VASCULAR STREAK DIEBACK OF COCOA AND ITS MANAGEMENT

By
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THESIS

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DECLARATION

I hereby declare that the thesis entitled Vascular streak dieback of cocoa and it s management is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, fellowship associateship or other similar title of any other university or society

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We the undersigned members of the Advisory Committee of **Mr Ajay Kumar, K M** a candidate for the degree of **Master of Science in Agriculture** with major in **Plant Pathology**, agree that the thesis entitled **Vascular streak dieback of cocoa and it's management** may be submitted by Mr Ajay Kumar, K M in partial fulfilment of the requirement for the degree

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Introduction

INTRODUCTION

Cocoa (*Theobromae cacao* L) one of the important beverage crops belonging to the family Sterculiaceae is a native of rainforests of tropical America Cocoa is widely cultivated in many South American African and South East Asian countries where the agroclimatic conditions are ideal for its growth In India commercial cultivation of cocoa was started in 1960 s Kerala is the principal cocoa growing state of India accounting for 80 per cent of the total area followed by Karnataka and Tamil Nadu The area under cocoa in Kerala is about 15 000 hectares with annual production of 8 300 tonnes

Diseases affecting cocoa act as an important factor responsible for the low yield of this crop in Kerala Among the diseases vascular streak dieback (VSD) has now become a devastating one This disease was first recorded from Papua New Guinea and it was shown by Keane *et al* (1972) and Prior (1978) to be caused by a new genus and species of fungus which was given the name *Oncobasidium theobromae* (Talbot and Keane 1971) Since then the occurrence of the disease has been reported from many South East Asian countries

In India, first record of this disease was made by Abraham (1981) and Chandramohanan and Kavaeriappa (1982) Abraham and Ravi (1991) after conduct ing a detailed survey observed that this disease was present in almost all districts of Kerala except Thrissur and Palakkad However recent survey indicated that the disease is spreading at an alarming rate in all the cocoa gardens of Kerala including Thrissur and Palakkad districts

In general the pathogen *O theobromae* grows very slowly in culture and has not so far been reported to sporulate in axenic culture Because of this difficulty screening for resistance was done by exposing the test plants to natural inoculum in the field Further the pathogen has got a long incubation period According to Keane and Prior (1992), *O theobromae* is a highly specialised near obligate parasite of cocoa and is the only known wind borne leaf penetrating basidiomycete vascular pathogen

This disease can be easily identified by its characteristic symptoms like yellowing of leaves with green islets defoliation enlargement of lenticels on the stem production of sporophores on the fallen leaf scar vascular streaking and finally the death of the infected parts Since the disease causes considerable damage to the grown up plants and is found to be lethal in seedlings a better understanding of the disease is essential for proper monitoring and management of the disease In view of the serious nature of the disease in Kerala and also a potential threat to cocoa growing areas of neighbouring states the present investigation was carried out with the following objectives

1 Standardisation of isolation techniques using different plant parts and media
2 Identification of the causal organism based on morphological characters
3 Critical study of the symptomatology of the disease
4 Histopathological studies of the infected stem
5 Studies on the transmission of the pathogen through vegetative propagation and seeds
6 Evaluation of cocoa types for host resistance/tolerance against VSD
7 Effect of fungicides in preventing the incidence of VSD

Review of Literature

REVIEW OF LITERATURE

A destructive dieback disease of cocoa distinguishable from other types of dieback induced by environmental factors and insect attack was recognised in Papua New Guinea in the early 1960 by Shaw (1962) and Bridgland *et al* (1966a 1966b 1967) This disease was referred as "vascular streak dieback" (VSD) to distinguish it from other types of dieback by Keane *et al* (1972)

Keane *et al* (1972) established the causal organism of the disease as a new genus and species of fungus and was given the name *Oncobasidium theobromae* (Talbot and Keane 1971)

Subsequently the disease was reported from West Malaysia (Keane and Turner 1971) Sabah (Liu and Liew 1975) Indonesia (Turner and Shepheard 1978) Hainan Island of China (Turner and Keane 1982) Sarawak (Tiong and Kueh 1984) Burma (Lai 1985) and Southern Thailand (Keane and Prior 1992)

In India Abraham (1981) and subsequently Chandramohan and Kaveriappa (1982) reported the occurrence of VSD of cocoa from Kottayam district of Kerala Abraham and Ravi (1991) reported that the maximum occurrence of the disease was in Kottayam district followed by Ernakulam Thiruvananthapuram Kozhikkode Idukky and Pathanamthitta districts of Kerala They reported that this disease was not noticed in Thrissur and Palakkad districts However further disease survey conducted during 1993 revealed the incidence of the disease in Thrissur district of Kerala (KAU 1993)

Byrne (1976) reported 25 50 per cent yield loss in certain regions of Papua New Guinea due to VSD According to Keane (1981) the disease was found to be most damaging in seedlings less than 10 months old Tan (1982) showed a significant negative correlation between yield and incidence of VSD in Papua New Guinea Keane and Prior (1992) observed that the disease was causing yield loss upto 80 per cent in young plantings Varghese *et al* (1992) reported about 70 per cent mortality in immature plants of cocoa due to VSD

2 1 Isolation and pure culturing of the pathogen

Isolation of the VSD pathogen of cocoa has been attempted from infected plants by planting cut pieces of stem tissue in Water agar medium (Keane *et al* 1972 Prior 1977) A new technique giving a higher percentage of frequency of isolation of VSD fungus was developed by planting sections of lamina containing midrib of leaves exhibiting the symptoms on Water agar medium (Varghese *et al* 1981)

In general the fungus grows very slowly in culture medium and has not been reported to sporulate in axenic culture Keane *et al* (1972) reported that sub cultured mycelium grew fastest in Sweet potato sucrose agar but the growth was much slower compared to that from diseased cocoa wood The fungus showed growth in dual culture with cocoa callus tissue (Prior 1977 Varghese *et al* 1981) Prior (1980) remarked that growth in culture was slow erratic and inadequate for any cultural studies Varghese *et al* (1981) studied the growth of *Oncobasidium theobromae* on Corn meal agar Czapek Dox agar Water agar Lima bean agar Nutrient agar and Corticium culture medium They observed maximum growth of

the fungus in Corticium culture medium followed by Czapek Dox agar Water agar Potato dextrose agar Corn meal agar Lima bean agar and Nutrient agar in descending order

Attempts to induce sporulation in culture were unsuccessful except in one instance when the fungus was grown with cocoa callus for 3 6 weeks and then exposed to open sky during night for 3 5 days which yielded few spores (Prior 1982) Musa (1983) recommended coconut water as culture medium for *Oncobasidium* Lam *et al* (1988) was successful in inducing sporulation and basidiospore production *in vitro* by growing the fungus initially on Corticium culture medium and then on Water agar by incubating at 25 $\pm$ 2°C for 2 weeks followed by passage of saturated moist air with relative humidity above 96 per cent However, there were no reports on the production of disease by using the spores produced artificially

2 2 Pathogenicity test

Keane *et al* (1972) reported that symptoms developed in six months old cocoa seedlings after an incubation period of two to three months by shedding the basidiospores of the pathogen collected from the naturally infected plants on very young seedlings But they were not successful in developing the disease symptoms by inoculating the spores or the mycelium of the fungus into young seedlings

Prior (1978) succeeded in developing the symptoms of the disease on seedlings by inoculating basidiospores collected from the naturally occurring sporophores of the fungus Keane (1981) stated that in the absence of easy sporulation of the fungus in culture it was necessary to inoculate with spores

produced by sporophores from infected plants He was able to produce symptoms of the disease on seedlings after an incubation period of 3-4 months

2 3 Causal organism

Talbot and Keane (1971) were the first to describe the pathogen causing VSD of cocoa as a new genus and species of Basidomycotina (Tulasnellales Ceratobasidiaceae) and named the fungus as *Oncobasidium theobromae* Talbot and Keane They described the fungus collected from Papua New Guinea fruit bodies white membraneous to subhypochnoid occurring as small effused adherent patches on leaf scars and adjoining stem of cocoa with mycelium emerging from xylem vessels Basal hyphae thin walled but firm hylaine to yellowish smooth not encrusted without clamp connections with prominent dolipores more or less horizontal and upto 10 μm wide long celled (upto 200 μm) branching at a wide angle ascending hyphae narrower upto 5 6 μm diameter shorter celled hyaline binucleate Cystidiate structures absent Basidia arising from clustered moniloid hyphae holobasidiate at first often broad obovate later elongating and becoming capitate-clavate (rarely sub cylindrical) with a subcylindrical base 6-8 μm wide and a more or less abruptly inflated apex (10-) 12 16 μm wide the whole metabasidium (18 23)26-36 μm long Sterigmata constantly 4 stout conical straight or curved 6-12 μm long upto 4 μm wide at the base Basidiospores repetative smooth hyaline thin walled not amyloid, often multiguttulate broad ellipsoid with one side flattened (12)15 25 x (5)6 5 8 5 μm conidal and sclerotial states not known to occur

The morphological characters of VSD pathogen occurring in Malayasia and Indonesia were studied by many workers (Zainal Abidin 1982 Lam *et al*

1988 and Pawirosoemardjo *et al* 1990) They reported similar morphological characters as described Talbot and Keane (1971) from Papua New Guinea Keane and Prior (1992) reported that basidia collapsed immediately after the shedding of spores because of which only very few basidia were visible

2 4 Symptomatology

Symptomatology of VSD was first described by Keane *et al* (1972) from Papua New Guinea VSD had very characteristic symptoms which were similar whether the disease occurred on the main stem of a seedling or on a branch of an older tree The first symptom was chlorosis of one leaf usually second or third flush from the tip The pattern of chlorosis was very distinctive with islets of tissue remaining green and by 2 3 days the chlorotic leaf fall down and subsequently the leaves above and below it turn chlorotic in the same way and are shed in a distinctive pattern where the youngest and oldest leaves are intact while all the middle leaves fall down Enlarged lenticels were seen on the stem immediately below the petiole of the infected leaf causing roughening of the bark Leaf scars resulting from the fall of diseased leaves showed three blackened spots when the dry surface was scrapped off

The disease spread to the lateral branches particularly those formed by the growth of axillary buds on the diseased stem and on such branches the leaves turned chlorotic and dropped off in succession from the base Leaves in the latest flush of the diseased seedlings or branch often showed interveinal necrosis (oak leaf pattern) characteristic of calcium deficiency Eventually leaf fall occurred right to the growing tip followed by the death of the branch From such a branch the disease spread to other branches or the trunk and finally killing the whole tree

Leaf scars resulting from the fall of chlorotic leaves were sometimes covered by a white effused adherent fruiting bodies of the fungus These fruiting bodies were found only on the leaf scar and adjacent bark When the bark of the diseased stem was peeled the cambium seen turned brown abnormally fast and the underlying xylem were discoloured by brown streaks Similarly when the infected stem was split into two brown streaks were readily seen

