I. STUDIES ON CERTAIN CHEMICAL CONSTITUENTS OF BANANA LEAVES IN RELATION TO INCIDENCE OF LEAF SPOT DISEASES.

II. A NOTE ON THE FUNGI OCCURRING ON BANANA

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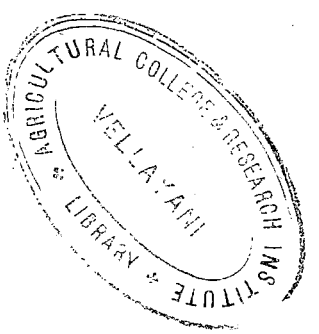

THESIS

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C E R T I F I C A T E

This is to certify that the thesis herewith submitted contains the results of bona fide research work carried out by Shri M.K.Chandrasekharan Nair, under my supervision. No part of the work embodied in this thesis has been submitted earlier for the award of any degree.

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C O N T E N T S

INTRODUCTION

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Banana is the most important fruit crop in Kerala, occupying an area of over one lakh acres.

This crop, as any other crop, is susceptible to a number of diseases of fungal, viral and bacterial origin. Of the diseases, "Bunchy top" caused by a Virus is the most destructive one affecting banana in this State. Next in importance are the leaf spots caused by a number of fungi, to which all the varieties of banana are susceptible to a lesser or greater extent.

One of the interesting features of all leaf spot diseases of banana is that they are generally confined only to the older leaves, irrespective of the variety and age of the plants. This phenomenon occurs in certain other crops as well. Another feature is that there is a marked variation between the varieties in their susceptibility to these diseases, though no variety is completely free from infection. The reasons for these phenomena are not clearly understood. It was thought likely that the chemical composition of the leaves may to some extent influence the susceptibility of plants to infection. There are ample instances to support this assumption. Tannin, for which banana is well known, has been reported to have fungitoxic properties against certain fungi. The C/N ratio of the leaves is also known to influence infection by fungi.

An attempt was, therefore, made to study whether some of the chemical substances present in banana leaves, namely, tannin, sugars, total carbohydrates and total nitrogen have any influence on the incidence of diseases and on varietal susceptibility.

Though leaf spot diseases occur only on the older leaves, the stage of development on the leaf at which infection can take place is not known. In the case of Cercospora musae, Stahel (1937) showed that only the two youngest leaves were very susceptible to infection and the successive older leaves were infected less readily. But as the first symptoms took a considerable time to develop, the young leaves appeared to be quite free from the disease, even though they were infected. Inoculation experiments with Cordana musae were done to study whether this fungus also could infect young leaves and remain latent as Cercospora musae.

Except Cordana musae, the other fungi which commonly occur on banana in Kerala have not been properly identified and studied. An attempt was, therefore, made to list out the common fungi that occur in and around the Agricultural College, Vellayani.

During the course of the present investigation a leaf spot caused by a species of Leptosphaeria was also noticed and described. This disease is new to India.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Chemical composition of the plants has been known to influence the incidence of diseases. While certain chemical constituents of the plants are toxic to the pathogens others favour their development and hence resistance and susceptibility of plants are often governed by these substances.

Tannin and some phenolic derivatives of tannin present in the cell sap, have been considered as important substances in checking the growth of some fungi. Tannin has also been reported to inactivate viruses like Tobacco Mosaic Virus and Tobacco Ring spot Virus (Thresh 1956). On the other hand there are also instances about fungi capable of utilising tannin.

Bavendamm (1927) reported that corrosion fungi were capable of utilising tannin. A marked feature of the tannin culture was the formation of fair sized deep brown to black halo round and below the mycelium, which he interpreted as an indication of the alteration of tannins by the fungi. Rippel and Keesling (1930) reported that Penicillium spp., Aspergillus spp.and Citromyces spp. isolated from soil were able to grow with tannin as the sole source of carbon. D'Oliveira (1935) found that Endothella gyrosa a fungus which infects the cork layer of cork oak, was capable of growing in

media containing tannic acid upto a concentration of 2.5 per cent. But the rate of growth was inversely proportional to the concentration of tannic acid in the media. Baens and Yenko (1936) observed that the activity of Aspergillus niger and Penicillium glaucum on the tanning liquors extracted from betelnuts, bark of Acacia decurrens, Terminalia edulis and Pithecolobium dulce decreased the tannin content of the extracts.

Cook and Taubenhaus (1911) showed that Gloeosporium musarum was to some extent resistant to tannin. The conidia showed complete germination in a 0.6 per cent tannin solution but would not germinate in higher concentrations. They suggested that fungal growth may sometimes be stimulated by low percentage of tannin. Offord (1940) found from a study of the tannin in species of Currant (Ribes) which markedly differed in their susceptibility to Cronartium ribicola that the quantities present in the cells gave no measure of their relation to the rust. But qualitative differences were found and nothing was known regarding them. Butler and Jones (1961) reported that it was hard to get positive evidence of the action of tannins. They reported that Fomes ignarius causing the 'Esca' disease of grape vine penetrated the wood of the host plant after tannin had accumulated in it. Young ones with little tannin content appeared to be immune from the disease. But if tannin content was increased or if the variety

was one naturally rich in tannin, infection might pursue a more rapid course.

De villifers (1929) concluded from the investigations on keeping quality of grapes that grapes having a high acidity or tannin content in the sub epidermal layers were less liable to injury from fungi. Walker and Link (1935) found that the presence of protocatechuic acid in red onions prevented the infection by Botrytis cinerea and Colletotrichum circinans. Barnell and Barnell (1945) showed that there was nearly four times as much tannin in the skin, as in the flesh of freshly harvested bananas and during storage, the amount fell in both regions at the phase of incipient ripening. They suggested the existence of a causal relationship between tannin content of the skin and Gloeosporium infections, since the 'anthracnose' spotting did not occur until the tannin concentration was very low. Johnson and Schaal (1952) showed that the presence of phenols in certain potato varieties was a factor for the resistance to scab disease by Streptomyces scabies. Chakravarthi (1957) found that the juice of green skin of banana had an inhibitory effect on the germination of spores of Gloeosporium musarum possibly due to the presence of tannin which disappeared to some extent as the fruit ripened. From inoculation experiments she found that Gloeosporium musarum penetrated the cuticle of

the host by mechanical means and in the young fruit the germ tube remained in an inactive condition in the subcuticular regions until the fruit reached a certain state of ripeness. Then it grew and produced typical 'anthracnose' lesions. She also observed that the juice of the ripe fruit skin may have some stimulatory effect on conidial germination. Grossman (1958) reported that addition of tannin even in very low concentration caused a marked inhibition of the pectolytic activity of the culture filtrate of a highly pathogenic strain of Fusarium oxysporum, f.lycopersici. He further suggested (1962) that the importance of oxidised phenols for the disease resistance was based partly on the inactivation of pectolytic and other extra-cellular enzymes of the pathogens. Echandi and Fernandez (1962) reported that the presence of phenols in coffee plants prevented infection by Ceratocystis fimbriata. Baruah et.al. (1963) reported that polymerised condensed tannin from Acacia arabica was to a limited degree toxic to the spores of Piricularia oryzae. Bordoli et.al. (1964) found that polymerised condensed tannin from Cassia fistula and Acacia catechu was fungitoxic on the spore germination of Colletotrichum falcatum at higher concentrations. Mahadevan (1964) reported that the phenol content of the cucumber variety susceptible to Cladosporium cucumerinum increased upto 48 hours after inoculation and afterwards declined with the appearance of symptoms. But the phenols in

the resistant showed little change after inoculation. Extracts in water and ethanol from the resistant inhibited fungal growth more than those from the susceptible. Resistance was associated with decreased production and activity of pectinolytic enzymes and decreased growth of the pathogen. Boyer (1964) found that healthy white pine tissue contained phenolic substances only in the vacuoles. But following infection by Cronartium ribicola, phenols were detected histochemically and chromatographically in the cytoplasm and on the cell walls. In young leaves, infection caused the vacuoles to fragment although the released phenols did not appear to affect the growth of the pathogen. He suggested that, later tonoplast might collapse and release the phenols which are toxic to the mycelium, but the reaction was local and not sufficient to prevent systemic development of the pathogen.

The nitrogen and carbon contents of the plants also have been known to influence the susceptibility of plants to diseases.

Coons and Klotz (1925) found from chemical analysis that celery leaves infected by Cercospora apii and Septoria apii contained a lesser percentage of nitrogen than healthy leaves. Gassner and Franke (1938) found wide

variation in the nitrogen content of diseased and healthy leaves of wheat plants infected by leaf rust. The total nitrogen content of the leaf was considered to exert some influence on the infection by this fungus. They observed that the older leaves of wheat plants had lesser nitrogen content and were generally, severely infected by leaf rust. Bonzintzky's (1940) biochemical studies showed that leaves of sugar beet severely affected by Cercospora beticola had a lesser content of total soluble and alubuminous nitrogen. Tani and Naito (1957) found no significant difference of nitrogen between diseased and healthy Hibiscus esculentus. Finkner et.al. (1962) reported that protein was degraded in the leaves of sugar beet infected by Cercospora beticola and some of the degraded proteins were translocated to the roots. Chakrabarti (1964) reported that there was apparently no relationship with the level of infection by Cercospora oryzae and nitrogen content of rice plant.

Leach (1937) reported that roots of normally felled trees of tea have a high carbohydrate content, but if they were depleted of starch before felling by ring barking to prevent the passage of carbohydrates from the foliage to the roots, they would be less susceptible to Armillaria mellea. Grainger (1956) reported that the most important

factor influencing infection by Helminthosporium avenae causing leaf spot of oats, and Phytophthora infestans causing late blight of potato was the total carbohydrate present in the leaves. He found a greater carbohydrate content in the diseased plants than the healthy. Ashworth (1959) found that high sugar concentration in the seedlings of Persian melon, predisposed them to infection by Macrophomina phaseoli. Gaumann (1950) suggested that susceptibility of pine roots to mycorrhizal fungi increased with increasing sugar content. Damodaran and Ramakrishnan (1963) observed that Gloeosporium musarum produced small lesions with early sporulation in varieties of banana with a high reducing sugar content, where as it produced larger lesions with delayed sporulation in varieties low in sucrose content. Subramaniam (1963) observed from a comparison of root carbohydrate content of 3 varieties of pigeon pea susceptible to Fusarium wilt that the most susceptible variety contained a very high amount while the less susceptible variety registered a very low amount. When inoculated, depletion of carbohydrate with progression of disease was evident in all the 3 varieties.

Mortimore and Ward (1964) reported that maize plants with high levels of sugars in the pith at maturity were resistant to root and stalk rot caused by various fungi

and bacteria. Plants grown under high population densities and with late defoliation were predisposed to stalk rot owing to the reduction in sugar content whereas resistance was increased by preventing kernel development.

Nagel and Leonard (1940) studied the chemical composition of healthy, diseased and pruned plants of Beta vulgaris. Pruning was done to simulate defoliation caused by the fungus. They observed that the percentage of sugars decreased in the diseased plants, while the amount of total nitrogen increased in the roots and crown of the diseased plants. The roots and crowns of infected plants grown in green house and field had higher soluble nitrogen than the corresponding pruned and healthy plants. Nightingale (1963) found that a relatively low carbohydrate and high nitrogen content was correlated with susceptibility and the reverse to resistance to blight by Erwinia amylovora in apple. Rajan (1964) believed that C/N ratio may be one of the factors influencing the susceptibility of older leaves of tapioca and relative resistance of younger ones to infection by Cercospora henningsii. The younger leaves contained more nitrogen and less of total carbohydrates while the older ones contained less of total nitrogen and more of total carbohydrates. Rangaswamy and Natarajan (1966) reported

that in banana C/N ratio of healthy leaf widened due to reductions in nitrogen content and increase in carbohydrate content, with age. They found that with fungal infections the C/N ratio of the leaf narrowed down. The diseased tissues contained more total nitrogen than the healthy tissues of diseased leaf and of healthy leaf.

Cordana musae is the most predominant organism occuring on banana in and around Vellayani. The leaf spot caused by this organisms was first reported by Zimmermann (1902) from Java, who named it as Scolecotrichum musae Zimm. Von Hehnel (1923) renamed the pathogen as Cordana musae (Zimm.) Von Hohn. In India this fungus was first reported by Subramanniam (1957). Elizabeth (1964) reported this fungus from Kerala and described the symptoms, morphological characters of the fungus, varietal susceptibility etc. Stahel (1934) found that the fungus could be easily brought into culture. However, the production of conidia was retarded and never abundant. Elizabeth (1964) also got similar results. Stahel (1934) made a detailed investigation of the entry and infection of banana leaves by this fungus. When conidia were placed on living leaf pieces in moist Petri dishes, the fungus penetrated into the inner tissues of the first young leaf only. It reached the lower surface in about 3 days, the result

being a large brown spot. He found that the pathogen readily entered the epidermis of other leaves upto the 13th but the layer of large watercells apparently acted as a barrier to further penetration. Field infection experiments yielded similar results through the effect on the youngest leaf was less evident. In the older leaves, the contents of infected epidermal cells became yellow, then brown and pink. Elizabeth (1964) reported that the infection hypha penetrated directly into the young and old leaf tissues, in moist Petri dishes. However, she reported that infection occurred only on the lower most two to three leaves, when inoculation was done on potted plants.

MATERIALS AND METHODS

MATERIALS AND METHODS

Four varieties of banana viz., Ennabaniyan, Pachanadan, GrosMichel and Neypoovan were used for the estimation of tannin. The varieties Ennabaniyan and Neypoovan were used for the estimation of total nitrogen, total sugars, crude fibre and total carbohydrate. The variety Ennabaniyan is considered highly susceptible to leaf diseases while the variety Neypoovan is comparatively more resistant. The varieties GrosMichel and Pachanadan are moderately resistant to the leaf diseases (Elizabeth, 1964). The plants were grown in a plot of land under identical cultural and manurial conditions.

Estimation of moisture.

The percentage of moisture was determined by drying 20 gm of the leaf samples at 105°C for 6 hours in a hot air oven. The oven dried leaf material was powdered and stored in separate glass stoppered containers inside a desiccator, for the estimation of nitrogen and crude fibre.

Estimation of tannin.

Collection of samples for analysis:- Four plants from each variety were used for analysis and 4 samples were collected from different positions of each plant. The top most fully

opened leaf formed the first sample, the third leaf from the top formed to second sample, the fifth leaf from the top formed the third sample and the bottom most green leaf formed the fourth sample.

The first and second samplings were done when the plants were five and seven months old respectively, when they had seven to eight fully unfolded leaves. About 45 gm of the leaf blade was cut out and used from the middle of each leaf. Since the older leaves were invariably affected by leaf spots, care was taken to cut out only the healthy portions from such leaves. They were immediately put in polythene bags and brought to the laboratory. The leaf material was separately weighed into two, 20 gm lots for the estimation of tannin and dry weight.

Tannin estimation was done as per method described in A.O.A.C. 1960 and Allen's organic commercial Analysis Vol. V.

Reagents used:-

1. Iodine solution, containing 5.2 gm of iodine and 7.6 gm of potassium iodide per litre.
2. 10 per cent sodium bicarbonate solution (aqueous)
3. Carbon disulphide.
4. Gelatin solution—25 gm of gelatin was soaked in saturated

sodium chloride solution for one hour and then heated until gelatin dissolved. Then it was cooled and diluted with saturated sodium chloride solution to 1 litre.

