm or more in length; gills and undersurface of the pileus white; gills up to 12 in number, adnate; spores hyaline, oval or elliptical, 9-10 × 4 microns.

Cardinal and optimum temperatures for this species were identical with those of *M. sacchari* var. *hawaiiensis*.

The fungus was identified as *Marasmius graminum* (Lib.) Berk. var. *brevispora* Dennis. The identification was confirmed by Mr. D. Pegler of the Royal Botanic Gardens, Kew.

(c) Distribution and Importance

In field trials and surveys disease incidence was determined by counting the number of stalks with the characteristic white fungous growth on basal leaf sheaths. This was done by plucking the basal leaf sheaths from the plants in order to expose some or all of their underground portions. A separate tally was kept of plants showing obvious stalk rot symptoms.

By far the majority of records of *M. sacchari* var. *hawaiiensis* on maize have been made on the Atherton and Evelyn Tablelands south-west of Cairns, but it also occurs at Parada (west of Mareeba) and in the lower Burdekin district situated in the dry tropics nearly 300 miles south of Cairns.

It is widely distributed in maize-growing soils on the Tablelands but the incidence varies from paddock to paddock and farm to farm. In a survey carried out in 1966, 38 fields were systematically sampled and the percentage of stalks with basal leaf sheaths colonized by *M. sacchari* var. *hawaiiensis* varied from a nil incidence to 86. Populations were low on new land or land recently cropped to maize for the first time and in paddocks where maize had been rotated with peanuts. Highest counts were recorded in paddocks with a continuous history of maize culture or on farms where a grass-legume pasture had been part of the rotation.

M. graminum has so far only been recorded from the maize areas of the Atherton Tableland.

(d) Host Range in the Field

M. sacchari var. *hawaiiensis* has been found on teosinte (*Euchlaena mexicana*) and nut-grass (*Cyperus rotundus*) at various sites on the Atherton Tableland. Sporophores have been seen on these hosts and the pathogen has been

isolated. In addition, typical symptoms have been recorded in the field on grain sorghum (*Sorghum vulgare*), Johnson grass (*Sorghum halepense*) and guinea grass (*Panicum maximum* var. *typica*).

M. graminum var. *brevispora* has so far only been found on maize.

III. EXPERIMENTAL RESULTS

(a) Varietal Susceptibility

Over a period of time 78 maize lines, including introductions and local lines (hybrids and inbreds), have been screened for resistance to *M. sacchari* var. *hawaiiensis*. This work was done using high and low levels of wheat grain inoculum, namely 3 oz and ½ oz per 2 gal pot. None of the lines has consistently shown sufficient promise to warrant further testing.

The two hybrids most widely grown in North Queensland (QK37 and GH128) were equally susceptible in these tests but observations in maize paddocks suggest that QK37 has greater field resistance than has GH128.

(b) Host Range

In pot trials employing a soil inoculation technique similar to that used in the pathogenicity tests, stem and root lesions were produced on soybean, cotton, cowpea, rice, green panic (*Panicum maximum* var. *trichoglume*) and Black Winter rye.

(c) Fungicide Tests

(1) *In vitro*.—For the *in vitro* screening of fungicides a range of dilutions from 0 p.p.m. to 1,000 p.p.m. active ingredient of various commercial formulations was prepared in P.D.A. and poured into petri dishes (10 ml/plate). A small cube of inoculum from a P.D.A. culture of the organism was introduced to the centre of each plate. Measurements of the diameters of the resulting colonies were made when the majority of the 0 p.p.m. plates were completely covered—an interval ranging from 3 to 5 days. In most cases the colony shape was sufficiently regular to require only one diameter measurement.

Eight fungicides were screened against *M. sacchari* var. *hawaiiensis*. A mean colony diameter was calculated from the five plates poured for each concentration and the results expressed as percentages of the mean colony diameter in the 0 p.p.m. concentration. These appear in Table 1.