Symptomatology of the disease from Papua New Guinea India Malayasia and Indonesia were described by many workers (Prior 1980 Abraham 1981 Zainal Abidin 1982 and Abraham and Ravi 1991 KAU 1995)

Prior (1980) reported that maximum disease occurrence was seen 3 5 months after seasonal rainfall Zainal Abidin *et al* (1981) suggested that there were different isolates of the fungus present in Papua New Guinea and Malaysia but similarity of morphology of Malayasia isolate to *O theobromae* showed that both fungi belong to same genus Varghese *et al* (1981) reported some difference in symptom of VSD occurring in Malaysia Wood and Lass (1985) noted that in Malaysia occurrence of intervenal necrosis was rather more common compared to yellow leaves with green islets as that in Papua New Guinea

Studies conducted at Cadbury KAU Cocoa Research Project also revealed some variation in symptoms of VSD from those reported from Papua New Guinea (KAU 1995)

2 5 Histopathology

Keane *et al* (1972) observed the presence of hyphae of *Oncobasidium theobromae* in the xylem vessels of main vein lateral viens and petiole of infected leaf Prior (1985) reported the presence of tyloses and deposition of brown phenolic material in the infected vessels of cocoa stem

Microscopic examination of longitudinal sections of the xylem vessels of the split infected branches revealed that the fungus was present in at least 50 per cent of xylem vessels of the major fan branches and extended right through the trunks and down to the main tap roots (Dennis and Keane 1992)

2 6 Transmission studies

Keane (1972) showed that infection of *Oncobasium theobromae* passes through the approach grafts and no successful establishment of the grafts Chan and Syed (1976) reported that cuttings and all bud patches taken from infected branches failed to establish in healthy root stock

There was no evidence that the disease was transmitted through seed (Chan and Syed 1976) Prior (1985) traced hyphae of the fungus to the placenta of few pods growing on infected branches but could not find hyphae in seeds or any transmission of the disease through seeds Keane and Prior (1992) germinated 2000 seeds from pods formed on infected branches but did not see any transmission of the disease

Studies conducted at the Cadbury KAU Cooperative Cocoa Research project revealed that buds and grafts taken from infected plants failed to establish (KAU 1993)

2 7 Screening for host resistance

In the preliminary screening trial conducted at Papua New Guinea wide range of field resistance was seen The Trinitario clones originated from natural hybridisation of criollo and forestro types and clonal selections (designated as KA or K) included some very susceptible and other very resistant types against VSD (DASF 1963 1967)

In Malaysia Amelonado cocoa was found more susceptible than upper Amazon cocoa to VSD (Keane and Turner 1971 Chan and Lee 1973 Chan and Syed 1976)

Chan and Syed (1976) observed that hybrid trees remained relatively free from infection in infected cocoa fields Prior (1978) reported that the main approach to control the disease was by propagation and planting of resistant cocoa clones or hybrid seedlings selected from the survivors of the epidemic occurred in Papua New Guinea He also reported that the cultivars of upper Amazon origin PA 7 appeared susceptible while KA 2 101 appeared to be especially resistant and was used as a standard in resistant trial Another report states that some of the Amelonado trees had some resistance to VSD (DPT 1984)

Tan and Tan (1987) reported that the inheritance of VSD resistance was stable and polygenically controlled

In Indonesia all widely planted cocoa types were at least moderately susceptible to VSD (Pawirosoemardjo *et al* 1990)

In India Abraham and Ravi (1991) reported that maximum percentage of incidence of VSD was noticed in cocoa types V 15/5 followed by V 4/8 and V 10/3 and minimum in type V 5/9 and G II 20/4

Keane and Prior (1992) stated that in South East Asia and Malaysia an important criteria in cocoa selection has been resistance to VSD Because of difficulty of artificial inoculation most resistance screening had involved exposure of the test plants to natural inoculum in the field They further stated that the exact mechanism of resistance to VSD was not understood and there was no detailed studies on the virulence of different isolates of the pathogen however there was strong evidence that this resistance was horizontal

2 8 Chemical control of disease

According to Prior (1980) protective fungicides were unlikely to be effective in controlling VSD of cocoa in Papua New Guinea However Keane and Prior (1992) suggested that systemic fungicides should have particular value for the control of the disease Inspite of the obvious ineffectiveness of both protectants and systemic fungicides in the control of VSD many studies were carried out to control the disease with fungicides

Zainal Abidin (1982) observed that bitertanol at 1500 ppm gave signifi cant reduction of VSD Chung (1983) also reported the effectiveness of bitertanol in controling the disease

In vitro screening studies with bitertanol triadimefon triadimenol propiconazole pp 969 (all belonging to triazole group) and benomyl showed

fungitoxicity against *Oncobasidium* (Donough 1984 Varghese *et al* 1985) Musa and Tey (1986) reported total inhibition of mycelial growth of *Oncobasidium* by benomyl at 5 ppm

Gurmit (1986) observed more than 90 per cent control of VSD in seedlings with soil drenching of triadimefon at monthly intervals From Papua New Guinea Prior (1985) reported the effectiveness of propiconazole as spray and stem painting in reducing VSD incidence in cocoa seedlings Sindhu (1987) reported that foliar spraying of traidimenol and PP 969 at weekly and fortnightly intervals provided seedling protection against VSD in nursery

Holderness (1990) noticed that systemic fungicides viz tebucanozole hexaconazole and triadimenol when given as monthly foliar spray gave good protection against VSD of cocoa but they resulted in stunting of seedlings

Abraham and Ravi (1991) reported that Bordeaux mixture and Kitazin as foliar spray were effective in checking the severity of VSD disease to a certain extent in older plants

Hee *et al* (1992) reported that monthly soil drenching of flutriafol at 25 200 mg ai per plant controlled VSD and seedlings were found vegetatively more vigorous compared to untreated plants Foliar spray of flutriafol at 200 ppm and triadimefon at 500 ppm also gave good disease control

Materials and Methods

MATERIALS AND METHODS

3 1 Location of the experiment

The field experiments were conducted at the cocoa field of Cadbury KAU Cooperative Cocoa Research Project Observations on the evaluation of host resistance against vascular streak dieback were taken from the cocoa types planted in the Kalaketty area of Kottayam district and Muttom and Vazhithala area of Idukki district Laboratory experiments were carried out at the Department of Plant Pathology College of Horticulture Vellanikkara Thrissur The above experiments were carried out during the year 1994 to 1996

3 2 Standardisation of isolation techniques

This experiment was conducted in order to find out a suitable medium and plant part to be used for easy and successful isolation of the pathogen The following different media were used to select the best medium for isolating the pathogen causing vascular streak dieback (VSD) of cocoa

1) Sweet potato sucrose agar
2) Czapek Dox agar
3) Nutrient agar
4) Potato peptone agar
5) Richards agar
6) Corticium culture medium
7) Potato dextrose agar

8) Water agar

9) Oat meal agar

10) Host extract dextrose agar

11) Woody plant medium

The composition of the above media are given in Appendix I

Five different plant parts were used for isolation of the pathogen viz hard stem tender stem petiole midrib and vein

Severely infected small twigs of cocoa were brought to the laboratory and washed in tap water The different category of plant parts mentioned above were cut into small bits of size 5 mm surface sterilised with 0 1 per cent mercuric chloride solution for one minute and washed in three changes of sterile water These bits were then planted in sterile petridishes previously poured with the different media and incubated at room temperature Ten replications were maintained for each medium and each plant part When the growth of fungus was visible the mycelial bits were examined under the microscope and those mycelial bits resembling the pathogen *Oncobasidium theobromae* were transferred to slants of Potato dextrose agar and Corticium culture medium and incubated at room temperature

Observations on the per cent success of isolation of the pathogen with each medium and plant part were taken Observations on the per cent contamination during the study were also recorded Attempts were made to identify the contaminants based on their morphological characters

Another study was carried out in which the bark of the infected hard stem was removed longitudinally with a sterilised blade Such bark removed hard

stem pieces were cut into pieces of 5 mm size by a sterilised blade and directly placed in the sterile petridishes previously poured with the *Corticium* culture medium and water agar medium Bark retained hard stem bits served as control Twenty replications were maintained and dishes were incubated at room temperature Observations on per cent success was recorded after five days

Preparation of cocoa callus

Usefullness of cocoa callus for the isolation of the pathogen from different plant parts mentioned earlier was also studied

For preparing cocoa callus 6 8 cm long shoots with hardened leaves were collected from the fan branches The leaves were trimmed and the shoots were washed thoroughly in tap water drained using blotting paper and the dried shoots were swabbed with cotton dipped in 70 per cent alcohol In the laminar air flow cabinet these shoots were segmented into explants Leaf bits about 0 5 cm^2 or inter nodal segments of 0 5 1 cm long were used as explants These were then surface sterilised in freshly prepared chlorine water for 3 minutes followed by 2 3 washings with sterile distilled water These explants were allowed to dry for 10-15 minutes in sterilised blotting paper contained in a petri dish and then cultured in MS/WPM containing any auxin @ 1 2 mg l^{-1}

3 3 Pathogenicity

An attempt was made to prove the pathogenicity of the fungus from the isolated mycelium The mycelium of the fungus resembling *O theobromae* obtained in the study was inoculated on young seedlings For inoculation mycelial discs of size 5 mm were placed on the upper surface of young leaves and also on the axils of

leaves with and without pm pricks and covered with moist cotton The inoculated plants were kept under high humid conditions and observed for the development of the disease

3 4 Growth of the pathogen on different media

Media which showed promising results in the isolation studies were selected for this study viz Corticium culture medium Poatato dextrose agar and Water agar

It is well established that the pathogen causing VSD of cocoa is a near obligate parasite which required nutrients from the original piece of xylem even after subculturing (Keane and Prior 1992) It was observed that subculturing of the pathogen immediately after isolation resulted in poor growth Hence for this study infected plant part as such was used as the inoculum

Since isolation from bark removed stem yielded dense and fast growth of the pathogen such bits were used in the study These bark removed stem bits were cut into pieces of 5 mm size and placed directly at the centre of the medium Sufficient replications were maintained for each medium The inoculated dishes were incubated at room temperature Observations on the growth of the fungus were taken daily for a period of 10 days

3 5 Morphological characters of the pathogen

Morphological characters of the pathogen were studied by microscopic examination of the sporophore occurring naturally on the infected trees and also by examining the growth of the fungus obtained during the isolation

The sporophores were collected during early hours of the day and brought to the laboratory From the sporophore observations on the morphological characters such as length and breadth of moniloid hyphae basidia sterigmata and spores were recorded Similarly length and breadth of hyphae and moniloid hyphae from the culture were also recorded These observations were taken using Olympus research microscope and measurements were taken after calibrating with micrometer Microscopic drawings of the fungus were done using a camera lucida Photomicrographs of the pathogen were also taken

3 6 Symptomatology

Symptomatology of the vascular streak dieback occurring in Kerala was studied under natural conditions by periodic close examination of severely infected trees and seedlings in different cocoa gardens of Kerala