5. Acid sodium chloride solution—975 ml of saturated sodium chloride solution was acidified with 25 ml of concentrated sulphuric acid.

5. Powdered kaolin.

The leaf material was cut into small bits and immediately boiled in about 75 ml of distilled water in a 250 ml beaker for 30 minutes. The solution was decanted to another 250 ml beaker. The residue was again boiled for 30 minutes after adding about 25 ml of distilled water and the extract was again decanted to the same beaker. The decanted extracts were then filtered through a piece of muslin cloth and the filtrate was made upto 100 ml by adding distilled water.

10 ml of this extract was taken in a 100 ml conical flask to which 30 ml of 10 per cent sodium bicarbonate was added. 2-3 ml of carbon disulphide was added as indicator. This was titrated against standardised iodine solution filled in a microburette. The iodine solution was added drop by drop, with constant stirring until a blue colour appeared. The titration was repeated thrice and the average quantity of iodine solution was calculated.

Since the leaf extract contains tannin like substances also, in addition to tannin, the titration was repeated after precipitating tannin. The precipitation of tannin was done as follows. 50 ml of the extract was mixed with 50 ml of acid sodium chloride solution, 25 ml of gelatin solution and 5 gm of powdered kaolin. The mixture was shaken for 10 minutes in a mechanical shaker and then allowed to settle. After settling it was filtered through Whatman No.I filterpaper. 25 ml of this filtrate, (which is equivalent to 10 ml of leaf extract) was taken in a conical flask and titrated against iodine solution as per the method mentioned above and the average quantity of iodine solution required was calculated. The difference between the first and second litre values ie. before and after precipitation of tannin, was calculated in order to find out the volume of iodine solution required to oxidise tannin in 10 ml of the extract. From this, the quantity of tannin present in the leaf was calculated in terms of tannic acid (1 ml of iodine solution=1.42 mg of tannic acid).

Estimation of total nitrogen, sugars, crude fibre and total carbohydrate.

Seven months old plants were used for this. Three samples were taken from each of the 4 plants, from different positions. The top most fully opened leaf formed the first

sample, the middle leaf formed the second sample and the bottom most green leaf formed the third sample.

All precautions taken in the collection of samples for the estimation of tannin were taken for this also.

Estimation of total nitrogen.

Total nitrogen was estimated as per method given by Piper (1950).

One gram of the oven dry, powdered leaf material was taken in a 800 ml Kjeldahl Flask. About 10 gm of potassium sulphate and 1 gm of copper sulphate were added to it followed by 25 ml of concentrated sulphuric acid. The contents were mixed properly. The flask was placed on a Kjeldahl digestion stand and heated first gently and then strongly until the contents became clear. The contents were cooled and diluted with about 200 ml of distilled water. Few glass beads were put in the flask and it was then fitted upon a distillation stand making all the necessary connections. 40 ml of 0.1 N sulphuric acid was taken in a conical flask to which 2 drops of methyl red were added as indicator and placed underneath the condenser of distillation apparatus so that the end of the condenser dipped under the acid. About 75 ml of 40 per cent sodium hydroxide solution was added to the flask and distilled until about 150 ml of the distillate was collected in the conical flask. The excess sulphuric acid

was back titrated with 0.1 N sodium hydroxide. From the volume of 0.1 N sulphuric acid required the quantity of nitrogen present in the sample was found out from the following factor.

1 ml of normal acid = 0.014 gm of nitrogen.

Estimation of total sugars.

10 gm of the green leaf was cut into small bits and taken in a 250 ml Erlenmeyer flask to which was added 180 ml of distilled water and 20 ml of concentrated sulphuric acid. The flask was provided with a reflex condenser and the contents were boiled for 2½ hours, cooled and neutralised with sodium hydroxide. Then it was filtered through Whatman No.I filter paper into a 250 ml measuring flask and the volume was made upto the mark by adding distilled water.

10 ml of Fehling solution (5 ml of A + 5 ml of B) was measured into a 150 ml conical flask, brought to boiling and the sugar extract was added from the burette. When a faint precipitate was observed 2-3 drops of methylene blue were added and the addition of sugar extract was continued till the colour of methylene blue disappeared. From the volume of sugar extract required to react with 10 ml of Fehling's solution, the quantity of total sugar present in each sample was calculated.

Estimation of crude fibre.

2 gm of dry, powdered leaf material was taken in a 250 ml Erlenmyer flask. About 200 ml of boiling sulphuric acid (0.125 per cent by weight) was added to it and the contents were boiled for 30 minutes. Then the contents were filtered through a muslin cloth supported in a fluted funnel and the residue was washed free from acid with boiling distilled water.

The residue on the muslin cloth was transferred to a flask to which 200 ml of boiling sodium hydroxide (0.125 per cent by weight) was added and the contents were boiled for 30 minutes. Filtration was done as above and the residue on the muslin cloth was washed free of alkali by using boiling distilled water. Final washing was done with a small quantity of alcohol. The residue was transferred to a silica dish. The last traces of the residue were washed down into the dish with a fine jet of water. It was evaporated to dryness over a water bath, dried in a hot air oven at 105°C for about 10 minutes, cooled in a desiccator and weighed. Then the residue was ignited in a muffle at 600°C to white ash and the weight of the ash was taken. The difference between the two weights ie. before and after ignition gave the weight of crude fibre in the sample.

Estimation of total carbohydrates.

For the estimation of total carbohydrates, the total sugars and crude fibre estimated separately were added and represented as per cent.

Isolation and pure culture of Cordana musae.

Isolation of Cordana musae was made from bits of naturally infected banana leaves. They were first washed well with tap water to remove dirt etc. The infection spots were then cut into small bits of about 5x5 mm size. Only spots showing early stages of infection were used for isolation. The leaf bits were surface sterilised with 1:1000 mercuric chloride solution for one minute and washed with 3 changes of sterile distilled water, to remove traces of mercuric chloride. They were then placed in sterile Petri dishes previously poured with potato dextrose agar medium and incubated at room temperature. When the fungus growth was visible to the naked eye bits of mycelium were carefully removed from the Petri dish by means of sterile inoculation needle and transferred into P.D.A. slants. After sporulation, single spore isolations were made by the dilution plate method. The culture of the fungus was maintained on P.D.A. slants (Potato 200 gm, dextrose 20 gm, agar-agar 20 gm, distilled water 1000 ml).

The fungus was also grown on leaf extract dextrose agar medium with the following formula--leaf extract of

200 gm of fresh leaf; dextrose 20 gm, agar-agar 20 gm and the volume made upto 1000 ml).

Effect of tannic acid on the growth of Cordana musae in culture.

The fungus was grown in potato dextrose agar medium containing 0.125 per cent, 0.250 per cent, 0.50 per cent, 0.75 per cent and 1 per cent tannic acid. Since tannic acid gets hydrolysed by autoclaving, it was added only after sterilisation of the medium. Streptomycin sulphate at the rate of 30 μ gm per ml was added to the medium to prevent bacterial contamination. Media were poured into 9 cm Pyrex Petri dishes at the rate of 15-20 ml per dish. Circular discs of 4 mm diameter were then cut from a culture of the fungus grown in Petri dish by means of a sterile cork borer and placed in the centre of each dish containing solidified medium and incubated at room temperature. Potato dextrose agar medium was used as control. Since tannic acid was added without sterilisation there was contamination by other fungi. To overcome this, sufficient number of dishes were used for each treatment, to get at least 4 dishes without contamination.

The radial growth of the fungus was measured every 24 hours. The final observation was taken on the 15th day

by which time, the fungus in the potato dextrose agar medium had nearly reached the edge of the Petri dish.

Germination of the spores of Cordana musae in different substrates.

Germination of the spores of Cordana musae was tested in distilled water, tannic acid in distilled water and extracts from top and bottom leaves of Ennabaniyan and Neypoovan varieties. The leaves were kept in the ice compartment of the refrigerator for 2-3 hours and then ground well by means of pestle and mortar. The crude extract was clarified by centrifuging for 15-20 minutes.

Spore suspension was prepared in the appropriate media so as to have 15-20 spores per microscopic field when viewed under the low power. Drops of spore suspension were placed on clean, microscope slides and each slide was inverted and placed over two pieces of glass rods inside Petri dish lined with moistened filter paper. The percentage of spore germination was recorded at intervals of 3, 6, 9 and 12 hours.

The length of germ tubes was also recorded 6 hours after placing the spores in hanging drops.

The pH of the leaf extracts from top and bottom leaves of Ennabaniyan and Neypoovan varieties was measured.

Inoculation studies.

Field inoculations were done to study whether young leaves could take infection. Spores required for the inoculation were obtained by growing the fungus on sterilised host leaf tissue.

The inoculations were done in the evening. Two methods of inoculation were tried. In the first method spores from 15 days old culture on sterilised host tissue were scraped with a sterile needle and placed at different places on the leaf surface and covered with moistened cotton wool.

In the second method a heavy spore suspension was prepared in sterile water and it was sprayed on both the surfaces of leaves by means of an atomiser.

The inoculated leaves were covered with polythene bags for 48 hours, after which they were removed. Control plants were also given similar treatments except that they were sprayed with sterile water instead of spore suspensions.

RESULTS - PART I

RESULTS

PART I

Tannin content.

The tannin content of the leaves decreased as the leaves became older in both five and seven months old plants of all the varieties. This was true on dry as well as fresh weight bases. On dry weight basis the average tannin content of the top and bottom leaves was 0.85 per cent and 0.63 per cent in the case of five months old plants, while it was 0.83 per cent and 0.66 per cent in the case of seven months old plants. On fresh weight basis, the average tannin content of the top and bottom leaves was 0.169 per cent and 0.147 per cent in five months old plants and 0.174 per cent and 0.161 per cent in seven months old plants, respectively. The difference in tannin content at different stages of maturity of leaves, was found to be significant (Table I and Appendices A, B, C & D).

On dry weight basis, the variety Pachanadan had the maximum tannin content and the variety Neypoovan the minimum, when the plants were five months old. The average quantity was found to be 0.751 per cent for Pachanadan and 0.708 per cent for Neypoovan. No significant difference was found between the tannin content of the varieties Pachanadan and GrosMichel, GrosMichel and Ennabaniyan and Ennabaniyan and Neypoovan, when the plants were five months old.

There was no significant difference between the tannin content of the four varieties, when the plants were seven months old. (Table I and Appendices A and B).

On fresh weight basis the variety Pachanadan had the maximum tannin content and Ennabaniyan the minimum when the plants were both five and seven months old. The average quantity was found to be 0.165 per cent in Pachanadan and 0.153 per cent in Ennabaniyan, when the plants were five months old, where as it was 0.171 per cent and 0.165 per cent in seven months old plants. There was no significant difference between the tannin content of the varieties Pachanadan and GrosMichel and Neypoovan and Ennabaniyan in five months old plants. In seven months old plants the difference between Pachanadan and Neypoovan and Ennabaniyan and GrosMichel was not significant.(Table I and Appendices C and D).

Total nitrogen, total sugars and total carbohydrates.

The young leaves contained more nitrogen which decreased gradually as the leaves became old. This was true for Neypoovan as well as Ennabaniyan varieties. The average nitrogen content was 2.57 per cent and 2.17 per cent in the top leaf of Neypoovan and Ennabaniyan varieties, respectively, on dry weight basis, while, it was only 1.64 per cent and 1.61 per cent in the bottom leaf of the same varieties.

Table I

Percentage of tannin in different varieties, on dry and and fresh weight bases (average of 4 plants).

Five months old plants

Variety	Average percentage of tannin (in terms of tannic acid)							
	I		II		III		IV	
	Dry wt.	Fresh wt.	Dry wt.	Fresh wt.	Dry wt.	Fresh wt.	Dry wt.	Fresh wt.
Neypoovan	0.77	0.155	0.73	0.156	0.67	0.150	0.66	0.152
Ennabaniyan	0.86	0.173	0.72	0.154	0.68	0.150	0.60	0.135
Pachanadan	0.89	0.176	0.78	0.167	0.72	0.164	0.64	0.153
GrosMichel	0.86	0.172	0.75	0.163	0.67	0.156	0.62	0.148

Seven months old plants.

Variety	Average percentage of tannin (in terms of tannic acid)							
	I		II		III		IV	
	Dry wt.	Fresh wt.	Dry wt.	Fresh wt.	Dry wt.	Fresh wt.	Dry wt.	Fresh wt.
Neypoovan	0.83	0.177	0.73	0.175	0.70	0.170	0.68	0.160
Ennabaniyan	0.80	0.172	0.77	0.169	0.70	0.163	0.64	0.158
Pachanadan	0.83	0.178	0.76	0.171	0.70	0.169	0.68	0.167
GrosMichel	0.85	0.172	0.75	0.166	0.69	0.164	0.65	0.162

I. Topmost fully opened leaf
II. Third leaf from the top
III. Fifth leaf from the top
IV. Bottommost green leaf.

On fresh weight basis, nitrogen content was found to be 0.634 per cent and 0.603 per cent in the top leaf of Neypoovan and Ennabaniyan varieties respectively while it was only 0.463 per cent and 0.424 per cent in the bottom leaf of the above varieties. The variety Neypoovan had a slightly higher nitrogen content than Ennabaniyan (Tables II and III).

On the other hand, the younger leaves of both varieties contained less of total sugars and total carbohydrates and these constituents increased as the leaves became older. This was true when calculation was made both on dry and fresh weight bases.

The average total carbohydrate content was 50.08 per cent and 48.58 per cent in the top leaf of Neypoovan and Ennabaniyan varieties respectively, on dry weight basis, while it was 58.66 per cent and 58.72 per cent in the bottom leaf of the above varieties. On fresh weight basis the average total carbohydrate was found to be 11.98 per cent and 11.51 per cent in the top leaf and 16.17 per cent and 15.35 per cent in the bottom leaf of the varieties Neypoovan and Ennabaniyan respectively (Table II and III).

The carbohydrate:nitrogen ratios (C/N ratios) were 19:1 and 36:1 in top and bottom leaves respectively for both the varieties, while the C/N ratio of the middle leaf was 24:1

Table V

Radial growth of Cordana musae in P.D.A. with and without tannic acid (in mm)

Time	Media					
	P.D.A. (control)	0.125% tannic acid	0.25% tannic acid.	0.50% tannic acid.	0.75% tannic acid.	1% tannic acid
After 72 hrs.	15.75	11.50	9.00	6.25	No growth	No growth
" 96 "	19.90	14.40	13.12	9.90	5.00	"
" 120 "	23.75	17.38	15.25	11.75	5.40	"
" 144 "	28.50	20.50	17.62	14.38	6.02	"
" 168 "	32.00	24.00	20.10	17.25	7.20	"
" 192 "	37.00	28.00	23.00	17.50	7.80	"
" 216 "	42.75	32.62	26.60	21.25	8.25	"
" 240 "	48.50	36.62	30.25	24.12	9.30	"
" 264 "	53.75	41.12	33.38	26.90	10.00	"
" 288 "	58.25	44.00	37.25	30.25	10.95	"
" 312 "	62.75	48.12	40.25	32.12	11.80	"
" 336 "	68.75	52.25	44.38	34.38	12.75	"
" 360 "	75.50	56.10	47.12	36.12	13.50	"

Table II

Percentage of sugars, crude fibre, total carbohydrates and total nitrogen in banana leaves.