TABLE 1

GROWTH OF *M. sacchari* VAR. *hawaiiensis* ON P.D.A. PLATES AMENDED WITH DIFFERENT FUNGICIDES

Fungicide	Concentration (p.p.m. Active Ingredient)					
	1	5	10	100	500	1,000
Bayer 6059	98*	98	95	26	13	9
BAS 2201 F	35	33	29	20	0	0
Chloroneb	70	47	32	13	6	0
Captafol	47	13	13	6	6	6
Pennsalt TD 5056 ..	38	7	0	0	0	0
Thiabendazole	100	40	22	0	0	0
Carboxin	45	4	2	0	0	0
Benomyl	30	0	0	0	0	0

* Percentage growth compared with unamended plates.

Benomyl was the most efficient fungicide. Growth was completely inhibited at concentrations of 5 p.p.m. and greater.

(2) *Pot trial.*—Seed was treated with benomyl (50% a.i.) either as a dust at the rate of 6 oz/bus of seed or as a 24 hr dip in a 500 p.p.m. (a.i.) suspension. When applied as a soil treatment the fungicide was intimately mixed with the potting medium (as used in the pathogenicity tests) at the rate of 110 p.p.m. (a.i.) prior to the addition of the wheat grain inoculum and the seed.

There were 4 replicates of each treatment and 24 seeds were planted per pot. Results were assessed by the counting and removal of obviously diseased seedlings during a 3-week period after planting and by an examination of the roots and mesocotyls of surviving seedlings at the end of this period. Results expressed as mean percentage seedlings infected were as follows:—soil treatment 20·5%; seed dip 100%; seed dust 94·5%; untreated 100%. Seed treatments were ineffective but soil treatment was responsible for an appreciable reduction in infection.

(3) *Field trials.*—In small field trials benomyl has since been tested against *M. sacchari* var. *hawaiiensis* as a seed dressing (6 oz/bus), as a soil drench applied to the drill at planting and as a combination of both treatments. No effective control resulted.

(d) Moisture Relationships

A pot trial was carried out to investigate the effect of soil moisture levels on the incidence of *M. sacchari* var. *hawaiiensis*. The soil used was a volcanic clay loam typical of the Atherton Tableland maize soils and was sterilized and inoculated prior to the sowing of the seed as in the pathogenicity tests. The soil was maintained at field capacity prior to emergence and after emergence the following moisture treatments at ambient temperatures were compared:—

Daily watering;

Watering at 3-day intervals;

Watering at 6-day intervals;

Watering carried out only when plants were wilted.

Seven pots (9 in.), 5 of which were inoculated, were used for each treatment. Plant populations were either 5 or 6 per pot. Disease counts were made when the plants commenced to tassel.

The results (Table 2) indicated that there was an increase in stalk rot incidence amongst plants subjected to low soil moisture levels.

TABLE 2

EFFECT OF DIFFERENT SOIL MOISTURE REGIMES ON INFECTION OF MAIZE HYBRID GH128 BY *M. sacchari* VAR. *hawaiiensis* IN POT TESTS

Watering Treatment after Emergence	Percentage Infection	
	Inoculated*	Control†
Daily	21	0
Every 3rd day	40	0
Every 6th day	42	0
At wilting point only	72	0

* Based on mean of 5 pots.

† Based on mean of 2 pots.

(e) Effect of Cultural Treatments

In a continuing trial at Kairi Research Station designed to investigate the effects of various cultural treatments on disease incidence and yield, the application of a mixed fertilizer (15:7:0) at the rate of 4 cwt/ac has been shown to reduce infection by *M. sacchari* var. *hawaiiensis* in three out of five seasons. The data tabulated in Table 3 were obtained during the 1966-67 season. The variety used was the hybrid QK37.