3 7 Histopathology

Histopathological changes due to vascular streak dieback disease were studied by taking transverse and longitudinal sections of both healthy and infected stems of cocoa Sections of 25 μm thickness were taken by means of a sledge microtome and stained with safranin for few seconds The stained sections were examined under the microscope for studying the histopathological changes Photomicrographs of these sections were also taken

3 8 Studies on the transmission of vascular streak dieback

This experiment was conducted to study whether the disease was trans mitted by grafting and budding or through seeds

3 8 1 Transmission of the disease through grafting and budding

Two types of grafting viz side and wedge grafting and five types of budding methods were carried out to study the transmission of the disease The various budding methods used were as follows

1) Patch budding patch of 40 x 10 mm size
2) Patch budding patch of 20 x 5 mm size
3) Flap budding
4) T budding
5) Inverted T budding

Six months old healthy cocoa seedlings served as the rootstock For grafting scion showing severe incidence of the disease was used Similarly for budding severely infected twigs served as budwood

This experiment was conducted in January 1995 and in October 1995 with 30 and 25 plants in each treatment respectively The grafted and budded plants were kept in shade and observed for a period of six months for the development of the disease After six months the bud near the grafted/budded area was removed and observed for the presence of vascular streaking as an indication for the transmission of the disease

3 8 2 Transmission of the disease through seeds

For this study 500 seeds obtained from severely infected plants were sown in polythene bags containing standard potting mixture The germination

percentage was recorded after 15 days and further observation on the incidence of the disease was taken at periodic intervals upto a period of five months

3 8 2 1 Effect of fungicide as a precaution against possible transmission of vascular streak dieback through seeds

In this experiment seed treatment with six fungicides was done as precaution against possible transmission of disease through seeds The following fungicides were used for the study

Fungicide	Concentration
Bavistin	0 1%
Kitazin	0 1%
Calixin	0 1%
Contaf	0 1%
Bayleton	0 08%
Aureofungin	0 02%
Control	

Fifty seeds from pods of infected plants were soaked in each fungicide solution for 10 minutes Seeds dipped in water served as control After the treatment the seeds were sown in polythene bags containing standard potting mixture The germination percentage was recorded 15 days after sowing Further observations on height of seedlings number of leaves and incidence of the disease were recorded after first and fifth month of sowing

3 9 Evaluation of cocoa types for their resistance/tolerance against vascular streak dieback

The promising cocoa types selected from the ongoing breeding programmes of Cadbury KAU Cooperative Cocoa Research Project were planted as seed gardens at farmers field at Vazhithala and Muttam areas of Idukki district and Kalaketty area of Kottayam district These cocoa types were evaluated for their resistance/tolerance against the disease The details of cocoa types are given below

Cocoa types at Vazhithala (planted in 1988)

Cocoa types	Total number of plants
1) GI 15 5	3
2) GI 10 3	7
3) GI 5 9	6
4) GI 4 8	6
5) GII 20 4	6
6) GII 19 5	7
7) M 16 9	7
8) M 9 16	7

Cocoa types at Muttom (planted in 1990)

Cocoa types	Total number of plants
1) M 9 16	9
2) M 16 9	12
3) M 13 12	27
4) GI 4 8	2

5) GI 5 9	3
6) GI 9 6	21
7) GI 10 3	2
8) GI 15 5	9
9) GI 12 3	9
10) GII 19 5	7
11) GII 20 4	7
12) GIII 1 2	4
13) GIV 4 1	7
14) GIV 2 5	9
15) GIV 18 5	13
16) GIV 32 5	15
17) GVI 50	57
18) GVI 51	7
19) GVI 54	6
20) GVI 55	6
21) GVI 56	5
22) GVI 59	26
23) GVI 60	14
24) GVI 64	4

Cocoa type at Kalaketty (planted in 1991)

Cocoa type	Total number of plants
1) M 16 9	116
2) GI 15 5	92

Disease scale	Intensity of infection
0	No infection
1	< 25 per cent twig infected
3	> 25 to < 50 per cent twig infected
5	> 50 to 75 per cent twig infected
7	> 75 per cent twig infected
9	Mortality of the plant

All plants of different cocoa types planted at Vazhithala and Muttom were individually observed for recording the disease score Due to the large number of population in each cocoa types at Kalaketty only 10 plants of each types except M 13 12 were selected at random and the disease score calculated

Further observations on the incidence and severity of the disease of 122 collection of vegetatively propagated cocoa types in germplasm VI of Cadbury KAU Co operative Cocoa Project were also recorded during October 1995

3 10 Effect of fungicides in preventing the incidence of vascular streak dieback

The comparative efficacy of five fungicides in preventing the incidence of VSD in cocoa seedlings were tested by a pot culture experiment during June 1995 to March 1996 The details of the experiment are given below

Design	CRD
Replications	26
Treatments	6

Treatments	Fungicides	Concentration
T_1	Bordeaux mixture	1%
T_2	Indofil M-45	0 3%
T_3	Calixin	0 1%
T_4	Kitazin	0 2%
T_5	Bavistin	0 1%
T_6	Control	

Forty five days old healthy cocoa seedlings raised in standard potting mixture in polythene bags were kept under severely infected cocoa plants so as to expose them to natural infection during the onset of monsoon in June 1995 The fungicide treatments were given as spray at 15 days interval The first spraying was given immediately after the plants were kept under severely infected field The fungicide treatments were given till November 1995 (till the end of monsoon period) Observations on the incidence of the disease were recorded on the middle and end of each month till February 1996

Results and Discussion

RESULTS AND DISCUSSION

Vascular streak dieback (VSD) a destructive disease of cocoa now assumes alarming proportion in the different cocoa growing tracts of Kerala VSD affect all age group of plants and is found to be lethel in seedlings In view of the serious threat of the disease to cultivation of cocoa the present investigation was carried out to study etiology symptomatology histopathology and transmission of the disease and to evolve a viable management practice

4 1 Standardisation of isolation techniques

It is well known that VSD pathogen grows poorly on artificial media due to its near obligate parasitic nature Efforts were made to select the best suited medium for the isolation and also to find out the part of the plant to be used for getting maximum success in the isolation Isolation of the fungus was tried from hard stem tender stem petiole midrib and viens of the infected plants on eleven different media The results are presented in Table 1

The data revealed that the maximum percentage of successful isolation of the pathogen was obtained when petiole and midrib of the infected leaves were used followed by tender stem hard stem and veins of leaves

Among the different media used for isolation Corticium culture agar gave the maximum percentage of success of isolation closely followed by Water agar and Potato dextrose agar Success in isolation with Richards agar and Host extract agar were meagre while media like Sweet potato sucrose agar Czapek dox agar Nutrient agar, Potato peptone agar Oat meal agar Woody plant medium did

Table 1 Comparison of media and plant parts for successful isolation of vascular streak dieback pathogen (*O theobromae*

Medium		Percentage of successful isolation					
		Hard stem	Tender stem	Petiole	Midribe	Veins	Total
1	Sweet potato sucrose agar	0	0	0	0	0	0
2	Czapek dox agar	0	0	0	0	0	0
3	Nutrient agar	0	0	0	0	0	0
4	Potato peptone agar	0	0	0	0	0	0
5	Richards agar	0	0	10	10	0	4
6	Corticium culture medium	30	90	90	70	30	62
7	Potato dextrose agar	20	50	70	80	40	52
8	Water agar	30	80	80	90	10	58
9	Oat meal agar	0	0	0	0	0	0
10	Host extract agar	10	0	0	0	0	2
11	Woody plant medium	0	0	0	0	0	0
	Total	8 18	20 00	22 73	22 73	7 27	

not help in the isolation of the pathogen Results of the present study are in confirmity with the findings of Varghese *et al* (1981) They reported higher frequency of successful isolation of VSD pathogen from parts of infected leaves compared to stem pieces Further usefulness of Corticium culture medium followed by Czapek dox agar Nutrient agar Potato dextrose agar and Water agar for the satisfactory growth of the pathogen was also noticed by them However in the present study Czapek dox agar did not help in the isolation

It was also noticed that when Corticium culture medium Potato dextrose agar and Water agar were used for the isolation there was success in isolation with all the plant parts tried with varying percentages In Corticium culture medium maximum percentage success of isolation was noticed when petiole and tender stem were used In Potato dextrose agar and Water agar maximum success of isolation was observed by using midrib of infected leaves

Thus the present study indicated the efficiency of Corticium culture medium Water agar and Potato dextrose agar for the isolation of this near obligate fungal pathogen Further for easy isolation of the pathogen infected plant parts like midrib of leaves as well as tender stem could be used

During the isolation of the pathogen lot of contaminants associated with the plant parts were seen on the media Data on the extent of contamination are given in Table 2a and 2b It was noticed that the maximum contamination was by *Fusarium* sp followed by *Colletotrichum gloeosporioides* bacteria and other unidentified fungi Among the media tried the maximum contamination was recorded in the Potato peptone agar followed by Czapek dox agar Host extract dextrose agar Nutrient agar Sweet potato sucrose agar Woody plant medium and

Table 2a Percentage frequency of contamination in different media during the isolation of *O theobromae*

Medium	*Fusarium sp*	*Colletotrichum gloeosporioides*	Bacteria	Other unidentified fungi	Total
1 Sweet potato sucrose agar	28	40	4	10	82
2 Czapek Dox agar	74	22		2	98
3 Nutrient agar	46	12	20	6	84
4 Potato peptone agar	75	14	10		99
5 Richards agar	24	36			60
6 Corticium culture medium	10			4	14
7 Potato dextrose agar	24	2	4		30
8 Water agar	18				18
9 Oats meal agar	20	30	2	10	62
10 Host extract dextrose agar	78	4		6	88
11 Woody plant medium	44	10	20		74
Total	40 1	15 5	5 5	3 5	

Table 2b Percentage frequency of contamination from different parts of infected plants during the isolation of *O theobromae*

Plant part used	*Fusarium* Sp	*Colletotrichum gloeosporioides*	Bacteria	Other unidentified fungi	Total
1 Hard stem	70	10	10	1	91
2 Tender stem	49 1	20	10	2 7	82
3 Petiole	35	21 02	6 13	7 2	69 4
4 Midrib	31 4	23 2	1 12	4 24	60
5 Veins	15	3 03		2 1	20 1
Total	40 1	15 5	5 5	3 5	65

Oat meal agar In general media efficient for the isolation of VSD pathogen had less contamination percentage In all the media tried *Fusarium* sp emerged as the major contaminant except in Sweet potato sucrose agar Richards agar and Oat meal agar where *C gloeosporioides* dominated Maximum bacterial contamination was noticed in Nutrient agar and Woody plant medium followed by Potato peptone agar

Among the different plant parts used for isolation maximum contamination occurred on using hard stem followed by tender stem petiole midrib and secondary veins (Table 2b)