Variety	Plant No.	Position of leaf.	Dry weight				Fresh weight			
			Total sugars	Crude fibre	Total carbo-hydrate	Total 'N'	Total sugars	Crude fibre	Total carbo-hydrate	Total 'N'
Neypoovan	1	I	21.08	28.80	49.88	2.55	5.04	6.88	11.92	0.620
		II	22.77	29.25	52.02	2.24	6.26	8.02	14.28	0.605
		III	24.47	34.30	58.77	1.69	6.82	9.36	16.18	0.471
	2	I	20.70	27.25	47.95	2.56	4.92	6.49	11.41	0.619
		II	21.95	27.50	49.45	2.10	6.06	7.61	13.67	0.581
		III	24.45	32.80	57.25	1.63	6.82	9.15	15.97	0.455
	3	I	21.05	29.35	50.40	2.60	5.32	6.45	11.77	0.657
		II	22.77	30.10	52.87	2.29	6.37	7.42	13.79	0.606
		III	24.70	34.65	59.35	1.63	7.20	8.96	16.16	0.465
	4	I	22.32	29.75	52.07	2.56	5.77	7.07	12.84	0.660
		II	24.18	30.40	54.58	2.16	6.83	7.57	14.40	0.600
		III	25.06	34.20	59.26	1.61	7.40	8.96	16.36	0.460
Ennabaniyan	1	I	18.99	29.50	48.49	2.54	4.45	6.91	11.36	0.595
		II	20.64	33.50	54.14	2.17	5.28	8.45	13.73	0.555
		III	22.77	35.35	58.12	1.61	5.90	9.21	15.11	0.419
	2	I	19.81	29.30	49.11	2.55	4.75	7.02	11.77	0.611
		II	21.35	34.20	55.55	2.02	5.54	8.87	14.41	0.524
		III	22.71	35.50	58.21	1.66	6.06	9.47	15.53	0.442
	3	I	19.48	29.70	49.18	2.57	4.67	7.12	11.79	0.616
		II	19.40	34.45	53.85	2.22	5.05	8.96	14.01	0.578
		III	23.75	35.65	59.40	1.58	5.80	9.50	15.30	0.421
	4	I	19.37	28.15	47.52	2.54	4.52	6.56	11.08	0.592
		II	21.51	33.15	54.66	2.25	5.54	8.53	14.07	0.571
		III	23.45	35.70	59.15	1.57	5.94	9.49	15.43	0.416

I Topmost fully opened leaf
II Middle leaf
III Bottom most green leaf.

Table III

Average percentage of sugars, crude fibre, total carbohydrates and total nitrogen in the leaves of banana on dry and fresh weight basis (Average of 4 plants)

Constituents.	Neypoovan						Ennabaniyan					
	I		II		III		I		II		III	
	Dry wt.	Fresh wt.	Dry wt.	Fresh wt.	Dry wt.	Fresh wt.	Dry wt.	Fresh wt.	Dry wt.	Fresh wt.	Dry wt.	Fresh wt.
Sugars	28.79	5.26	29.31	6.38	33.99	7.06	29.16	4.60	33.83	5.35	35.55	5.93
Crude fibre	21.29	6.72	22.92	7.66	24.67	9.11	19.42	6.91	20.72	8.70	23.17	9.42
Total carbohydrates	50.08	11.98	52.23	14.04	58.66	16.17	48.58	11.51	54.55	14.05	58.72	15.35
Total nitrogen	2.57	0.634	2.20	0.598	1.64	0.463	2.55	0.603	2.17	0.557	1.61	0.424
C/N ratio	19:1		24:1		36:1		19:1		25:1		36:1	

I. Topmost fully opened leaf
II. Middle leaf
III. Bottom most green leaf.

for Neypoovan and 25:1 for Ennabaniyan. The C/N ratios of the two varieties were not significantly different, while they were significantly different in leaves at different stages of maturity. (Table III and Appendix E).

Growth of the fungus Cordana musae in solid media.

1. Potato dextrose agar.

The fungus grew well on this medium with the production of profuse aerial mycelium. The colony appeared fluffy and cottony white with entire margins. There was no diffusion of any colour in the medium. The sporulation was poor.

2. Leaf extract dextrose agar.

The fungus grew well in this medium also. The hyphae were partly submerged with comparatively less aerial growth. The mycelium was whitish and sporulation was better than in potato dextrose agar. However, there was no profuse sporulation.

Effect of tannic acid in the growth of Cordana musae, in culture.

The presence of tannic acid in the medium adversely affected the growth of the fungus. At concentrations of 0.125 per cent and 0.25 per cent of tannic acid, the growth of the fungus was fairly satisfactory. It produced some growth even at 0.75 per cent tannic acid concentration. The growth of the

fungus was totally inhibited, when the concentration of tannic acid was one per cent, in the medium. A brown halo was observed round and below the colony, when the fungus was grown in medium containing tannic acid (Table 5--Fig.1).

Germination of spores of Cordana musae in tannic acid.

Only 24 per cent of the spores germinated in 0.03 per cent tannic acid, while 95 per cent of the spores germinated in distilled water, by the end of 12 hours. No germination occurred at higher concentrations of tannic acid, tried (Table 6).

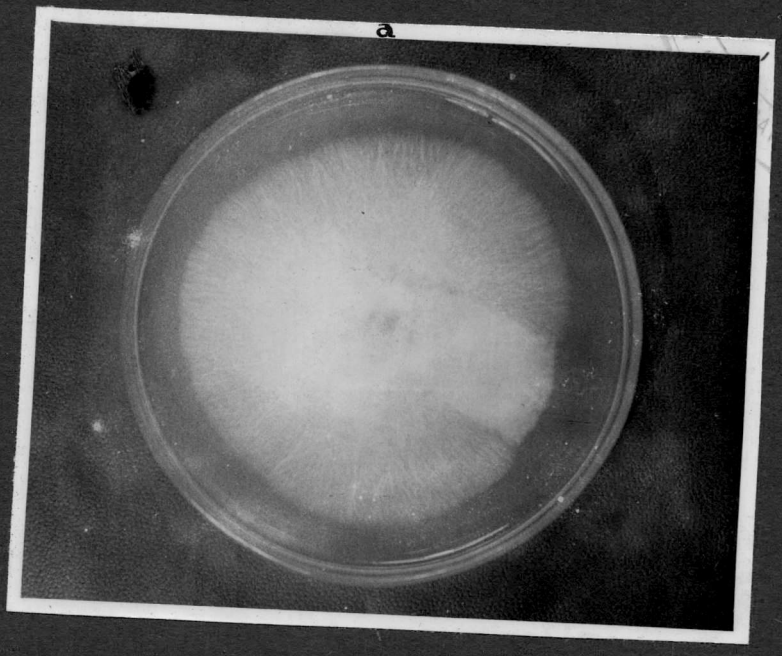
Germination of spores of Cordana musae in leaf extract.

The spores germinated readily in distilled water and also in concentrated and diluted leaf extracts. But the percentage of germination was lower in concentrated leaf extracts. At the end of 12 hours, the percentage of germination in distilled water was 92 per cent, while the percentages of germination were 74 and 76 in the concentrated leaf extracts of top leaf and 79 and 84 in the concentrated leaf extracts of bottom leaf of Nevpoovan and Ennabaniyan varieties respectively. There was no appreciable difference either between the varieties or between the top and bottom leaves, in the percentage of germination. When leaf extract was diluted, the percentage of germination was comparable to that of distilled water (Table 7).

Fig. 1. Growth of *Cordana musae* in media containing tannic acid:

a. P.D.A.
b. P.D.A.containing 0.125% tannic acid.
c. P.D.A.containing 0.250% tannic acid.
d. P.D.A.containing 0.50% tannic acid.
e. P.D.A.containing 0.75% tannic acid.
f. P.D.A.containing 1.00% tannic acid.

Fig. 1



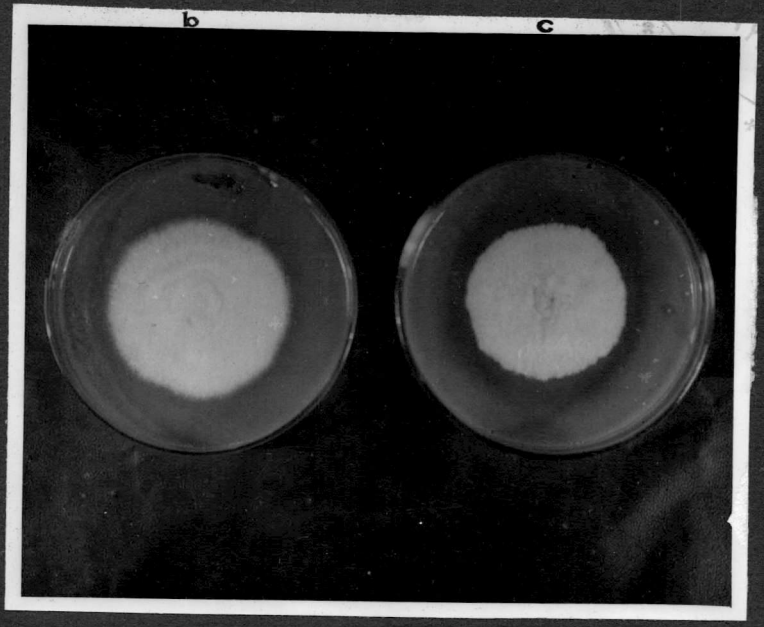
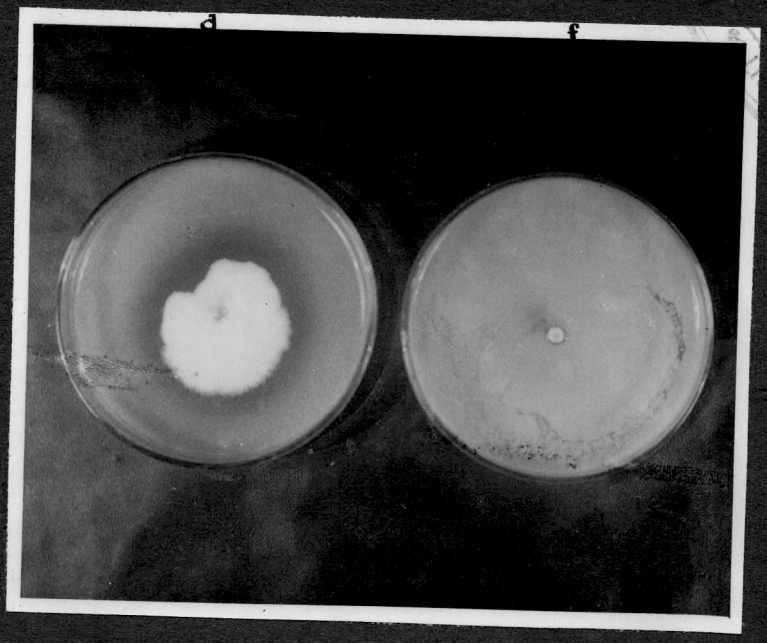


Fig. 1



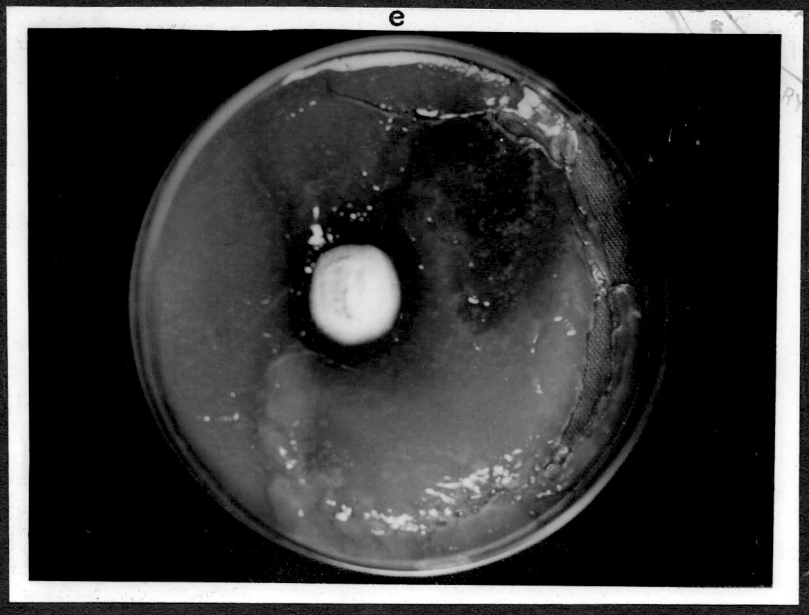


Table 6.

Percentage of germination of spores of Cordana musae in tannic acid.

Time	Distilled water	Concentrations of tannic acid(in %)				
		0.03	0.05	0.07	0.09	0.11
After 3 hrs.	33%	Nil	Nil	Nil	Nil	Nil
After 6 hrs.	95%	16%	"	"	"	"
After 9 hrs.	95%	21%	"	"	"	"
After 12 hrs.	95%	24%	"	"	"	"

Table 7

Percentage of germination of spores of Cordana musae in leaf extract

Time	Distilled water	Neypoovan						Ennabaniyan					
		Top leaf			Bottom leaf			Top leaf			Bottom leaf		
		1	2	3	1	2	3	1	2	3	1	2	3
After 3 hours	29	12	31	33	15	34	35	12	33	37	16	35	39
After 6 hours	91	52	84	90	56	86	93	53	82	87	59	79	91
After 9 hours	92	73	90	94	74	89	93	70	84	95	79	89	93
After 12 hours	92	74	90	95	79	91	93	76	91	95	84	91	93

1. Concentrated leaf extract
2. 1 part of leaf extract + 2 parts of water
3. ---do--- + 4 ---do---

Length of germ tubes of Cordana musae in leaf extracts.

The length of germ tubes was shorter in leaf extracts than in distilled water. In both the varieties, the germ tube attained a greater length in the extract of bottom leaf. At the end of 6 hours, the length of germ tube was 67 μ in distilled water, 26.4 μ and 23.2 μ in the concentrated leaf extract of top leaf and 34.3 μ and 30.07 μ in the concentrated leaf extract of bottom leaf of Ennabaniyan and Neypoovan varieties, respectively. When the leaf extract was diluted with water, there was stimulation for the elongation of germ tubes. When one ml of leaf extract was diluted with four ml of water, the average length of germ tubes was significantly higher than in water. It was observed that the germ tubes were slightly shorter in the leaf extract of Neypoovan than in Ennabaniyan (Table 8)

The extract of top leaf of Ennabaniyan had a pH of 6, while that of Neypoovan it was 6.5. The bottom leaf extracts of these varieties had a pH of 5.9 and 6.4 respectively.

Inoculation.

Five days after inoculation extremely small, dark-brown specks were noted on all the inoculated leaves. 3 to 4 spots developed on leaves sprayed with spore suspension. More number of spots developed on leaves inoculated with culture containing spores.