TABLE 3

EFFECT OF FERTILIZER APPLICATION ON STALK ROT (*Marasmius sacchari* VAR. *hawaiiensis*)

Treatment	Percentage Stalks with the Fungus on Basal Leaf Sheaths		Percentage Stalk Rot	
	Equiv. Mean	Transformed Mean*	Equiv. Mean	Transformed Mean*
Fertilized	15·41	0·403	0·52	0·072
Unfertilized	20·69	0·472	1·54	0·124
S.E.	..	0·014	..	0·014
Necessary differences for significance 5%	..	0·041	..	0·040
Necessary differences for significance 1%	..	0·055	..	0·054
Significant differences	..	Unfertilized > Fertilized	..	Unfertilized > Fertilized

* Inverse sine transformation.

IV. DISCUSSION

Marasmius sacchari Wakker was first recorded on sugar-cane in Java in 1896 (Saccardo 1917). In 1906 Cobb diagnosed *M. sacchari* var. *hawaiiensis* on the same host in Hawaii (Saccardo 1925), and Cottrell-Dormer (1924) reported a basal stem rot and sheath rot of sugar-cane in Queensland caused by *M. sacchari* Wakker. Other *Marasmius* species (*M. tritici, M. oreades, M. interstitians, M. semiustus* (*Marasmiellus inoderma*), *M. paspali*) have been found on gramineous plants (Sprague 1950). A root rot of maize in the United Arab Republic caused by *Marasmiellus inoderma* has been described by Sabet *et al.* (1970) and a species of *Marasmius* has recently been identified as the cause of a blight disease of American beachgrass (*Ammophila breviligulata*) by Lucas *et al.* (1971).

There has apparently been some doubt in the past as to the status of some of these species of *Marasmius* as parasites. For instance, earlier it was concluded that *Marasmius sacchari* was an important parasite of sugar-cane. However, active parasitism of the roots of sugar-cane by this fungus has never been demonstrated and it is now accepted that the fungus lives saprophytically on plant debris in the soil and ordinarily only attacks plants through wounds or parasitizes plants that are weakened by other diseases or adverse growing conditions (Rands and Abbott 1964).

The actual role of *Marasmius oreades* in the "fairy ring" disease of turf grasses was the subject of controversy until Filer (1965*a*) presented proof that the fungus actually parasitized grass roots and also showed (Filer 1965*b*) that it produced cyanogenic compounds which damaged turf grasses.

Marasmius tritici was recorded on wheat, rye, barley and two grasses (Young 1925), but that author found no evidence that it caused injury even though he believed that the fungus developed while the hosts were still green.

On the other hand, Sabet *et al.* (1969) conclusively demonstrated the pathogenicity of *Marasmius inoderma* to maize, and a species of *Marasmius* was proved by soil inoculations to be pathogenic to American beachgrass (Lucas *et al.* 1971).

The studies reported here have shown that *Marasmius sacchari* var. *hawaiiensis* and *Marasmius graminum* var. *brevispora* cause root and stalk rot of maize in North Queensland.

M. sacchari var. *hawaiiensis,* which is the major pathogen, has so far been recorded in the field only on gramineous hosts and nut-grass. Pathogenicity tests have shown that the fungus will infect other cultivated hosts and the host range could well be quite extensive.

The fungus has an optimum temperature for growth of over 30°C and disease incidence is increased by low soil moisture levels. These characteristics are remarkably similar to those described by Sabet *et al.* (1968) for *Marasmiellus inoderma* and their conclusion that *M. inoderma* "may become serious under hot, dry weather" appears to apply equally well to *M. sacchari* var. *hawaiiensis.*

It seems possible that factors which lessen plant vigour (and this would presumably include low soil moisture) could predispose maize to infection, since the incidence of *M. sacchari* var. *hawaiiensis* is reduced when soil fertility is increased. Sabet *et al.* (1968) found that *M. inoderma* also responded in this fashion when soil fertility was increased to a reasonable level.

Both of the fungi discussed in this paper are soil inhabitants and therefore disease incidence could be expected to be less in soil treated with an efficient fungicide. Benomyl, while effective in laboratory and plant-house experiments, did not give effective control in field trials. It is presumed that this was due to the difficulty of ensuring a uniform distribution of the fungicide within the rapidly expanding root zone of the maize plant.

V. ACKNOWLEDGEMENTS

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