It is well established that contaminants interferes in the isolation of plant pathogens Reports of contamination during isolation of VSD pathogen especially by *Fusarium* sp *Phomopsis* and *Botryodiplodia theobromae* were there and these were assumed to be common inhabitants of outer layer of cocoa plants (Keane *et al* 1972) Further they reported that *Fusarium* had some association with dried bark by forming sprodochia in the enlarged lenticels Varghese *et al* (1981) also reported the interference of contaminants in the isolation of VSD In the present study in addition to *Fusasium* sp *C gloeosporioides* the pathogen causing pod rot and leaf disease in cocoa was invariably observed as contaminant It can be presumed that the major contaminants like *Fusasium* and *C gloeosporioides* might have either saprophytic or pathogenic association with the bark of cocoa plants

It was observed that the pathogen isolated from stem cuttings had dense and rapid growth while those from petiole midrib and veins were very sparse and slow But it was observed that the success of isolation using stem parts was less than that from leaves (Table 2b) due to higher surface contaminants probably from the outer bark of the infected stem Hence inorder to improve the success in isolation a

new method of isolation was tried where the bark of the stem was removed as mentioned in the Materials and Methods

From the data presented in Table 3 it was seen that the percentage success of isolation by removing the bark of stem was statistically significant compared to bark retained stem on both water agar as well as on Corticium culture medium

In Corticium culture medium successful isolation by removing the bark was 90 per cent while by retaining the bark it was only 20 per cent Similarly in Water agar bark removed stem pieces recorded 85 per cent isolation success whereas bark retained stem showed 30 per cent

Thus the study revealed that this method of isolation of VSD pathogen could be effectively utilized for routine laboratory isolation of the pathogen as it gave satisfactory growth of the fungus as well as fewer problem of contamination

Isolation on cocoa callus

Of the different plant parts used for isolation in cocoa callus none of them gave success This was found to be due to high fungal contamination mainly by *Fusarium* sp and *C gloeosporioides* However success in growth in dual culture with cocoa callus tissue was reported by Prior (1977) and Varghese *et al* (1981)

4 2 Pathogenicity

Attempts to prove the pathogenicity by inoculating young cocoa seedling with mycelial bits was made as described in Materials and Methods None of the inoculated seedlings showed disease even after six months indicating that mycelium

Table 3 Comparison of successful isolation of *O theobromae* by retention and removal of bark of stem

Method of isolation	Corticium culture medium		Water agar	
	Number of successful isolation	Number of unsuccessful isolation	Number of successful isolation	Number of unsuccessful isolation
Stem bark retained	4(20)	16(80)	6(30)	14(70)
Stem bark removed	18(90)	2(10)	17(85)	3(15)
	X^2 = 19 7979794**		X^2 = 12 3785162**	

Figures in parenthesis refers to values on percentage bases

** Significant at 1% level

of the fungus was incapable of causing infection This observation is in confirmity with that of Keane *et al* (1972) Further during the present study no spore production was noticed in culture Hence inoculation with spores was not attempted

4 3 Growth of the pathogen on different media

Growth of the pathogen on three different media viz Corticium culture agar Potato dextrose agar and Water agar was studied to select a suitable medium which support good growth of the fungus as already mentioned These media were selected based on the success of isolation of the pathogen in the previous study and the results are presented Table 4

(1) Water agar medium

The fungus showed slow irregular radial growth Aerial mycelium was sparse except in the centre Mycelium on the surface of media was white in colour while the aerial mycelium produced towards the centre was slightly yellowish

(2) Corticium culture medium

The radial growth which was faster initially slowed down after six days Aerial mycelium was profuse yellowish and fluffy in nature and continued the growth in a petridish even after 240 h

(3) Potato dextrose agar medium

The radial growth was fast Aerial mycelium was less profuse when compared to Corticium culture medium yellowish fluffy in nature and completed the growth m a petridish with in 216 h

Table 4 Growth of vascular streak dieback pathogen on different media

Sl No	Medium	Colony diameter (cm) after									
		24 h	48 h	72 h	96 h	120 h	144 h	168 h	192 h	216 h	240 h
1	*Corticium* culture medium		1 4	2 2	2 9	3 2	4 1	5 5	6 0	7 2	8 3
2	Potato dextrose agar		1 2	2 5	2 8	3 0	4 7	6 5	7 7	9 0	9 0
3	Water agar		0 5	1 5	2 0	2 8	3 2	3 7	4 3	4 8	5 2

It is obvious from the data that the rate of growth of the fungus is almost similar both in the Potato dextrose agar and Corticium culture media However after 144 h the radial growth of fungus in Corticium culture medium slowed down and did not complete the growth even after 240 h while in Potato dextrose agar it was over after 216 h

Varghese *et al* (1981) also reported satisfactory growth of the fungus in Corticium culture medium but he reported comparatively poor growth in Potato dextrose agar medium However the present study indicated that both Corticium culture medium and Potato dextrose agar could be used for satisfactory growth of the fungus

4 4 Morphological characters of the pathogen

The morphological characters of the pathogen causing VSD of cocoa were studied and results are presented in Table 5

On Potato dextrose agar and Corticium culture medium the fungus produced yellowish fluffy aerial mycelium but on water agar it produced sparse mycelium except in the centre

The mycelium from the culture of the fungus was found branched hyaline and septate Somatic hyphal cells measured 3 to 6 μm (mean 3 7 μm) in breadth and 36 to 186 μm (mean 103 μm) in length Some of the hyphae were differentiated into swollen monilioid cells of 6 9 μm (mean 7 5 μm) breadth and 24 to 48 μm (mean 31 2 μm) length (Fig 1) Clamp connection absent No basidiospores production was observed in cultures

Table 5 Morphological characters of *Oncobasidium theobromae* causing vascular streak dieback of cocoa

Characters	Mean (μm)	Range (μm)
Length of spores	15 1	9 27
Width of spores	6 1	3 9
Length of sterigmata	8	6 12
Width of sterigmata	<3	<3
Basal width of basidium	4 5	3 6
Apical width of basidium	7 9	7 10
Length of monilioid hyphae from field	69 4	30-120
Width of monilioid hyphae from field	10 2	6 12
Length of monilioid hyphae in culture	31 2	24-48
Width of monilioid hyphae in culture	7 5	6 9
Length of hyphae in culture	103 3	36 186
Width of hyphae in culture	3 7	3 6

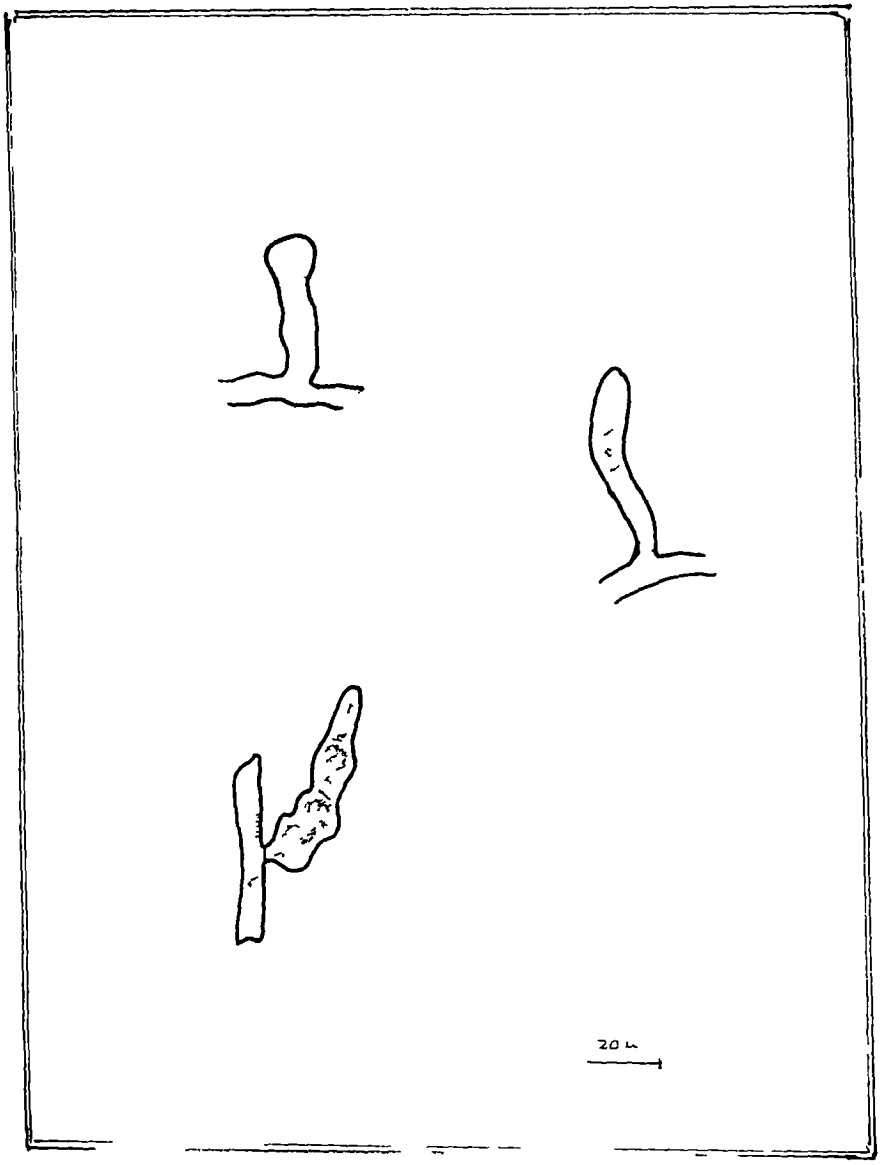


Fig 1 Moniliioid hyphae of *Oncobasidium theobromae* from culture

On host the fungus formed white effused adherent sporophores on leaf scars and adjacent bark Basidia was holobasidiate capitate clavate and measured 3 6 μm (mean 4 5 μm) basal width and 7 10 μm (mean 7 9 μm) in apical width (Fig 2) Sterigmata 4 conical straight or curved Length of sterigmata measured 6 12 μm (mean 8 μm) and less than 3 μm in width Moniliod hyphae seen on the host were comparatively larger than that seen in culture (Fig 3) Moniliod hyphae in the host measured 6-12 μm (mean 10 2 μm) breadth and 30-120 μm (mean 69 4 μm) m length (Plate 1) Basidiospores hyaline ellipsoid with one side flattened and 9 15 26 x 3 6-9 μm in size (Plate 2 and Fig 4)