Table 8

Measurement of the length of germ tubes of Cordana musae in leaf extract

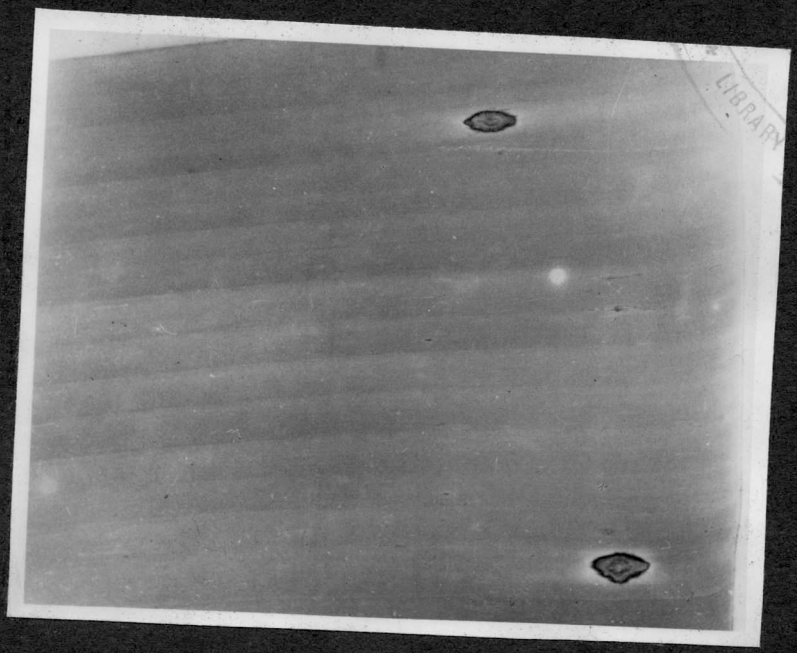
Variety	Length of germ tubes in μ after 6 hours (average of 3 observations)						
	Distilled water.	Extract of top leaf.			Extract of bottom leaf		
		1	2	3	1	2	3
Neypoovan	67.00	23.20	63.90	97.00	30.07	70.17	95.80
Ennabaniyan	67.00	26.40	63.00	103.40	34.30	68.60	98.10

1. Concentrated leaf extract
2. 1 part of leaf extract + 2 parts of water
3. --do-- + 4 parts of water

It was observed that development of spots on younger leaves was much slower than on older leaves. After one month the spots on the middle leaf (fourth leaf from the top) and bottom green leaf which were the top and middle leaves respectively at the time of inoculation measured 14 mm x 6.7 mm and 20-21 mm x 12-13 mm.

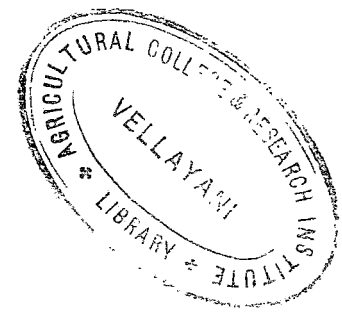
The spots had typical zonations and were surrounded by a yellowish band (Fig.2). Control plants remained healthy. It was further observed that the spots became larger at a faster rate, as the leaves became still older.

Fig. 2



Spots produced by Cordana musae on artificial inoculation on young leaf, after one month.

DISCUSSION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DISCUSSION

There is marked variation in the susceptibility of banana varieties to infection by leaf spot fungi. Under similar environmental conditions the variety Neypoovan is highly resistant and the variety Ennabaniyan is highly susceptible. The varieties Pachanadan and GrosMichel are moderately resistant. Under field conditions, the young leaves of all the varieties are apparently free from infection while the old leaves are infected.

From the estimation of tannin, it was found that there was no appreciable difference between the tannin content of the resistant variety Neypoovan and the susceptible variety, Ennabaniyan. It was also observed that the variety Pachanadan which is only moderately resistant had a higher tannin content than the other varieties including Neypoovan which is highly resistant. The difference between the tannin content of the varieties Pachanadan and Ennabaniyan, having the maximum and minimum tannin contents respectively was only 0-012 per cent in five months old plants and 0-006 per cent in seven months old plants on fresh weight basis. It was also found that the topmost fully opened leaf of all the varieties tested had the maximum tannin content which gradually decreased with age. The difference in tannin content

between the top and bottom leaves was only 0.022 per cent in five months old plants and 0.013 per cent in seven months old plants on fresh weight basis.

From the above facts, it appears that tannin is not a factor that can be correlated with the resistance of different varieties to leaf diseases. The difference between the young and old leaves is also so narrow that it is difficult to suggest the existence of a relationship between the tannin content at different stages of maturity of the leaves and fungal infections, unless all the fungi infecting the leaves are sensitive to narrow ranges of tannin concentration.

Cultural studies with Cordana musae, the predominant organism infecting banana in this locality, showed that it could grow fairly well in the medium containing 0.25 per cent tannic acid and produce some growth even at 0.75 per cent tannic acid concentration. A brown halo was formed around the colony, when the fungus was grown in medium containing tannic acid which suggested that the fungus was able to utilise small quantities of tannic acid in the presence of other nutrients. A similar observation was made by Bavendamm (1927) who reported that corrosion fungi were capable of utilising tannin. He found that these fungi produced a fair sized, deep brown to black halo around the mycelium, in tannin containing media and interpreted this as an indication of the alterations

of tannin by them. Rippel and Keesling (1930) reported that Penicillium spp., Aspergillus spp. and Citromyces spp. isolated from soil were able to grow with tannin as the sole carbon source. D'Oliveira (1935) found that fungus Endothiella gyrosa infecting the cork layer of cork oak was capable of growing in media containing tannic acid upto a concentration of 2.5 per cent. Cook and Taubenhaus (1911) showed that Gloeosporium musarum was to some extent resistant to tannin, the conidia showing complete germination in a 0.6 per cent tannin solution. Butler and Jones (1961) reported that grape vine with a higher tannin content was more susceptible to Fomes ignarius causing the 'Esca' disease.

On the other hand Devillifers (1929) reported the inhibitory effect of tannin in grapes against fruit infecting fungi and Barnell and Barnell (1945) in banana fruits against infection by Gloeosporium musarum.

Studies on the germination of spores of Cordana musae showed that in the undiluted leaf extracts the percentage of germination and also the elongation of germ tubes were significantly lesser than those in distilled water; but when the extract was diluted with distilled water the percentage of germination as well as elongation of germ tubes were found to be as good as in water. It was further noticed that while there was no appreciable difference in the

percentage of spore germination either between leaf extracts of resistant and susceptible varieties or between the extracts of top and bottom leaves, the length of germ tubes produced in the extracts of top leaves was slightly shorter than those produced in the extracts of bottom leaves. This shows that the tannin content of the leaf sap of susceptible and resistant varieties, at different stages of growth, does not apparently affect the spore germination of Cordana musae, Chakravarti (1957) found that the juice of green skin of banana had an inhibitory effect on the germination of spores of Gloeosporium musarum possibly due to the presence of tannin. This effect disappeared to some extent as the fruit ripened, when the tannin content was considerably reduced.

The reduction in the length of germ tubes produced in the extracts of younger leaves, may not solely be due to the presence of a slightly higher tannin content of young leaves. It is likely that other factors may also contribute to this phenomenon.

The analysis of total nitrogen, total sugars and total carbohydrates of the leaves of the two varieties at different stages of growth indicated that these factors could possibly be correlated with disease incidence. The younger leaves contained more of total nitrogen, and less of sugars and total carbohydrates, while the older leaves contained

less of total nitrogen and more of sugars and total carbohydrates. Hence it appears that the widening of carbohydrate: nitrogen ratio (C/N ratio) as the leaf matures may be one of the factors influencing the susceptibility of older leaves to infection by leaf spot fungi.

The effect of sugars and total carbohydrates on infection by fungi has been reported by various workers. Grainger (1956) reported that the most important factor influencing infection by Helminthosporium avenae causing leaf spot of oats and Phytophthora infestans causing late blight of potato was the total carbohydrate present in the leaves. He found a greater carbohydrate content in the diseased plants than healthy. Ashworth (1959) found that high sugar concentration in the seedlings of Persian melon predisposed them to infection by Macrophomina phaseoli. Subramaniam (1963) reported that the roots of Pigeon pea most susceptible to Fusarium wilt had a very high amount of carbohydrates, while the less susceptibles had a low amount. Rajan (1964) reported that the younger leaves of tapioca contained more of total nitrogen and less of total carbohydrates and reducing sugars, while the older ones contained less of total nitrogen and more of total carbohydrates and reducing sugars. He believed that the C/N ratio may be one of the factors influencing the susceptibility of older leaves and relative resistance of younger ones to infection by Cercospora henningsii. Rangaswami & Natarajan

(1966) reported that in banana the C/N ratio of healthy leaf widened due to reduction in nitrogen content and increase in carbohydrate content with age.

The analysis further showed that there was no significant difference in the C/N ratios of Neypoovan and Ennabaniyan varieties. Hence the varietal susceptibility could not be correlated with C/N ratio. However, it was observed that the variety Neypoovan which is resistant had a higher total nitrogen content in the leaves than the susceptible variety Ennabaniyan. Gassner and Franke (1938) observed that the older leaves of wheat plants had lesser nitrogen content and were generally severely infected by leaf rust. It is therefore possible that the higher total nitrogen content in the leaves of Neypoovan may be one of the factors contributing to its greater resistance to fungal infections.

A more detailed study of the total nitrogen, sugars, total carbohydrates and amino acids in the leaves of different varieties at different stages of maturity is likely to throw more light on these aspects. Steward et al. (1960) in their studies on the physiology of banana plants as influenced by nutritional deficiencies reported the presence of 15 amino acids. Rangaswamy and Natarajan (1966) detected 13 amino acids in the leaves of a banana variety

studied by them. They found that younger leaves contained more quantities of cystine which decreased with age and suggested that this amino acid may have an inhibitory effect on fungal mycelium and may be responsible for resistance to infection in young leaves. Papavizas and Davey (1963) suggested that cystine had an inhibitory effect on Aphanomyces causing root rot of peas. A detailed qualitative and quantitative study of the amino acids in the young and old leaves of different banana varieties would be necessary for obtaining a conclusive evidence on this aspect.

From artificial inoculation studies with Cordana musae, it was found that young leaves also took infection. Stahel (1934) also got similar results, though the effect on youngest leaf was less evident. Elizabeth (1964) reported that infection occurred only on the lower most two to three leaves, when artificial inoculation was done.

The development of spots on the young leaves was much slower than in older leaves. It took about one month for the lesions on the youngest leaf to become conspicuous, by which time, it had become the fourth leaf from the top. In nature also minute infection spots could be observed on the young leaves, which took considerable time to develop into mature spots. In this respect Cordana musae appears similar to Cercospora musae which according to Stahel (1937) mostly

infected the youngest leaves. But the symptoms took 24 to 27 days to develop and hence the young leaves appeared quite free from the disease.

A narrow C/N ratio, the presence of higher quantities of cystine in the young leaves (as reported by Rangaswamy and Natarajan, 1966) and presence of slightly higher quantities of tannin, together may be some of the possible factors contributing to the slow development of Cordana musae in the young leaves.

It is also probable that the young leaves of banana may produce certain antifungal compounds post infectionally which inhibit the development of the pathogen. Calpouzos (1962) suggested that the banana leaves might produce such antifungal compounds since three to four weeks elapsed from the time of penetration to the development of mature lesions in the case of Mycosphaerella musicola whereas mature sized fungal colonies were formed in six to seven days in vitro. Further study is necessary to establish the nature of the inhibitor, if any, involved.

RESULTS - PART II

PART II

A NOTE ON THE FUNGI OCCURRING ON BANANA

A large number of diseases caused by fungi have been reported on banana from different parts of the world. In Kerala, almost all the varieties of banana are found affected by leaf diseases. Diseases affecting fruits and pseudostem are also known to occur. But very little is known about the organisms involved. An attempt was, therefore, made to list out the common fungi that occur on banana in and around the Agricultural College, Vellayani.

1. Cordana musae (Zimm.) Von Hohn.

In India, the disease caused by this fungus was first reported from Madras by Subramaniam (1957). Elizabeth (1964) reported this disease from Kerala. A preliminary study of the disease was made by her and she found that the cultivated varieties of banana had varying degrees of susceptibility to this disease. The varieties Gnalipoovan and Ennabaniyan are highly susceptible while the variety Neypoovan is resistant.

The infected leaves developed small oval spots with delicate concentric zonations (Fig.3) which later on turned into large, pale brown patches. Brown, septate,

Fig. 3 Leaf spot caused by *Cordana musae*

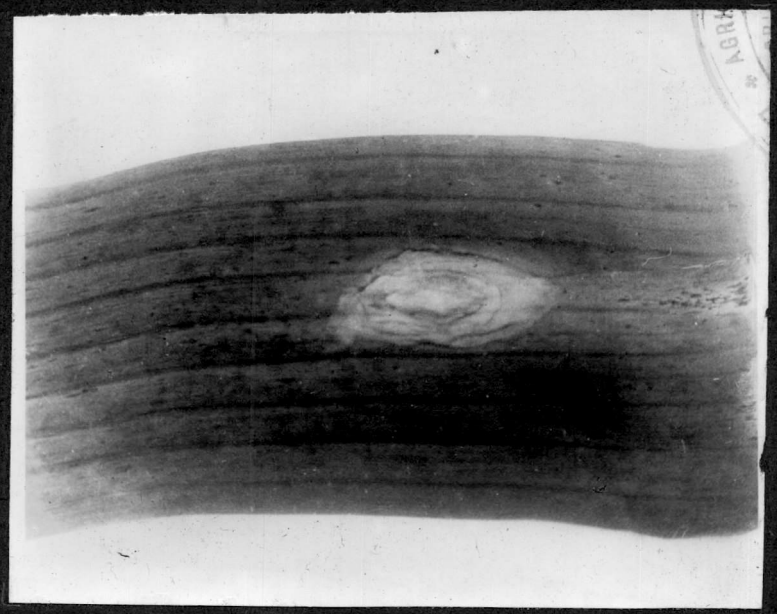
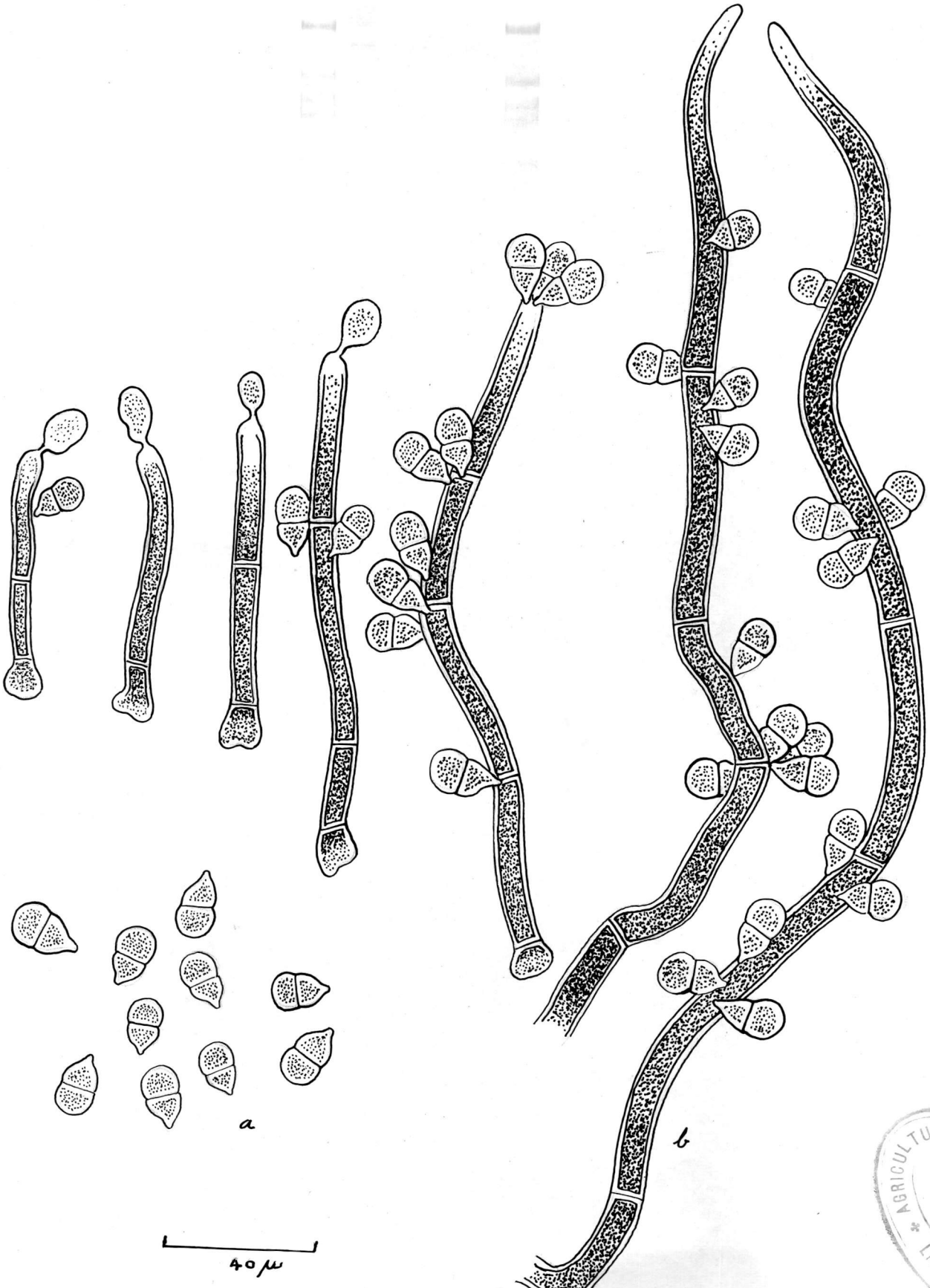
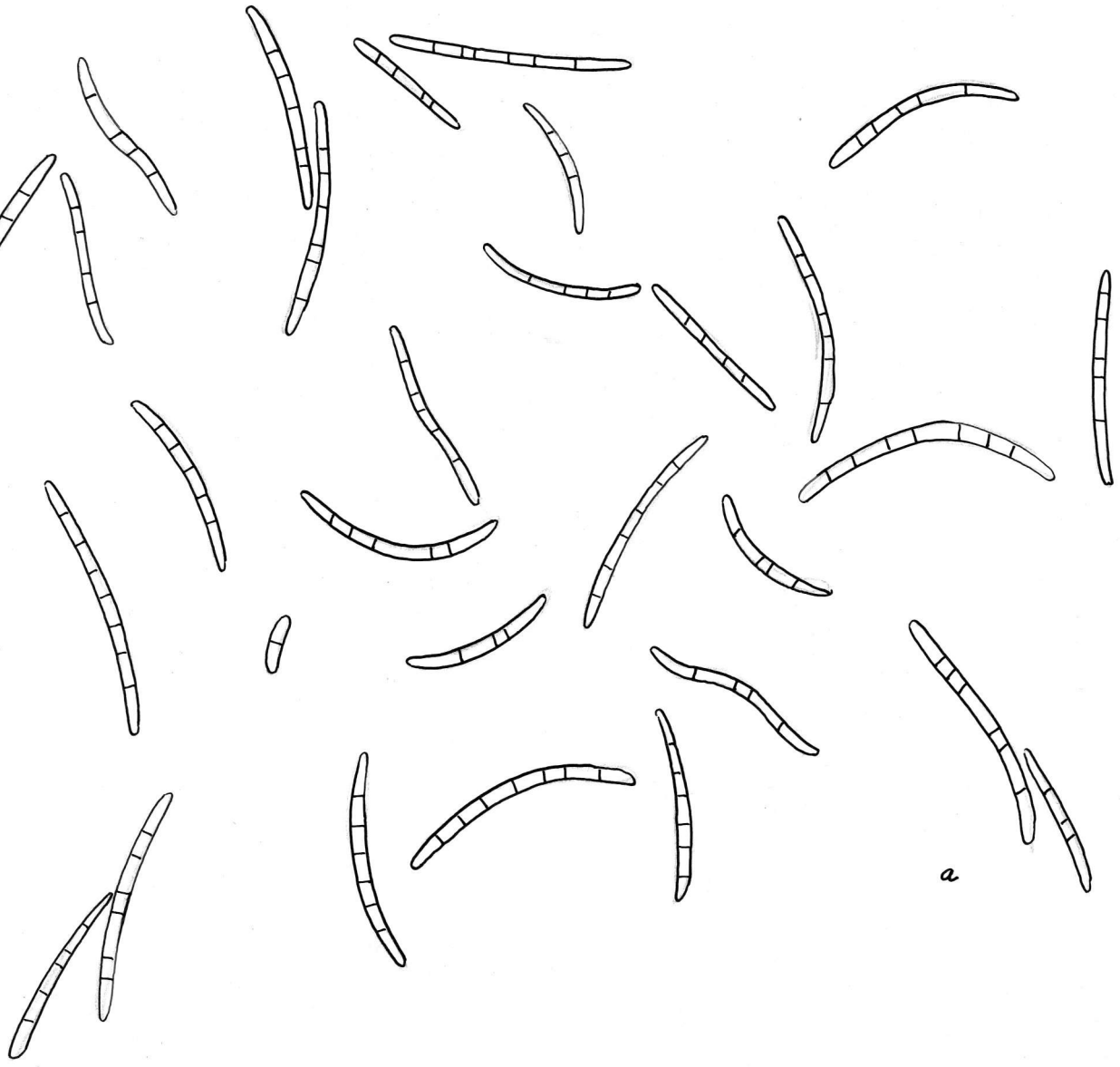


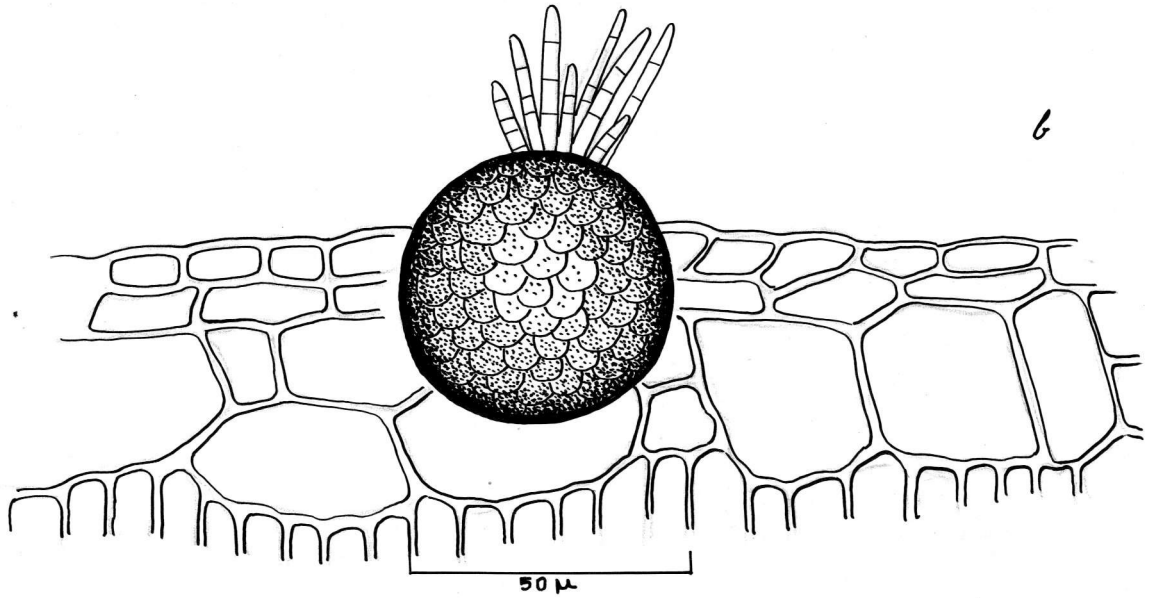
Fig. 5 Leaf spot caused by *Septoria keralensis*



Conidia and Conidiophores of *Cordana musae*
(camera lucida drawings)

a. Conidia





Pycnidium and Pycnidio Spores of *Septoria keralensis*
(camera lucida drawings)

a. Pycnidio Spores
b. Pycnidium

Fig 6

conidiophores were produced in large numbers on the underside. Two celled, lightly coloured conidia were produced singly or in clusters of 4-6 or more, mostly at the nodulations of the conidiophore. When young, conidia were hyaline and one called (Fig.4).

2. Septoria keralensis.

The leaf spot caused by this fungus was first recorded from Kerala by Elizabeth (1964). It has not been reported from anywhere else. From the preliminary study made by her, she reported that the variety Nendran was highly susceptible to the disease. However, in the present studies the variety Kappa (both red and white types) was found to sustain maximum damage due to the disease (Fig.5). The fungus produced numerous, extremely minute dark coloured pycnidia, mostly on the upper side of the infected regions. The pycnidia contained, hyaline, filiform, straight or slightly curved and 1 to 5 septate nconidia (Fig.6).

3. Macrophoma musae (Cke.) Berl. & Vogl.

Leaf freckle caused by Macrophoma musae was first reported in India by Butler (1905) and is now known to be widespread in the banana growing regions of the country. The disease is also reported to be responsible for the fruit disease known as "freckle". Only the "leaf freckle" stage of the disease was observed on the varieties Ennabaniyan, Pachanadan, Pevan, Monthan etc. in Vellayani area.

Symptoms.

Infections were characterised by the formation of very large number of pycnidia on the surface of leaves, rendering the surface hard. Pycnidia were formed on both the surfaces of the leaf. Frequently they were aggregated forming circular, raised, blackish spots (Fig.7). Individual pycnidia were also present. Wardlaw (1958) reported that only the older, debilitated leaves were attacked. But, here, otherwise healthy and green leaves were also found infected. However, the damage due to the disease was not severe.

Fungus. Pycnidia subconical, partly embedded, shining black. They are not beaked and the ostioles are not conspicuous. The conidia borne inside are densely granular and oval or irregularly shaped, single celled and with a thick, hyaline envelope (Fig.8).

Measurements:-

Pycnidia	Diameter	69-150 µ	Average 110 µ
	Height	69-115 µ	" 90 µ
Conidia	Length	10.4-19.0 µ	" 15.2 µ
	Width	6.9-10.4 µ	" 8.1 µ

The size and shape of pycnidia and conidia are in agreement with those reported by Carpenter (1918, 1919). The spermagonia, however, were not observed, along with Pycnidia as reported by him.

Fig. 7 Leaf spot caused by Macrophoma musae

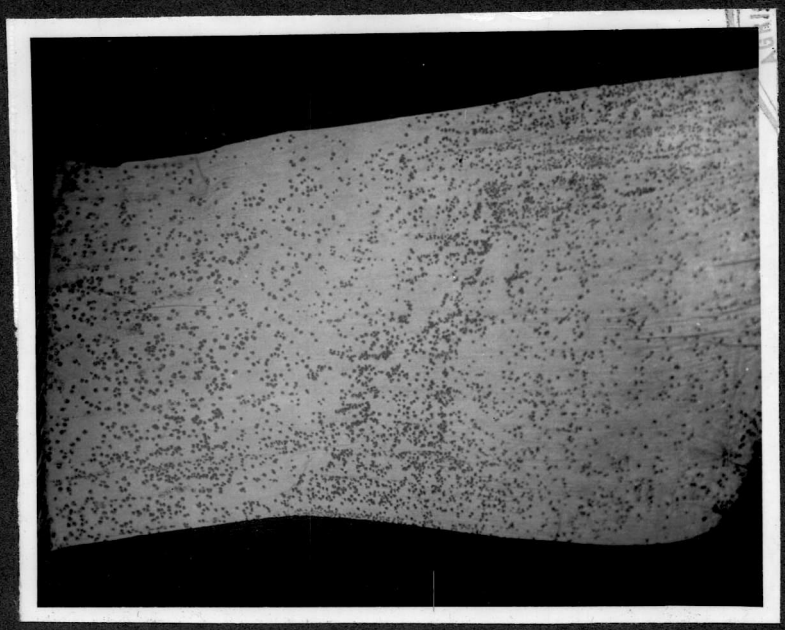
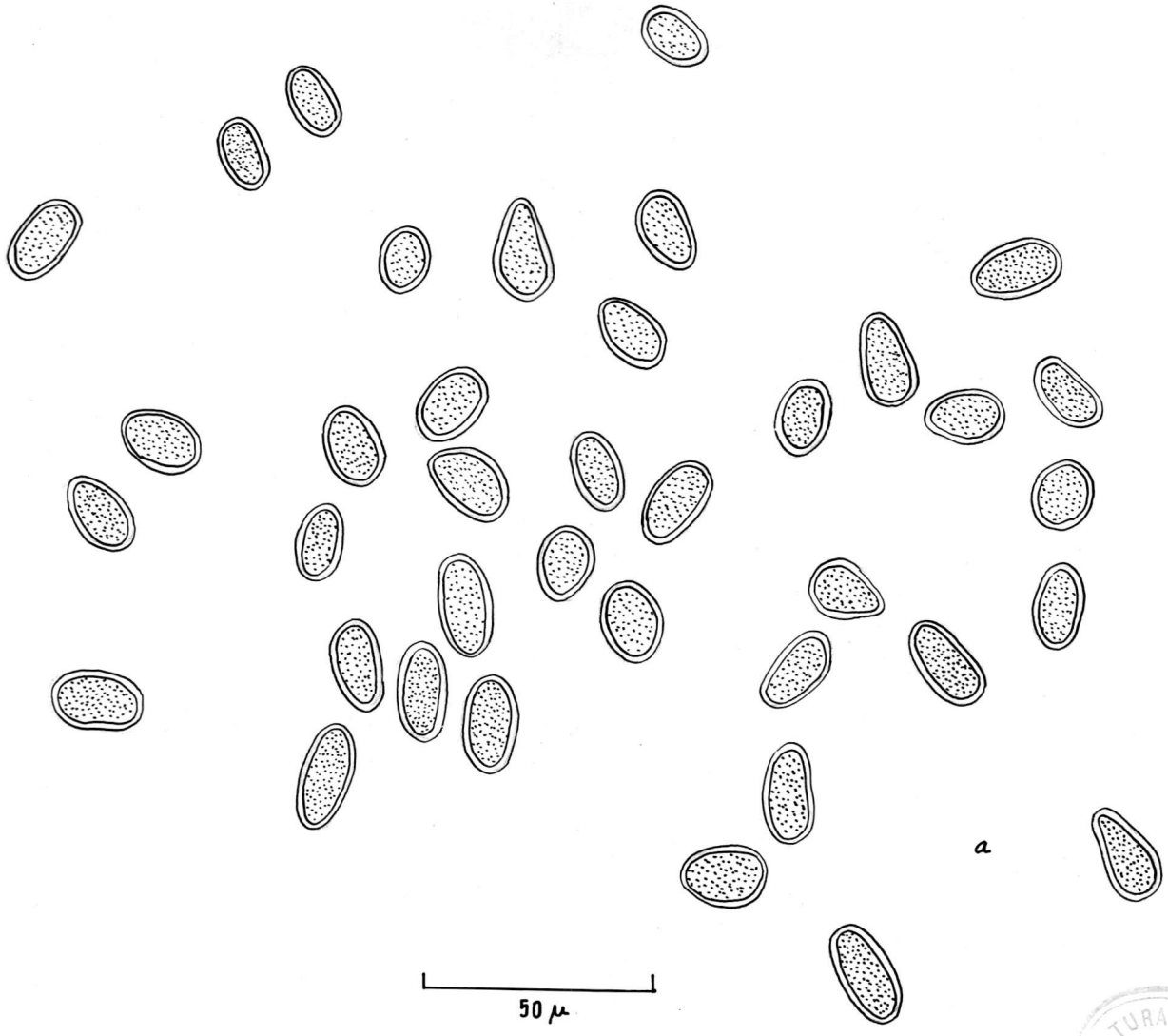
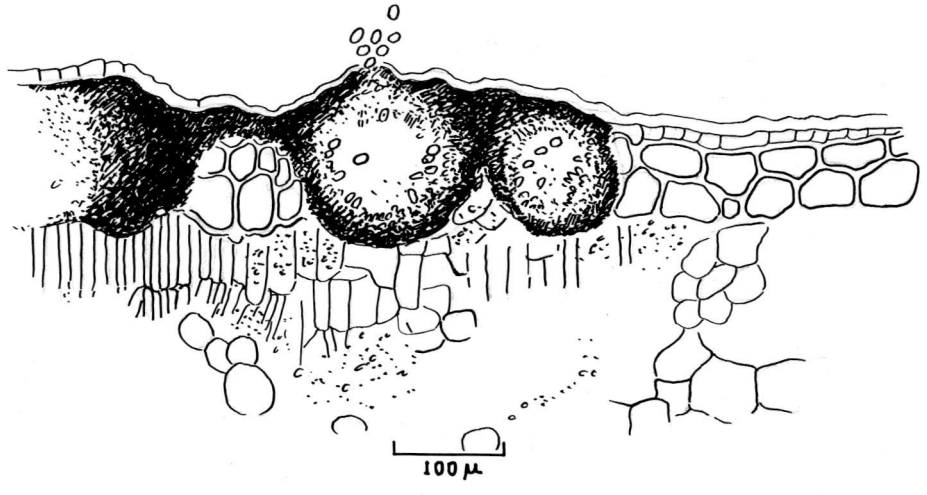