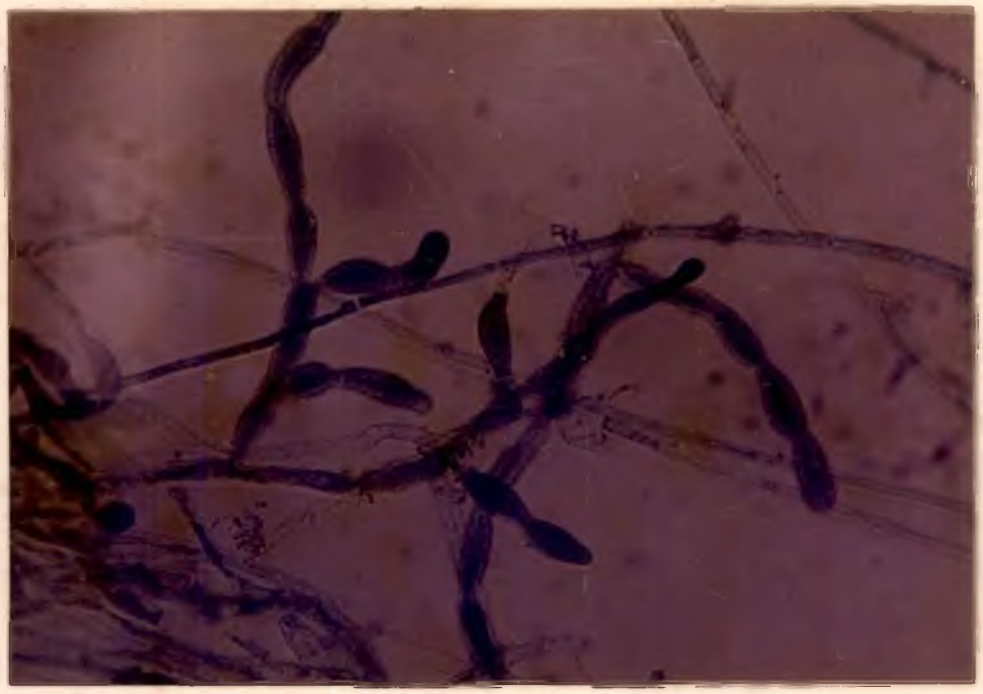
In culture the fungus produced whitish to yellowish mycelium and monilioid hyphae The morphological characters of the sporophores from the infected plants were found to be similar to those reported from Papua New Guinea and Malayasia (Talbot and Keane 1971 Zainal Abidin 1982 and Lam *et al* 1988) The morphological characters of hyphae monilioid hyphae basidia sterigmata and basidiospores were found to be almost similar to the original description of the pathogen by Talbot and Keane (1972) Only few basida were obtained in the study probably due to the reason suggested by Keane and Prior (1992) Hence based on the above morphological characters the fungal pathogen causing VSD of cocoa could be identified as *Oncobasidium theobromae* Talbot and Keane

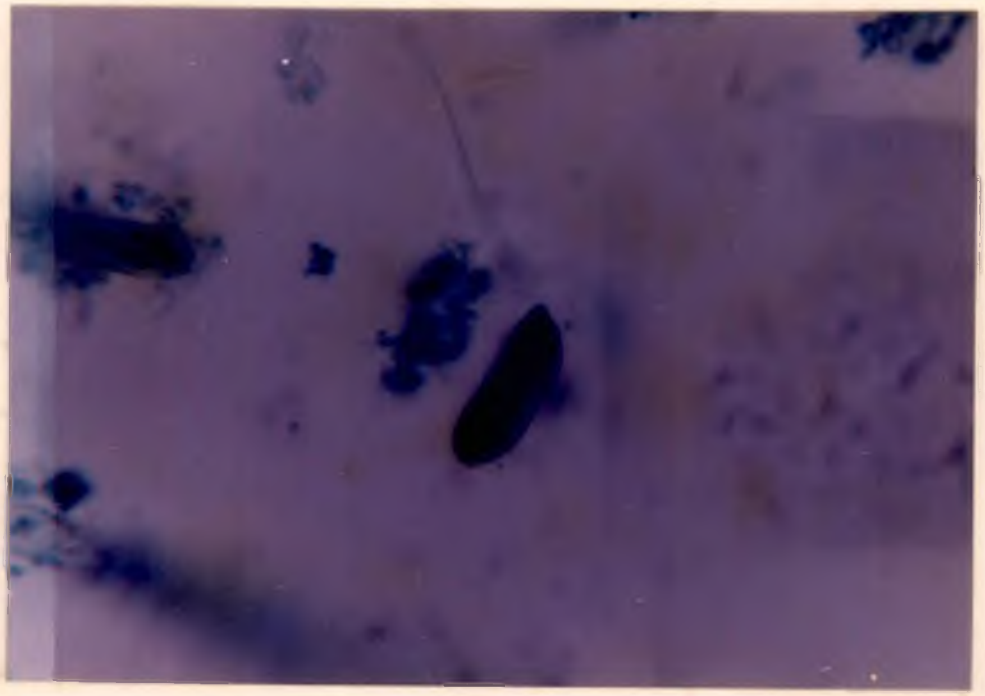
4 5 Symptomatology

Symptoms of VSD occurring in Kerala were studied m detail by periodical observations of severely infected cocoa gardens It was found that all age

Plate 1 Moniliod hyphae of *Oncobasidium theobromae* from the sporophores (400 x)

Plate 2 Basidiospore of *Oncobasidium theobromae* (1000 x)