Fig. 9 Bunch infection by Gloeosporium musarum





Pycnidium and Pycnidio Spores of *Macrophoma musae*
(camera lucida drawings)

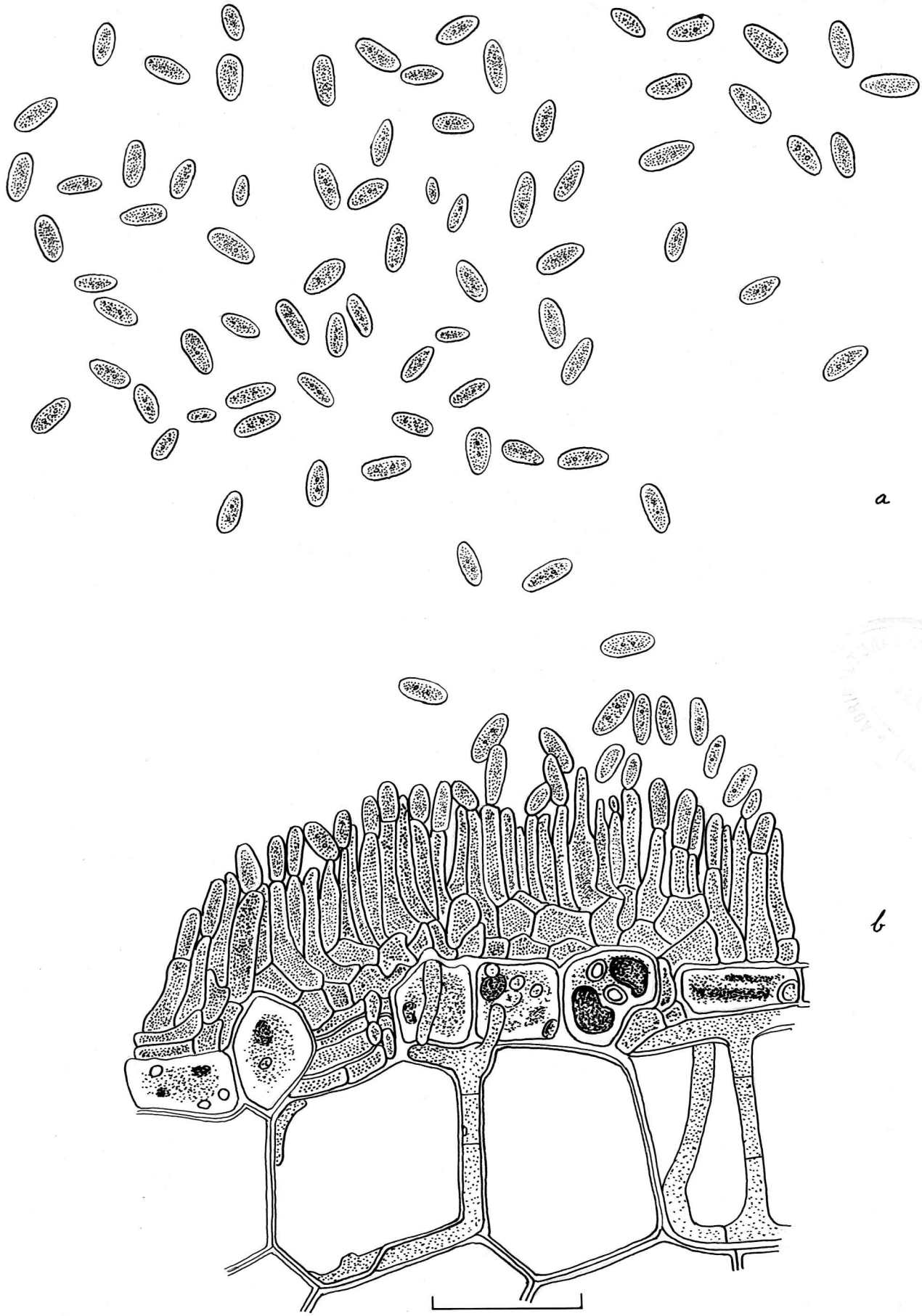
a. Pycnidio Spores
b. Pycnidium

Fig 8

4. Gleeosporium musarum Cke. & Massee.

This is mainly known to cause fruit rot of banana in storage. Dastur (1916) reported that it may also occur on immature fruits on plants causing 'anthracnose'. According to Andal and Seshadri (1965) this disease is widespread in our country and is known to cause serious damage during storage and transit of banana bunches.

In Vellayani area the disease was observed during the monsoon months affecting the bunches of Adakkakannan, Peykannan, Vennettumannan, Ennabaniyan and Vannan varieties. Young fruits, 2-4 weeks old were found affected, the infection starting from the distal end and slowly proceeding towards the proximal end (Fig.9). The skin turned black, the fruits becoming mummified and covered with characteristic pink acervuli. In severe cases the entire bunch was affected. In certain cases the main stalk of the bunch was also found affected, characterised by the black discoloration. Occasionally, the pseudostem of mature plants was also found affected by this fungus. Infection appeared as longitudinal dark coloured lesions on the pseudostem, under highly humid conditions. The presence of the fungus was detectable only at advanced stages when the pseudostem broke off, at the point of infection. When the outer sheaths of such infected plants were pulled out the infection could be seen to have penetrated deep into the pseudostem. At times mycelial growth of the



Acervulus and Conidia of *Gloeosporium musarum*
(camera lucida drawings)

a. Conidia
b. Acervulus

Fig 10

The only report of this fungus causing pseudostem infection is that of Chona (1933) from Punjab, who observed infection of young suckers.

Fungus.

The fungus produced pink acervuli on the fruit surface. Numerous closely packed conidiophores emerged from the acervulus on the tip of which conidia were borne. The spores were exuded in a sticky mass, when moisture was abundant. The spores were single celled, varying in size and shape, with a hyaline envelope and granular cytoplasm (Fig.10).

The measurements of spores:-

Average length	13.0 µ
Range	10.4-15.5 µ
Average width	5.5 µ
Range	4.3-6.9 µ

The measurement of spores agree with those given by Ashby (1931). When the spores were inoculated on young fruits after wounding, symptoms developed in 6-7 days.

5. Botryodiplodia theobromae Pat.

The pycnidia of a fungus resembling Botryodiplodia theobromae were observed on the banana fruits along with Gloeosporium infection.

Diplodia musae Died., was reported to occur on dead fruits of banana in Assam. Wardlaw (1961) believed that Diplodia musae reported from Assam was probably the same organism as Botryodiplodia theobromae, which is an important parasite of banana in storage.

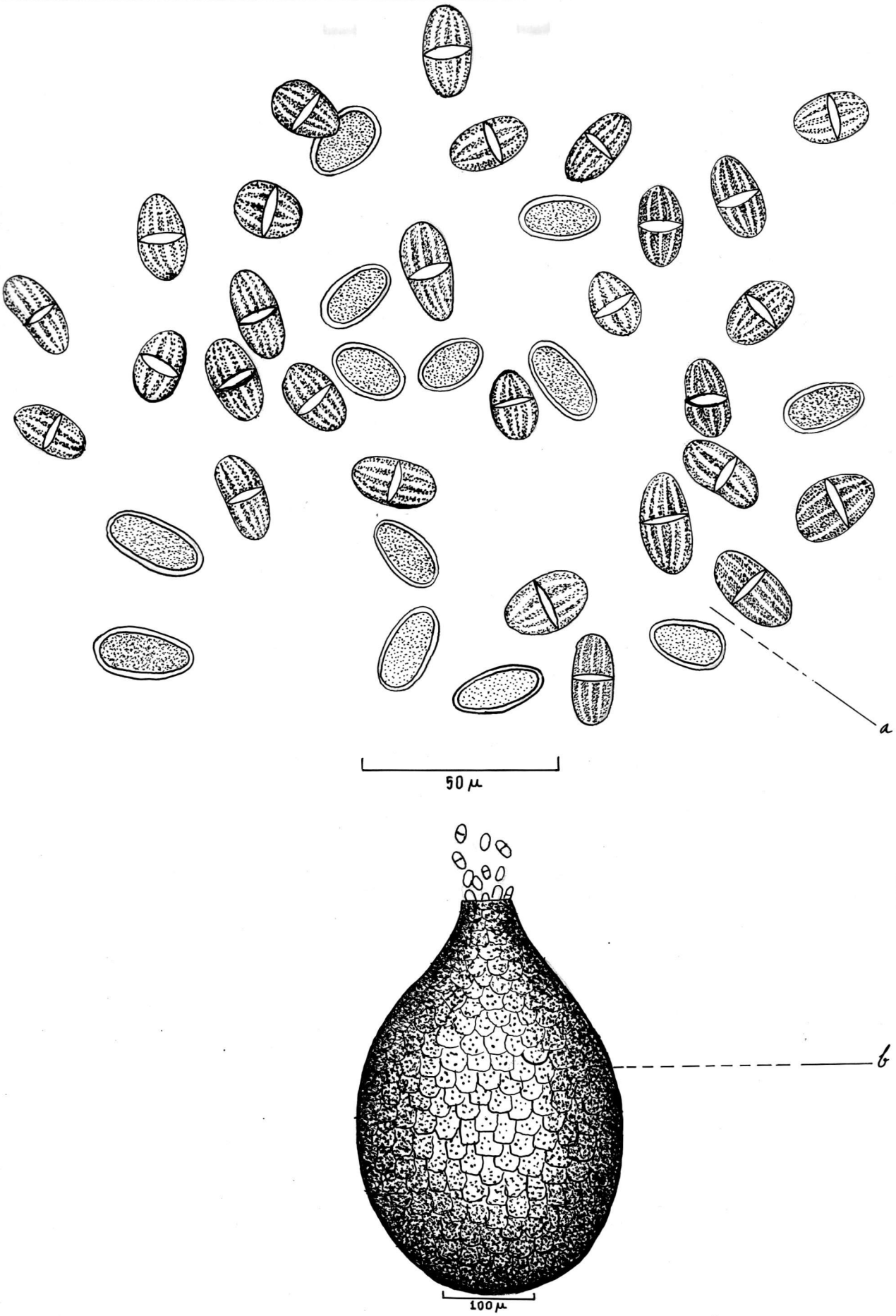
The fungus observed here had the following morphological characters.

Pycnidia dark coloured, flask shaped, ostiolate with a beak, almost superficial and densely gregarious with a diameter of 260-320 μ. Immature spores hyaline and one celled; mature spores 2 celled, darkish brown with longitudinal striations and measuring (average) 21 x 12 μ. (Fig. 11).

The morphological characters of the fungus under study are identical with that of Botryodiplodia theobromae and is therefore referred to this species.

6. Leptosphaeria sp.

A leaf spot of banana not previously reported in India was observed early in 1966, in the Agricultural College Farm, Vellayani. The variety Elavazhai was found to be mostly affected. Infection spots showed the presence of perithecia, asci and ascospores of a species of Leptosphaeria. On further examinations, pycnidia and spores of a species of Stagonospora were also observed in the same spots.


a
50 µ
b
100 µ

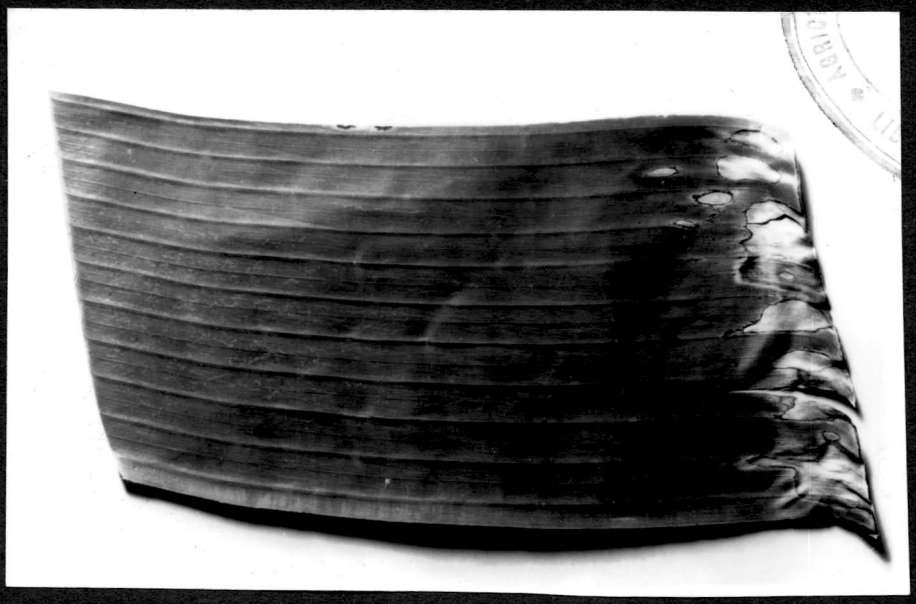
Symptoms.

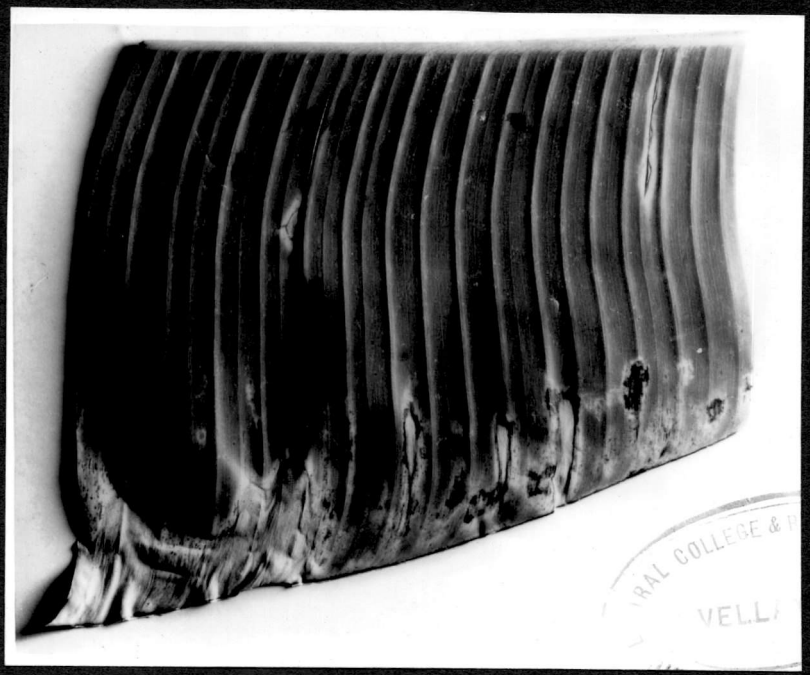
The infection started as water soaked, yellow discolouration mostly towards the margin of the lamina. This gradually became ashy grey in the centre, with a definite dark brown margin. A characteristic yellow halo could be observed around the young spots. Fully developed spots were oval to oblong measuring 10-25 mm in length and 5-15 mm in width. In severely affected leaves, the spots coalesced forming patches of diseased regions, eventually causing a downward curling of the leaf margins (Fig.12). The fructifications of the causal organism could be noticed as extremely minute, pinhead like protuberances on the upper surface of the infected lesions. These consisted of pycnidia as well as perithecia.

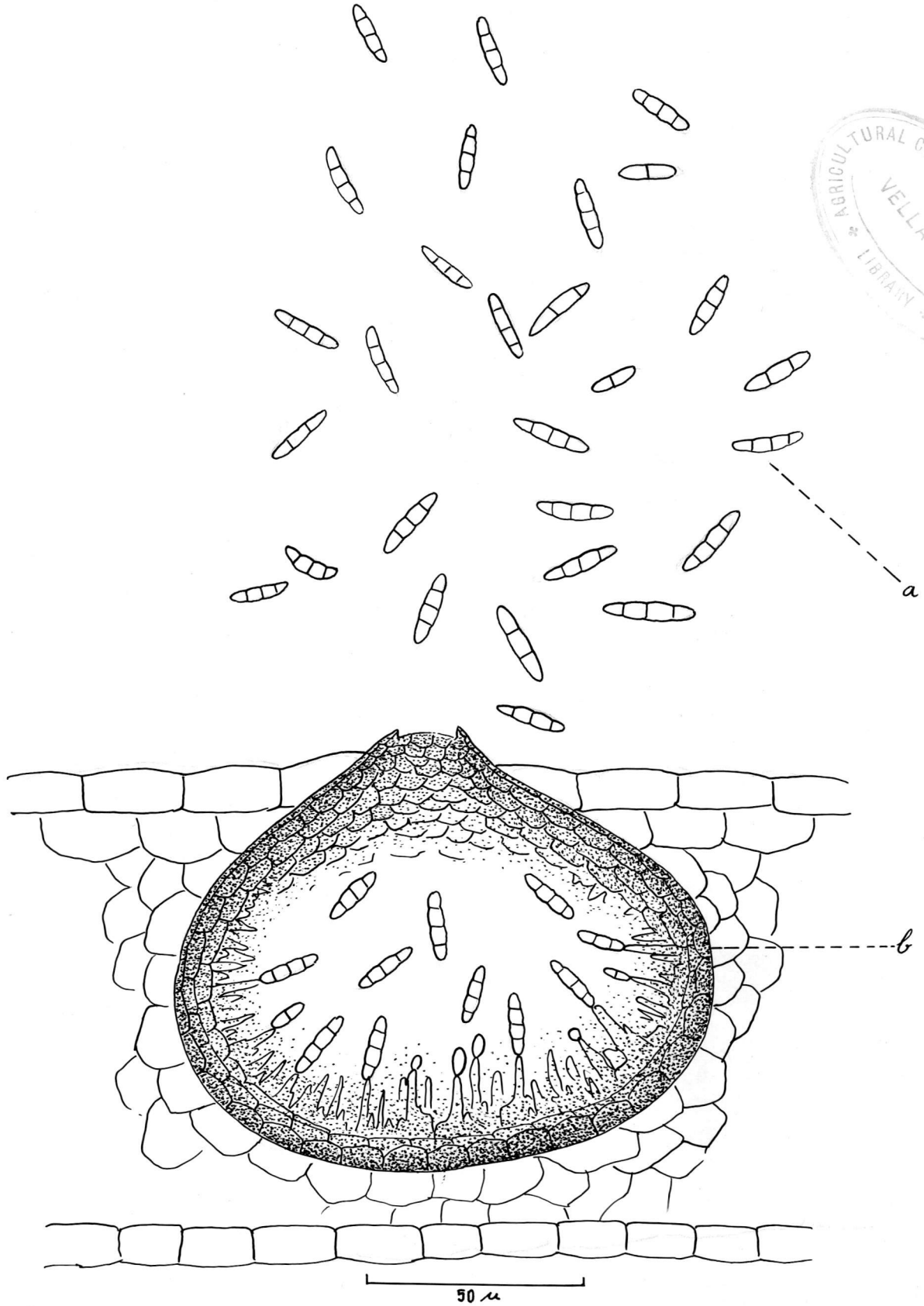
The causal organism.

The fungus was brought into pure culture on P.D.A. and the host leaf extract agar medium by single ascospore isolation. In P.D.A., the colony appeared whitish, gradually turning brown and in leaf extract agar, it appeared dark grey with profuse aerial mycelium. Minute, dark coloured fructifications of the fungus appeared in culture in about 12-15 days. These, when examined, were found to be pycnidia, containing spores. Pycnidium was dark coloured, flask shaped and provided with definite ostiole. Pycnidiospores were light yellow, oblong to elongate, 1 to 3 septate (mostly 3

Fig. 12 Leaf spot caused by Leptosphaeria.







Pycnidium and Pycnidio Spores of *Stagonospora*
(camera lucida drawings)

septate) and with definite constrictions at the septa. Young spores appeared hyaline and non-septate (Fig.13).

Peritheca began to develop, when the cultures were 2 months old. They were dark brown, spherical to sub-globose and provided with ostiole. Asci were hyaline, oblong to cylindrical, stipitate bitunicate, rounded at the top and containing 8 ascospores arranged approximately in a biseriate fashion. Slender, hyaline and filamentations paraphysis were also present interspersed with asci. Ascospores yellowish, 3 septate, fusiform, constricted at the septum and almost pointed at the extremities. The middle cells were slightly bigger and appeared swollen (Fig.14).

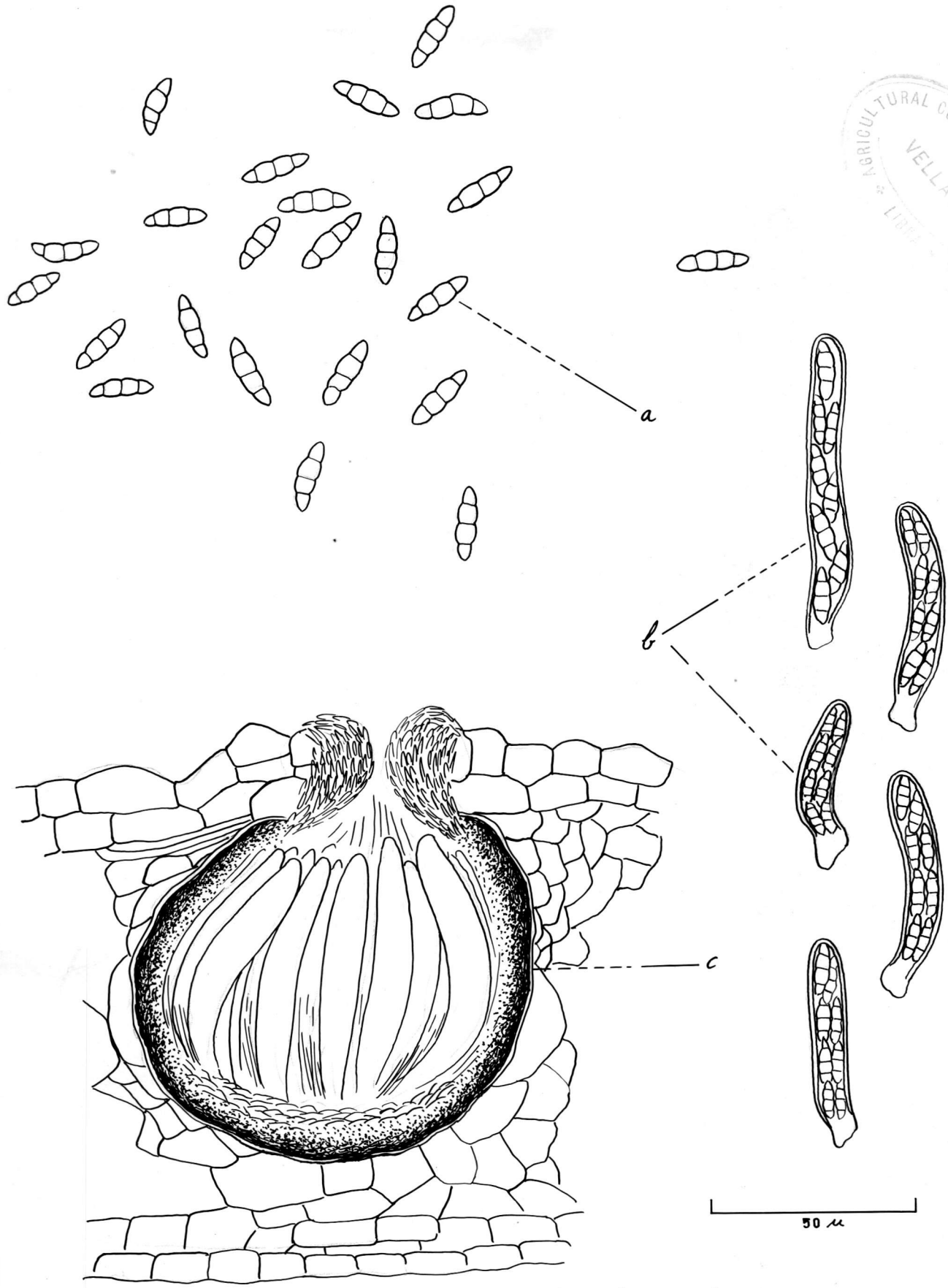
The measurements of the pycnidia, pycnidiospores, perethecia, asci and ascospores produced on the host as well as in culture are presented in tables.9 & 10.

Pathogenicity.

The pathogenicity of the fungus was proved by artificially inoculating the leaves of Elavazhai variety of banana with young cultures of the fungus. Typical symptoms, as those observed in nature developed on the leaves, within 7-10 days after inoculation.

Identity of the pathogen.

A leaf spot disease of banana was described by Cooke (1871) who named the causal organism as Hendersonia



Perithecium, Asci and Ascospores of *Leptosphaeria*

(camera lucida drawings)

a. Ascospores
b. Asci
c. Perithecium

Fig 14

Table 9

Stagonospora-Measurements of pycnidia and pycnidiospores.

Substrate	pycnidia				pycnidiospores			
	Diameter in µ		Height in µ		Length in µ		Width in µ	
	Range	Average	Range	Average	Range	Average	Range	Average
Host	90-120	102	105-165	130	10.4-20.7	15.2	2.6-4.3	3.7
Leaf extract agar	105-165	142	120-195	172	10.4-22.4	16.9	2.6-4.3	3.7

Table 10

Leptosphaeria-Measurements of perithecia, asci and ascospores.

Substrate	Perithecia				Asci				Ascospores			
	Diameter µ		Height µ		Length µ		Width µ		Length µ		Width µ	
	Range	Average	Range	Average	Range	Average	Range	Average	Range	Average	Range	Average

to the genus Stagonospora musae (Cke.) Sacc. Stevenson (1926) reported the occurrence of Leptosphaeria musarum Sacc. & Berl., on the leaves of Musa paradisiaca in Ceylon and Portugese St.Thomas. The morphological characters of Leptorphaeria musarum Sacc. & Berl., given by Stevenson (1926) are perithecia 166 μ in diameter; asci 60x10-12 μ and triseptate spores, 15-18 x 5-6 μ.

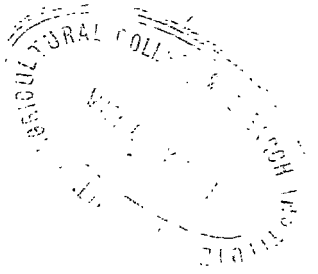
Simmonds (1933) noted two species of Leptosphaeria with 5 and 3 septate spores respectively and observed a close resemblance of the latter species to Leptosphaeria musarum Sacc. & Berl. Stahel (1937) reported the presence of perithecia of Leptosphaeria sp. having 4 celled spores and Pycnidia of Hendersonia sp. having 4-6 celled spores on banana leaves. Since both these spore forms gave rise to identical mycelia in pure culture, he reported that Leptosphaeria sp.and Hendersonia sp. are two stages of the same saprophytic fungus.

The measurements of the present fungus fall almost within the range quoted for Leptosphaeria musarum Sacc.& Berl. Therefore, based on the morphological characters of the fungus and its pathogenicity to banana leaves, it is tentatively identified as Leptosphaeria musarum Sacc. & Berl.

The spores of Hendersonia are dark coloured while that of Stagonospora are hyaline. Hendersonia sp. reported

by Stahel (1937) produced 4-6 celled spores. Stagonospora musae described by Saccardo (1884) produced hyaline, tri-septate spores, measuring 12x4 µ (average). The pycnidial stage of the present fungus on the leaf spot and also in culture produces hyaline to light yellow, 1-3 septate (mostly 3 septate) spores measuring 15.2x3.7 µ (average). It is therefore tentatively identified as Stagonospora musae (Cooke.) Sacc.

SUMMARY AND CONCLUSIONS



SUMMARY AND CONCLUSIONS

There is considerable variation between the varieties of banana, in their susceptibility to leaf spot diseases. But, in all the varieties, the young leaves are apparently free from infection while the older ones are infected.

There was no appreciable difference in the tannin contents in the leaves of susceptible and resistant varieties and hence it may not be a possible factor that could be correlated with varietal resistance. It is also not likely that the resistance of younger leaves to infection is due to the tannin content, since the difference between the tannin content of leaves at different stages of maturity was very narrow.