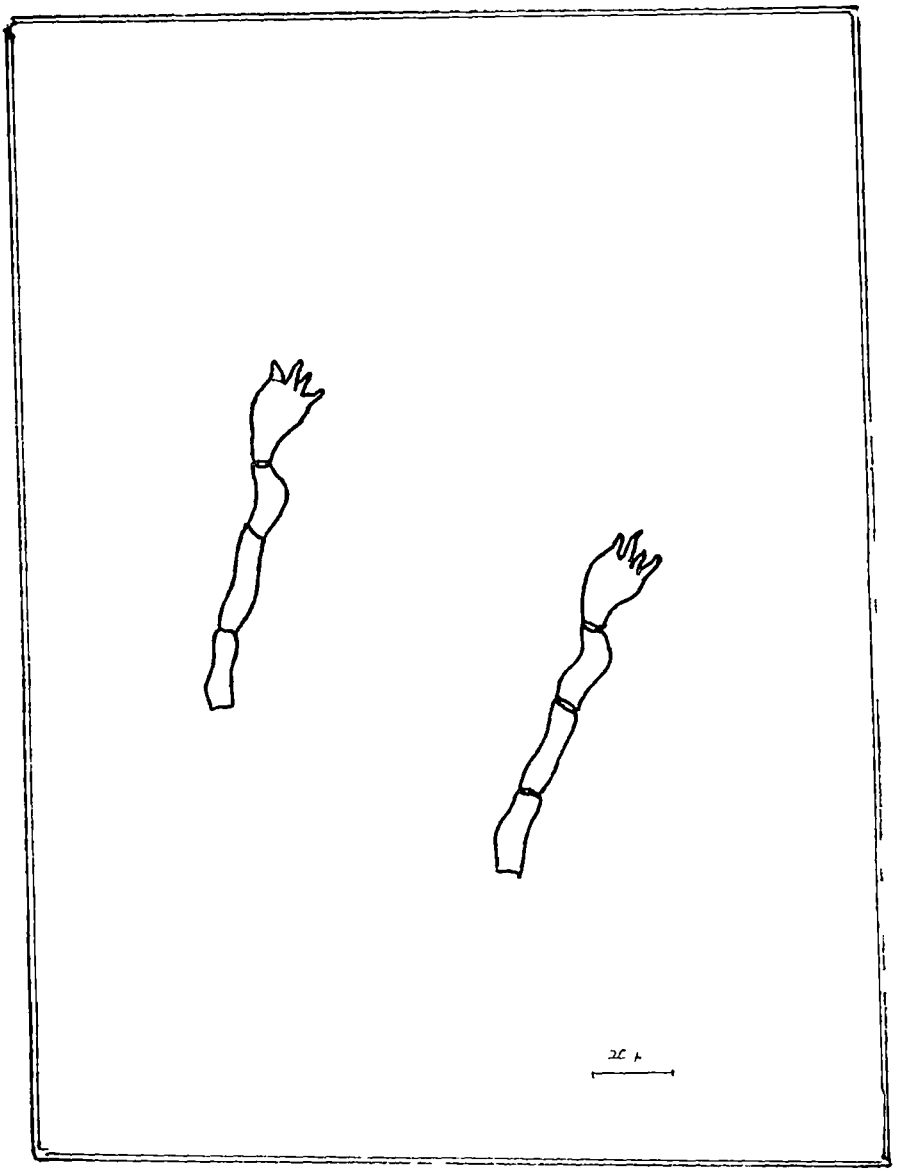


Fig 2 Basidia of *Oncobasidium theobromae*

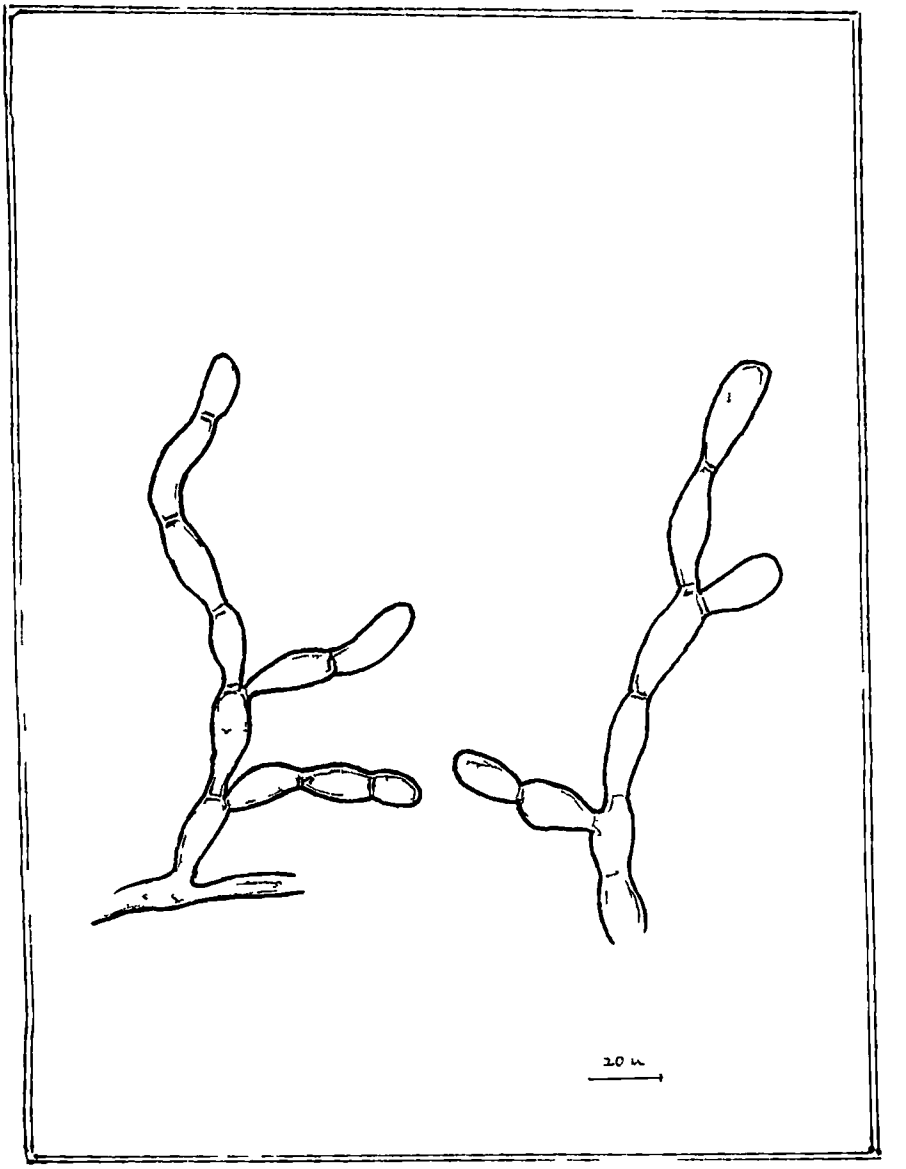


Fig 3 Moniliod hypahe on *Oncobasidium theobromae* from field

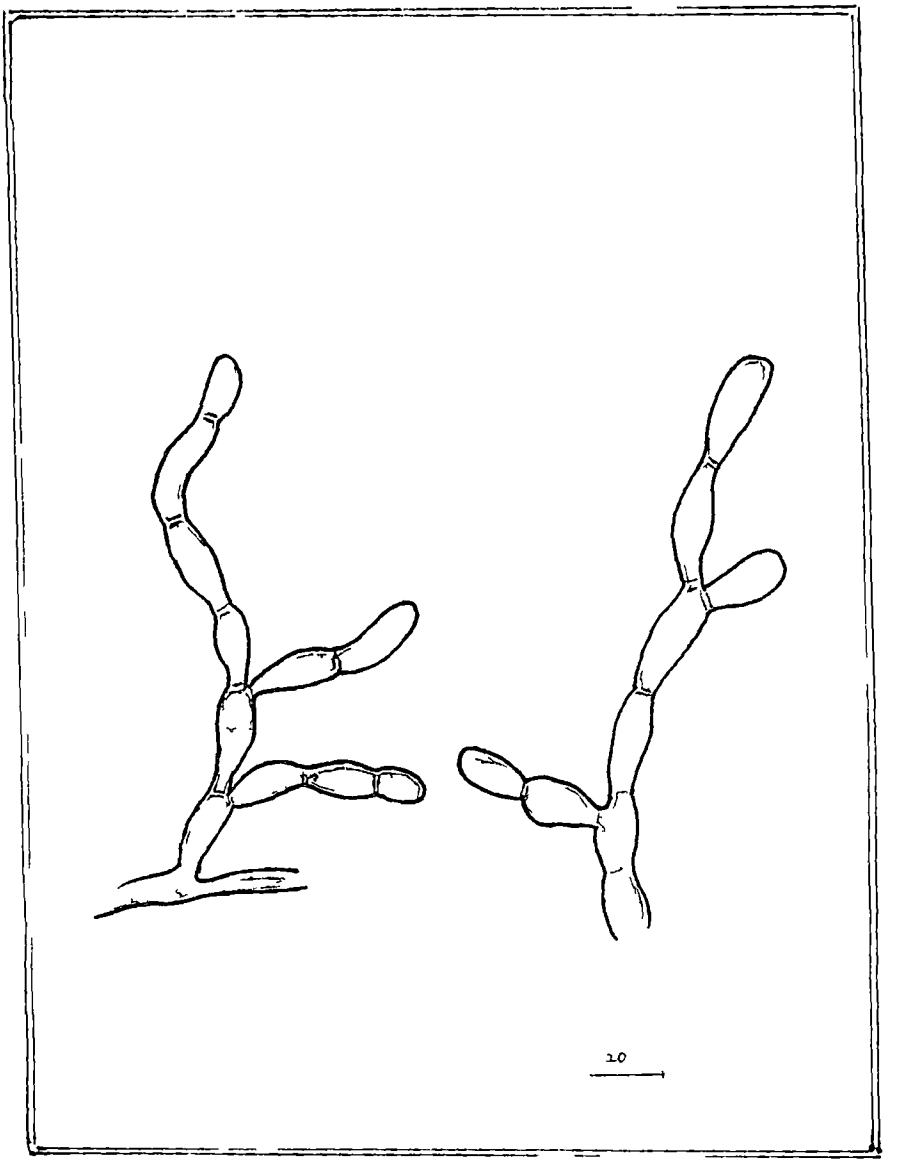


Fig 3 Monilioid hypahe on *Oncobasidium theobromae* from field

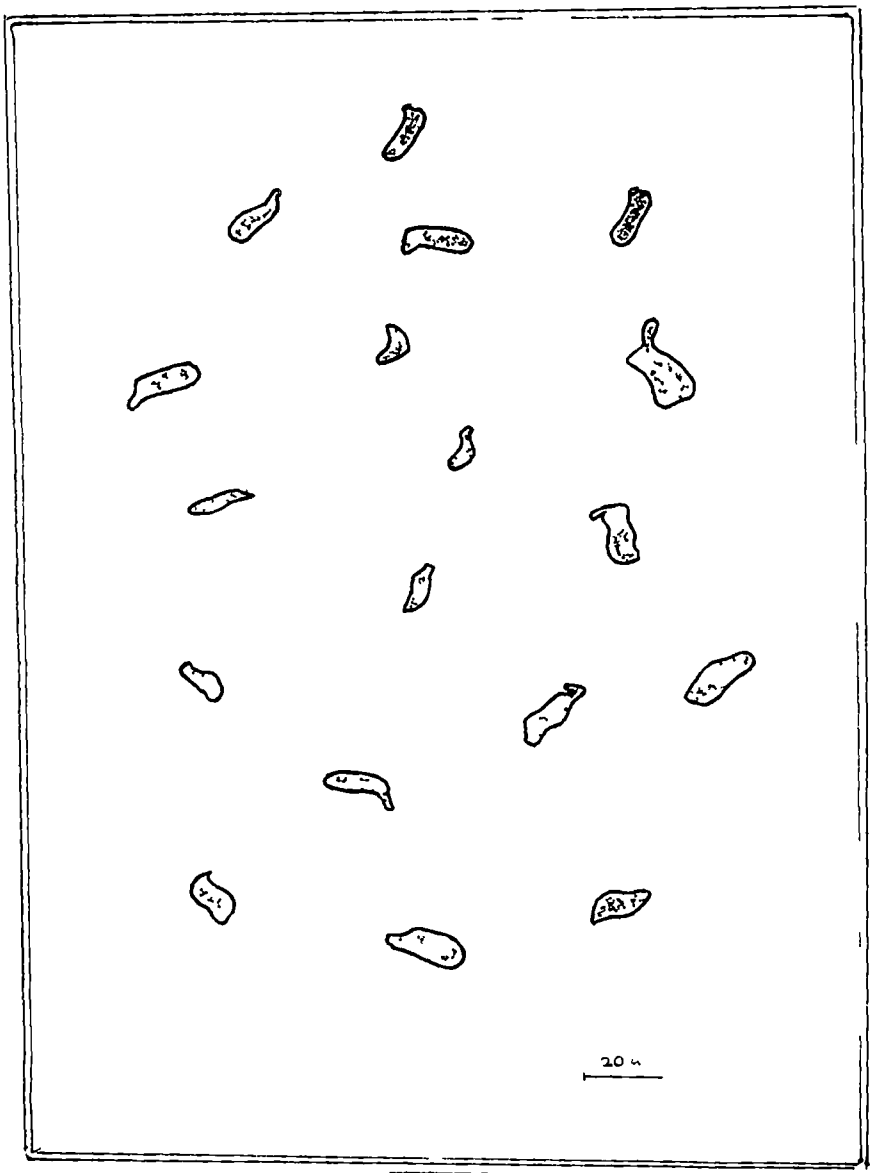


Fig 4 Different types of basidiospores of *Oncobasidium theobromae*

groups of plants were infected by the pathogen First symptom was the development of pale green colour intermingled with normal green areas starting from the proximal end of a leaf in the middle of the twig (Plate 3) Subsequently the pale green tissues turned yellowish and spread over the entire leaf lamina and the normal green areas developing into characteristic green islets (Plate 4) and such leaf fell off In certain cases the whole leaf did not turn yellowish but distal half retained the normal green colour (Plate 5) Leaves seen towards the upper and lower portions of the twig also developed similar symptoms and fell off retaining only the youngest and oldest ones

At times leaves with characteristic symptom remained attached to the twigs with yellow portions turning dark brown and green islets remaining as such (Plate 6) In certain cases severe marginal necrosis resembling potassium deficiency was observed in the leaves In some of the seedlings infected with VSD leaf lamina adjacent to the midrib and veins were green in colour while remaining portions turned yellow (Plate 7) These leaf symptoms were found to be different from those reported by Kean *et al* (1972) According to them the first symptom was the complete chlorosis of entire leaves with islets of green tissue such leaves fall off within two or three days However in this study it was found that the initial symptom was the development of pale green areas intermingled with green islets starting from the proximal end of the leaves Defoliation was not always noticed Some times leaves showing characteristic symptoms may remain attached to the twig with yellow area turning dark brown and green islets remaining as such Also in the present study it was found that the bark near the region of leaf fall remained normal in appearance (Plate 8) However in severe cases of infection the bark region was found to become rough due to the enlargement of lenticels (Plate 9) Unlike the

Plate 3 Initial symptom of VSD

Plate 4 Symptoms showing general yellowing with green islets

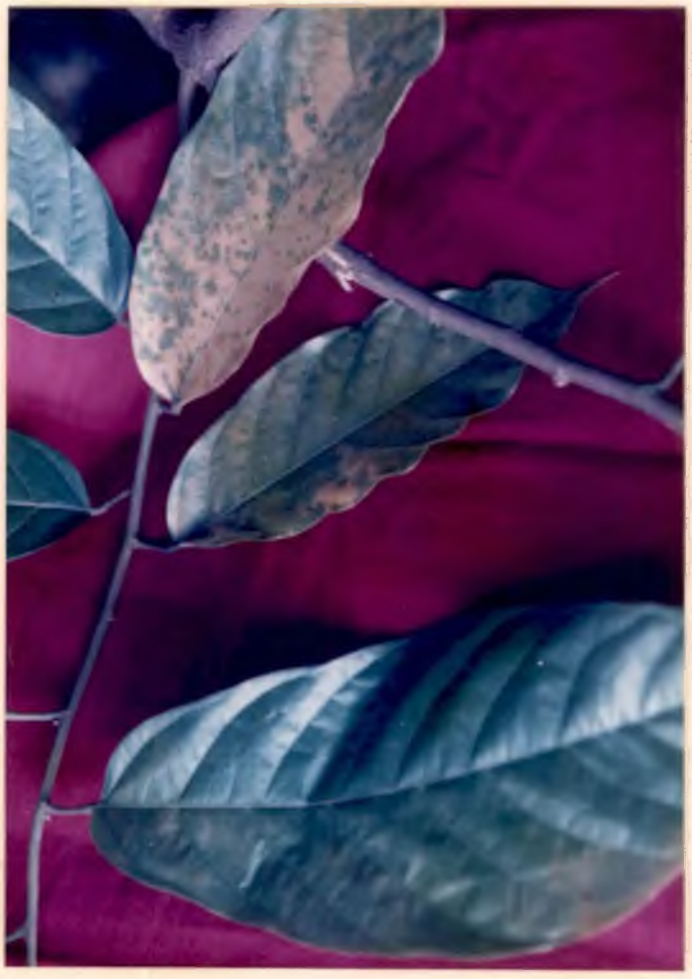


Plate 5. Leaf showing yellowing of the distal end

Plate 6. Yellow portions of the leaf turns to dark brown with green islets

Plate 7. Leaves of seedling with interveinal yellowing

Plate 8. VSD infected twig of cocoa plant without lenticel enlargement

symptoms described by others (Keane *et al.*, 1972 and Keane and Prior, 1992) enlargement of lenticels was not the earliest diagnostic symptom of VSD seen in Kerala.