The analysis of total nitrogen, total sugars and total carbohydrates of the leaves of two varieties, at different stages of growth showed an increase in the total carbohydrate content and a decrease in the total nitrogen content as the leaf became older, thereby resulting in the widening of the carbohydrate:nitrogen ratio. The widening of the C/N ratio coincides with the disease incidence in older leaves, possibly indicating a positive correlation between the two. There was no significant difference between the C/N ratios of the resistant and susceptible

varieties. However, the resistant variety had a slightly higher percentage of total nitrogen in the leaves, which may probably be one of the factors, contributing to its greater resistance.

Cordana musae grew fairly well on potato dextrose agar medium containing 0.25 per cent tannic acid and produced some growth even at 0.75 per cent tannic acid concentrations.

There was no appreciable difference between the percentages of germination of the spores of Cordana musae either in the leaf extracts of the resistant and susceptible varieties or in the extracts of the top and bottom leaves of them. But the length of germ tubes produced in the extracts of top leaves was slightly shorter than those produced in the extracts of bottom leaves.

Inoculation experiments with Cordana musae showed that it could infect even the youngest leaf. But the development of spots on the younger leaves was very slow, typical symptoms appearing only when such leaves became older. It is suggested that this may be due to the special nutritional requirements of the fungus, to the presence of certain unfavourable factors like cystine in higher concentrations or to the production of certain antifungal compounds, post-infectionally, in the young leaves.

Cordana musae, *Septoria keralensis*, *Gloeosporium musarum* and *Macrophoma musae* are the more commonly occurring fungi on banana in and around Vellayani. A leaf spot of banana, new to India, caused by a species of *Leptosphaeria* was recorded during the course of the study. From the morphological characters and pathogenicity to banana leaves, the fungus was tentatively identified as *Leptosphaeria musarum* Sacc. & Berl. and its pycnidial stage as *Stagonospora musae* (Cke.) Sacc.

REFERENCES

REFERENCES

ALLEN'S COMMERCIAL ORGANIC ANALYSIS, Vol. V.

ANDAL, A. & SESHADRI, K. (1965). Mycostatin for prevention of Anthracnose of bananas in storage. Indian Phytopath., 18, 367-372.

A.O.A.C. (1960). Official methods of analysis. Ed. 9. Assoc. Official Agri.Chem., Washington.

ASHBY, S.F. (1931). Gloeosporium strains. Trop. Agriculture, 8, 322-325.

ASHWORTH, L.J. (1959). The relation of total sugars to susceptibility of Persian melon seedlings to Macrophomina phaseoli. Abs. in Phytopathology, 49, 533.

BAENS, L. & YENKO, F.M. (1936). Effect of molds on some Philippine tanning liquors II. Philipp.J.Sci., Ixi, 417-426.

BARNELL, H.R. & BARNELL, E. (1945). Studies in tropical fruits. xvi. The distribution of tanning within the banana and the changes in their condition and amount during ripening. Ann.Bot., Lond., 9, 77-99.

BARUAH, J.N., RAO, P.R., JANARDHANAN, K.K. & GANGULY, D.(1963). Effects of polyphenolic extracts of Acacia arabica on the spore germination of Piricularia oryzae. Journ. and Proc.Ind.Inst.Chemists, 35, 303.

BAVENDAMM, W. (1927). Neue Untersuchungen uber die Lebensbedingungen holzzerstrendei pilze Ein Beitrag Zur Immunitatsfrage. Ber.Deutsch.Bot.Gesellsch., xlv, 357-367.

BOUZNITZKY, A.L. (1940). Control of pests and diseases in sugar beet-Main results of the scientific research work during 1938 of the Pan-Soviet Scientific Research Institute for the sugar Industry (VNIS) (Russian) 144-145.

BORDOLI, D.N., BARUAH, J.N., GANGULY, D. & RAO, P.R. (1964). Effect of polyphenolic extracts of Cassia fistula and Acasia catechu on the spore germination of Colletotrichum falcatum Went. Curr.Sci., 33, 408

BOYER, M.G. (1964). Studies on white pine phenols in relation to Blister rust. Can.J.Brit., 42, 978-987.

BUTLER, E.J. (1905). Pilzkrankheiten in Indien im Jahre.1903. Zeitschuft fur Pflanzen Krankheiten, 25, 48.

BUTLER, E.J. & JONES, S.G. (1961). Plant Pathology. Mac Millan and Co.Ltd., London.

CALPOUZOS, L. (1963). Inhibition of Mycosphaerella musicola by water extracts of susceptible banana leaves. Ren.Agric.Hort.Res.Sta. Bristol, 18, 111-115.

CARPENTER, C.W. (1918). Banana diseases. Hawaii Agric. expt. Stat.Rep. 1917, 40-42.

CARPENTER, C.W. (1919) Banana diseases. Hawaii agric.expt.Stat. Rep. 1918, 36.

CHONA, B.L. (1933). Preliminary investigations on the disease of bananas occurring in the Punjab and their method of control. Ind.J.agric.Sci., 3, 4.

COOKE, M.C. (1871). Hand book of Australian fungi, New York.

COOK, M.T. & TAUBENHAUS, J.L. (1911). The relation of parasitic fungi to the contents of the cells of the host plant (1. Toxicity of tannin). Del.coll.Agric.Expt.Bull.,91.

COONS, G.H. & KLOTZ,L.J.(1925). The nitrogen constituents of celery plants in health and disease. Journ.Agric. Res., 31, 287-300.

DAMODARAN, S. & RAMAKRISHNAN, K. (1964). Anthracnose of banana-1. Studies on the disease. Journ. of Madras Univ. Sect.B., 33, 249-279.

DASTUR, J.F. (1916). Spraying for ripe rot of the plantain fruit. Agric.J.India, 21.

DeVILLIERS, F.J. (1929). Studies of grapes in cold storage. S.African Journ.Nat. Hist., 6, 315-329.

D'OLIVEIRA, B. (1935). Phytopathological notes. Rev.agron.,23, 50-51.

ECHANDI, E. & FERNANDEZ, C.F. (1962). Relation between chlorogenic acid contents and resistance to coffee canker incited by Ceratocystis fimbriata. Phytopathology, 52, 541-547.

ELIZABETH, G.(1964). Studies on Cordana musae (Zimm.)Von Hohnel and a new Septoria leaf spot of banana. M.Sc.(Ag.) Thesis, University of Kerala (unpublished).

FINKNER, R.E., FARUS, D.E., OGDEN, D.B., DOXATOR, C.W. & HELMERICK, R.H. (1962). Chemical control of Cercospora leaf spot in sugar beets. J.Amer. Soc.Sug.Beet.Technol., 12, 43-52.

GASSNER, G. & FRANKE, W. (1938). Unter Suchungen uber den stickstoffhaus halt rostinfiziertei Cetreideblattei. Phytopathology, Z, 11, 517.

GAUMANN, E. (1950). Principles of Plant Infection. Crosby Lockwood & Son,Ltd., London.

GRAINGER, J.(1956). Host nutrition and attack by fungal parasites. Phytopathology, 46, 445.

GROSSMAN, F. (1958). Uber die Hemmung pectolytischer Enzyme von Fusarium oxysporium.f.lycopersici durch Gerbstoffe. Natur wissen schaften, 45, 113-114.

GROSSMAN, F. (1962). Studies on the inhibition of pectolytic enzymes of Fusarium oxysporum.f.lycopersici. III. Effect of some inhibitors in vivo. Phytopathology,Z, 45, 139-159.

JOHNSON, G. & SCHALL, L.A. (1952). Relation to chlorogenic acid to scab resistance in potatoes, Science, 115, 627-629.

LEACH, R. (1937). Observations on the parasitism and control of Armillaria mellea. Proc.roy.Soc.Ser.B., cxxi, 825, 561-573.

MAHADEVAN, S.A. (1964). Biochemistry of resistance in cucumber against Cladosporium cucumerinium. Phytopathology, 54, 886-913.

MORTIMORE, C.G. & WARD, G.M. (1964). Root and stalk rot of corn in South Western Ontario.III. Sugar levels as a measure of plant vigour and resistance. Can.J.Pl.Sci., 44, 451-457.

NAGEL, C.M. & LEONARD, O.A. (1940). The effect of Cercospora beticola on the chemical composition and assimilation of Beta vulgaris. Phytopathology, 30, 659-666.

NIGHTINGALE (ALICE A) (1963). Some chemical constituents of apple associated with susceptibility to fire blight. Bull.N.J.Agric.Exp.Stal., 613, 22.

OFFORD, H.R.(1940). The function of tannin in host parasite relationship with special reference to Ribes and Cronartium ribicola (Bull). U.S.Bur.Ent.E., 518, 27.

PAPAVIZAS, G.C. & DAVEY, C.B.(1963). Effect of sulphur containing amino compounds and related substances on Aphanomyces root rot of peas. Phytopathology, 54; 233-234.

PIPER,C.S.(1950). Soil and Plant analysis. Inter Science Publishers, New York.

RANGASWAMY, G. & NATARAJAN, S. (1966). Changes in the free amino acids and C/N ratios of banana leaves infected with fungal pathogens. Indian Phytopath., 19, 59-63.

RAJAN, K.M.(1964). Observations on the incidence of leaf spot of tapioca with special reference to certain chemical constituents of the plant. M.Sc.(Ag.) Thesis, University of Kerala (unpublished).

RIPPEL, A. & KEESLING, J.(1930). On tannic decomposing organisms. Arch.fur.Mikrobiol., 1, 60-77.

SACCARDO, P.A.(1884). Sylloge fungorum, 3.

SIMMONDS, J.H. (1933). Banana leaf spot. Pamphlet No.6,Dep.Agric. and Stock Div.of Entomology and Plant Path., Qi.

STAHEL, G. (1934). The banana leaf disease in Suriname. Trop.Agriculture, 11, 138-142.

STAHEL, G.(1937). Banana leaf spot (Cercospora musae). Trop. Agriculture, 14, 256-260.

STAHEL, G. (1937). Notes on Cercospora leaf spots of bananas. Trop.Agriculture, 14, 257-264.

STEVENSON, J.A. (1926). Foreign plant diseases. U.S.Dep.Agric.

STEWARD, F.C., HULME, A.C., FREIBERGE, S.R., HEGARTY, M.P., POLLARD, J.K., RABSON, R. & BARR, R.A.(1960). Physiological investigations on the banana plant. I. Biochemical constituents detected in the banana plant. Ann.Bot.N.S., 24, 83-116.

SUBRAMANIAM, S. (1963). Fusarium wilt of pigeon pea, changes in host metabolism. Pros.Ind.Acad.Sci., 57, Sect.B, 182.

SUBRAMANIAM, T.V. (1957). Diamond leaf spot of banana. Madras Agric.J., 44, 102.

TANI, T. & NAIRO, N. (1957). On the nitrogen content of plants infected by several rust fungi. Tech.Bull.Kagawa agric.Coll., 7, 141-143.

THRESH, J.M. (1956). Some effects of tannic acid and of leaf extracts which contain tannins on the infectivity of Tobacco Mosaic and Tobacco Necrosis Viruses. Ann.appl. Biol., 44, 608-618.

WALKER, J.C. & LINK, K.P. (1935). Toxicity of phenolic compounds to certain onion bulb parasites. Bot.Gaz., 96, 466-484.

WARDLAW, C.W. (1961). Banana diseases including plantains and abaca. Longmans, Green and Co. Ltd., London.

ZIMMERMANN, A. (1902). Uber einige tropischer kulturpflanzen beobachtete Pilze. Central Bakt. Abt., 2, 219.

APPENDICES

Appendix A

Analysis of variance of data in Table I

(Tannin content in five months old plants on dry weight basis).

Source	Sum of squares.	d.f.	Variance	'F'ratio calculated(0.05 level)	'F' ratio from Tables (0.05 level).	Whether significant or not
Total	0.57	63				
Variety	0.02	3	0.007	3.5*	2.81	Significant
Position	0.40	3	0.133	65.0**	2.81	Significant
Position x variety	0.04	9	0.004	2.0	2.11	Not significant.
Error	0.11	48	0.002			

Mean tannin content of varieties

Variety	Mean.
Neypoovan	0.708
Ennabaniyan	0.715
Pachanadan	0.751
GrosMichel	0.725

C.D.(0.05 level)=0.032

Mean tannin content in different positions

Position	Mean.
I	0.85

Appendix B

Analysis of variance of data in Table I (Tannin content in seven months old plantain dry weight basis)

Source	Sum of squares.	d.f.	Variance	'F'ratio calculated(0.05 level).	'F' ratio from Tables (0.05 level).	Whether significant or not
Total	0.380	63				
Variety (V)	0.005	3	0.0017	0.7	2.81	Not significant.
Positition (P)	0.250	3	0.0800	32.0**	2.81	Significant
P x V	0.005	9	0.0006	0.24	2.11	Not significant.
Error	0.120	48	0.0025			

Mean tannin content in different positions

Positions	Mean
I	0.83
II	0.75
III	0.70
IV	0.66

Appendix C

Analysis of variance of data in Table I (Tannin content in five months old plants on fresh weight basis

Source	S.S.	d.f.	V	'F' ratio	'F' ratio from table	Whether significant or not
Total	0.011	63				
Variety (V)	0.002	3	0.0007	8.75**	2.81	Significant
Position (P)	0.004	3	0.0013	16.25**	2.81	Significant
P x V	0.001	9	0.0001	1.25	2.11	Not significant.
Error	0.004	48	0.00008			

Mean tannin content varieties

Variety	Mean
Neypoovan	0.153
Ennabaniyan	0.152
Pachanadan	0.165
GrosMichel	0.160

C.D.(0.05 level)=0.0064

Mean tannin content in different positions

Position	Mean.
I	0.169
II	0.160
III	0.155
IV	0.147

C.D. (0.05 level) = 0.0064

Appendix D

Analysis of variance of data in Table I (Tannin content in seven months old plants on fresh weight basis)

Source	S.S.	d.f.	V	'F' ratio	'F' ratio from table	Whether significant or not
Total	0.0032	63				
Variety (V)	0.0004	3	0.00013	6.5**	2.81	Significant
Position (P)	0.0015	3	0.00050	25.0**	2.81	Significant
P x V	0.0003	9	0.00003	1.5	2.11	Not significant.
Error	0.0010	48	0.00002			

Mean tannin content of varieties

Variety	Mean.
Neypoovan	0.170
Ennabaniyan	0.165
Pachanadan	0.171
GrosMichel	0.166

C.D. (0.05 level) = 0.0032

Mean tannin content in different positions

Positions	Mean.
I	0.174
II	0.171
III	0.167
IV	0.161

C.D. (0.05 level) = 0.0032

Appendix E

Analysis of variance of data in Table III (C/N ratio)

Source	S.S.	d.f.	Variance	'F'ratio calculated.	'F' ratio from tables (0.05 level)	Whether significant or not
Total	1225.18	23				
Variety (V)	2.30	1	2.30	1.90	4.49	Not significant.
Position (P)	1199.93	3	399.98	336.60**	3.24	Significant.
P x V	3.87	3	1.29	1.09	3.24	Not significant.
Error	19.08	16	1.19			

Mean C/N ratios of positions

Positions	Mean.
I	19.28
II	24.51
III	36.19

C.D. (0.05 level) = 1.15