Another symptom of the disease was the presence of three brown marks on the leaf scars of the fallen leaves. These brown marks were visible when such dried portion was scraped off (Plate 10). Profuse axillary bud growth was also seen on the diseased twigs but majority of them dried up sooner or later. Dark brown vascular streak could be seen when the bark was peeled off or stem was split open. Cambium in the diseased region turned rusty brown faster than the healthy region when exposed to air (Plate 11).

New flushes arising from infected twigs sometimes showed interveinal necrosis resembling symptoms of calcium deficiency. Some of them showed downward or upward curling as a result of interveinal necrosis coupled with severe marginal necrosis. In the case of mild infection, the axillary buds of the infected twigs continued to grow with reduced internodal length and leaf size after the fall of infected leaves. In rainy season, whitish growth of the causal fungus from the scars of fallen leaves was seen on the infected twig (Plate 12). Finally, the infected twigs started drying slowly. The infection becomes lethal if it occurred on young plants. In mature plants flowering and pod formation were drastically reduced due to the infection by the pathogen.

The variations in symptoms noticed in the present study from those reported from Papua New Guinea (Keane *et al.*, 1972) may be due to the presence of a different isolate of the pathogen or due to climatic factors. Variation in symptoms of VSD occurring in Malaysia and Kerala were reported (Varghese *et al.*,

Plate 9. Cocoa twig with enlargement of lenticels (A. Healthy; B. Diseased)

Plate 10. Brown marks on the leaf scars

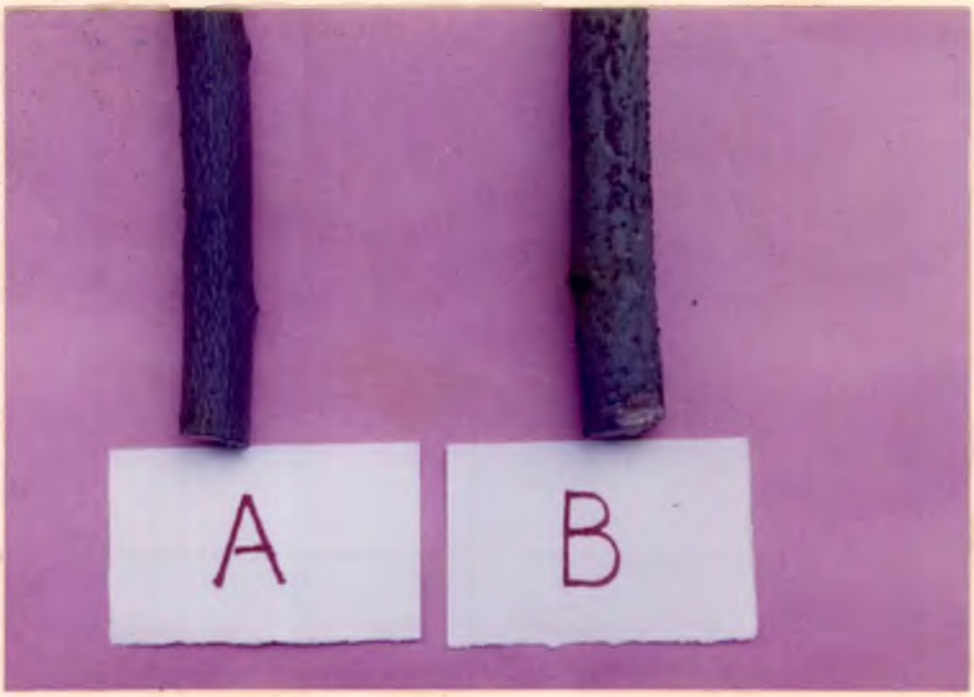
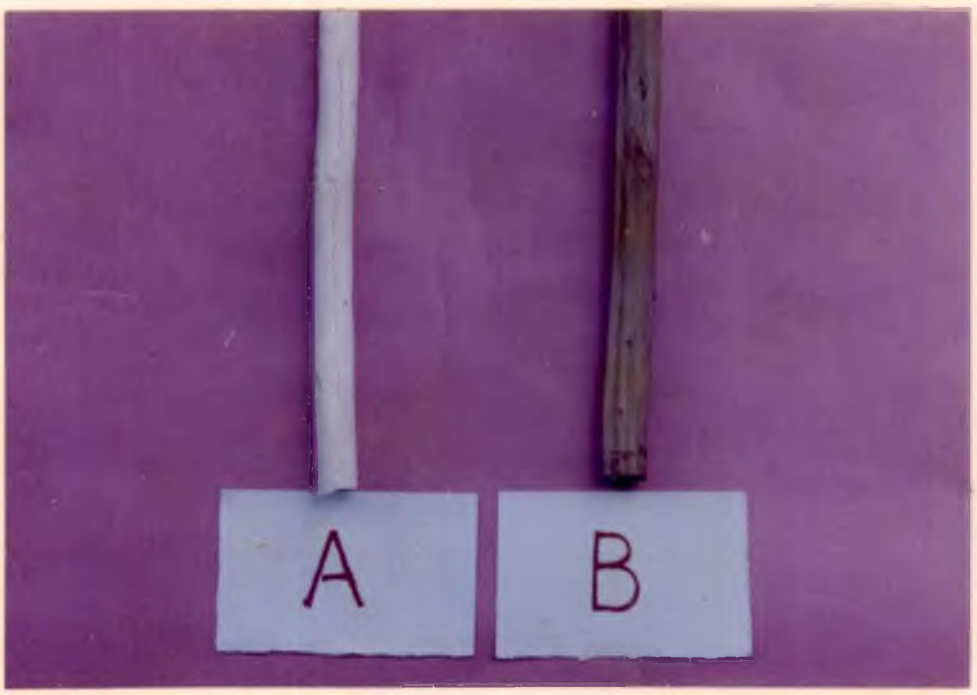

A
B

Plate 11. Rusty brown discolouration and vascular streaks on bark peeled off cocoa twig (A. Healthy; B. Diseased)

Plate 12. White sporophores on the scars of fallen leaves


A
B

1981; KAU, 1995). Zainal Abidin *et al.* (1981) suggested that there were different isolates of the pathogen present in Papua New Guinea and Malaysia but morphologically both the isolates were found to be similar. Further detailed studies are necessary to confirm whether the symptom variation in Kerala is due to the difference in the pathogen or due to any other factors.

4.6 Histopathology

Comparison of anatomy of infected and healthy stem of cocoa revealed noticeable difference (Plate 13 and 14). The longitudinal and cross section of infected stem revealed the presence of fungal hyphae in many of the xylem vessels (Plate 15a and 15b) as reported by Prior (1985), Dennis and Keane (1992).

Due to infection, the xylem vessels were found clogged, which might have disrupted the flow of water and nutrients resulting in typical symptom development. It was also observed that infected cells and tissue adjacent to fungal infection were deeply stained possibly due to release of phenolic compound and their subsequent oxidation. Such deposition of brown phenolic in the infected vessels of cocoa stem was reported earlier (Prior, 1985). The secondary xylem tissues were found to loose their integrity and were damaged.

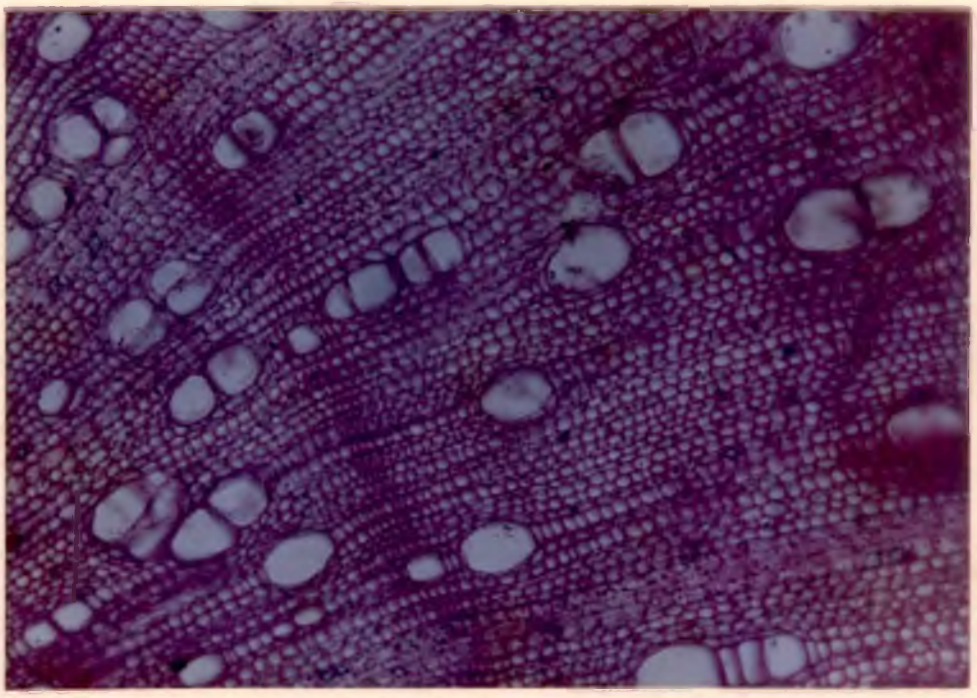
4.7 Transmission studies

4.7.1 Transmission through grafting

This experiment was undertaken to find out whether the disease is transmitted by the vegetative methods or through seeds. Two methods of grafting were tried with infected scion and healthy rootstock. No establishment of grafts was noticed when the study was conducted in both January and October, 1995. Further

Plate 13. Cross section of the healthy cocoa stem (100 x)

Plate 14. Cross section of the diseased cocoa stem (100 x)



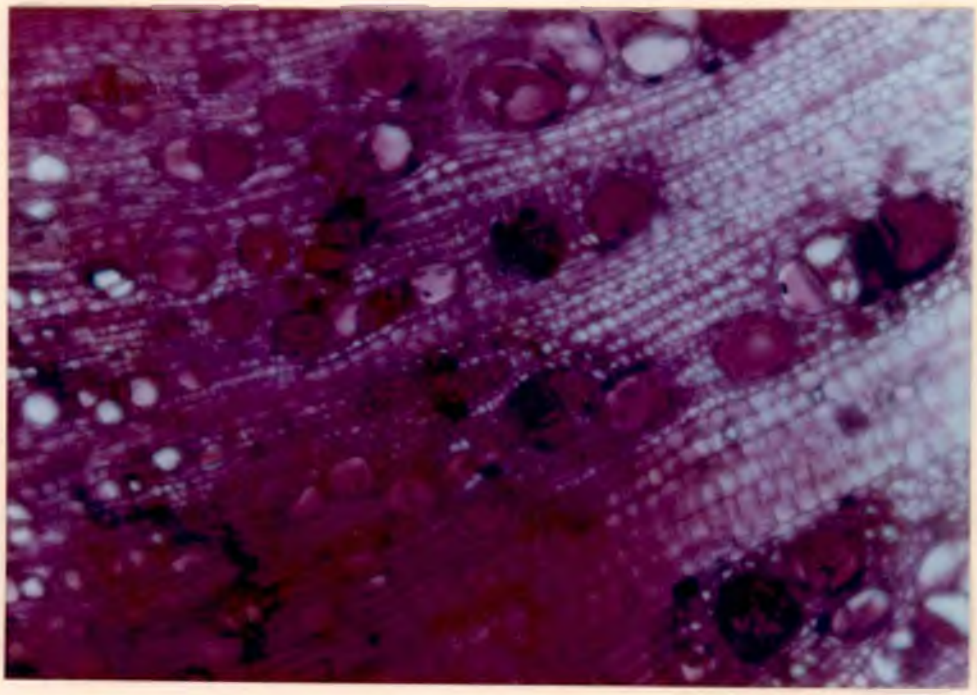
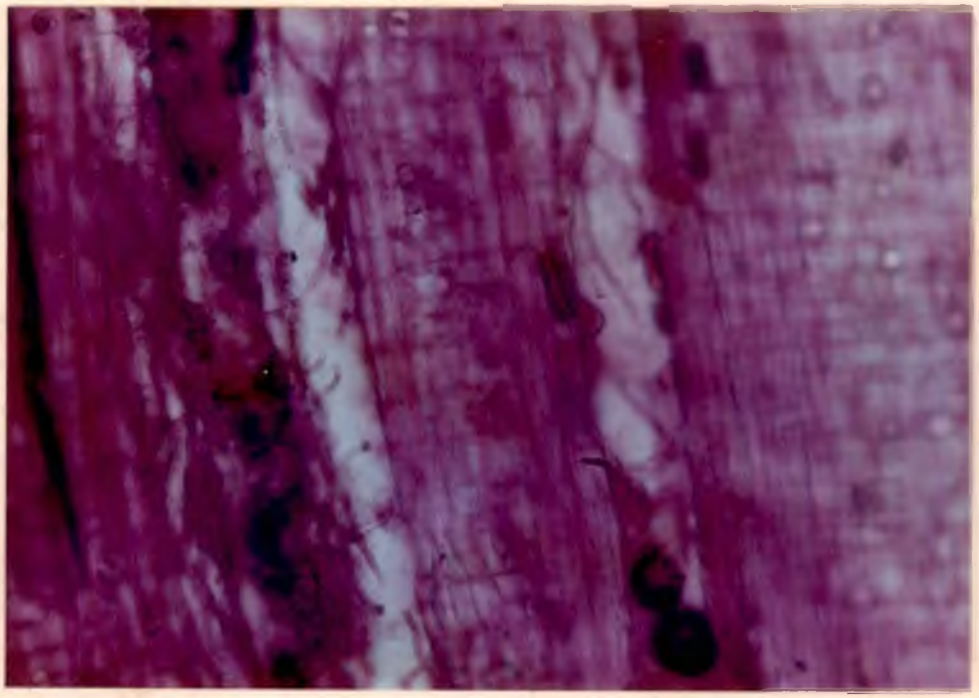


Plate 15a. L.S. of diseased cocoa stem with hyphae of *Oncobasidium theobromae* in xylem vessels (Low power) (100 x)

Plate 15b. L.S. of diseased cocoa stem with hyphae of *Oncobasidium theobromae* (High power) (400 x)



observations on the transmission of the disease to the grafted rootstocks were recorded as described in Materials and Methods

In root stocks grafted in January only wedge grafted ones showed vascular streaks (Table 6a) while those grafted in October vascular streaks was observed in both methods of grafting (Table 6b) Among the two types of grafting tried wedge grafted rootstock showed higher percentage of disease transmission compared to that of side grafted rootstock

So the result of the study indicated that VSD is transmissible through grafts The transmission was more when the grafting was done during October The reports on failure in the establishment of the grafts by using infected scions by Keane *et al* (1972) and KAU (1993) were in confirmity with the present study Further Keane *et al* (1972) reported transmission of disease through approach grafting with diseased plant The higher percentage of transmission in wedge grafted rootstock might be due to the larger area of the infected scion coming in contact with the rootstock

4 7 2 Transmission through budding

This study was also conducted during the months of January and October 1995 There was no successful establishment of the buds during these periods Data regarding the observations on the transmission of the disease to the rootstock are presented in Table 7a and 7b

The results of the study during January in general revealed compara tively lower percentage of transmission of the disease Only patch budding with larger patch of bud flap budding and inverted T budding showed transmission of

Table 6a Effect of methods of grafting on the transmission of vascular streak dieback during January 1995

Type of grafting	No of plants showing vascular streak	No of plants not showing vascular steak
Side grafting	0(0)	30(100)
Wedge grafting	3(10)	27(90)

Table 6b Effect of methods of grafting on the transmission of vascular streak dieback during October 1995

Type of grafting	No of plants showing vascular streak	No of plants not showing vascular streak
Side grafting	2 0(8 0)	23 0(92 0)
Wedge grafting	4 0(16 0)	21 0(84 0)

X^2 = 0 0909091 (NS)

Figures in parentheses indicate value in percentage

Table 7a Effect of methods of budding on the transmission of vascular streak dieback during January 1995

Type of budding	Number of plants showing vascular streak	Number of plants not showing vascular streak
Patch budding (large patch)	1(3 33)	29(96 6)
Patch budding (small patch)	0	30(100)
Flap budding	1(3 33)	29(96 6)
T budding	0	30(100)
Inverted T budding	1(3 33)	29(96 6)

Table 7b Effect of methods of budding on the transmission of vascular streak dieback during October 1995

Type of budding	Number of plants showing vascular streak	Number of plants not showing vascular streak
Patch budding (large patch)	6 0(24)	19 0(76)
Patch budding (small patch)	4 0(16)	21 0(84)
Flap budding	4 0(16)	21 0(84)
T budding	2 0(8)	23 0(92)
Inverted T budding	4 0(16)	21 0(84)

X^2 – 1 3809524 (NS)

Figures in parenthesis indicate value in percentage

the disease during that period But during October rootstocks budded by all the different methods showed symptoms of vascular streaking indicating transmission of the disease However there was no significant difference in the per cent transmission of the disease The maximum percentage of vascular streaking was observed in the case of patch budding with larger patch bud where as it was minimum in T budding

The results of the present study on the failure of bud establishment is in conformity with those of Chan and Syed (1976) and KAU (1993) As in the case of grafting favourable conditions during October may be the reason for the comparatively higher percentage of transmission of the disease Further the method of budding also influenced the transmission of the disease

4 7 3 Transmission through seeds

Inorder to find out the transmission of VSD through cocoa seeds seeds from pods of severely infected plants were sown and observed for the incidence of the disease Out of the 500 seeds sown 95 per cent of the seeds germinated None of the germinated seedlings developed symptoms of the disease even after 6 months Thus this study gives the evidence for the least possible transmission of the disease through seeds Further the seeds showed 95 per cent of germination which is comparable to that of seeds from healthy pods Chan and Syed (1976) and Prior (1985) also reported that the disease is not transmitted through seeds

4 7 3 1 Effect of fungicide treatment on cocoa seeds as a prophylatic measure against VSD transmission

Six fungicides were used for seed treatment against seed transmission of

VSD Since none of the germinated seedlings showed any symptoms of the disease the efficacy of the fungicide could not be worked out in preventing the possible transmission of the disease Even though the fungicides had no significant effect on seed germination there was noticeable effect on the further growth of seedling

4 7 3 1 1 Effect of fungicides as seed treatment on germination

From the result given in the Table 8a it was evident that fungicide treatments had no significant influence on percentage of germination Prior (1985) also observed that seed treatment with metalaxyl and propiconazole had no significant effect on germination percentage of cocoa seeds

4 7 3 1 2 Effect of fungicides as seed treatment on height of cocoa seedlings

Seed treatment with different fungicides had significant effect on the height of the seedlings during the initial stages of growth while it was not significant five months after sowing (Table 8b)

One month after germination Kitazin treated seeds showed maximum height and was on par with the Bavistin treated seeds and these were superior to all other treatments Bayleton treated seeds recorded the minimum height and was significantly inferior to all other treatments It was also observed that seeds treated with Bayleton Contaf and Aureofungin showed lesser height compared to that of control Further five months after germination seedlings from all fungicide treated seeds were taller than the control though not significant

The beneficial effect of Kitazin and Bavastin in promoting the growth of crop plants had been documented (Nene and Thapliyal 1979) The fungicides

Table 8a Effect of seed treatment with fungicides on germination

Fungicides	Number of germinated seeds	Number of ungerminated seeds
Kitazin	49(98)	1(2)
Bayleton	48(96)	2(4)
Aureofungin	48(96)	2(4)
Bavistin	49(98)	1(2)
Contaf	48(96)	2(4)
Calixin	46(92)	4(8)
Control	48(96)	2(4)

X_6^2 0 4583333 (NS)

Figures given in parenthesis indicates value in percentage

Table 8b Effect of seed treatment with fungicides on the height of cocoa seedlings

Fungicide	Height of seedlings (cm)	
	1 month after sowing	5 month after sowing
1 Kitazin	17 0 a	43 4
2 Bayleton	11 3 d	41 1
3 Aureofungin	14 1 bc	40 2
4 Bavistin	16 4 a	43 2
5 Contaf	13 5 c	40 2
6 Calixin	14 6 b	43 2
7 Control	14 7 b	38 0
CD	S	NS

NS Not significant S Significant
CD in Appendix II

Table 9c Evaluation of cocoa types against vascular streak dieback in seed garden at Kalaketty

Sl No	Cocoa type	Disease score of individual plants										Average disease score
		1	2	3	4	5	6	7	8	9	10	
1	M 16 9	1	3	3	3	1	3	1	3	5	1	2 4
2	GI 15 5	7	7	7	7	9	9	9	7	9	7	7 8
3	GI 5 9	3	3	3	3	5	5	7	3	3	5	4 0
4	GI 10 3	9	9	9	9	7	5	7	5	5	7	7 2
5	GI-4 8	7	7	7	7	5	7	5	7	5	5	6 2
6	GI 9 6	5	3	3	3	1	1	1	3	1	1	2 2
7	M 9 16	5	5	7	5	7	5	5	3	3	3	4 8
8	GII 20 4	3	4	4	7	3	3	3	3	5	3	4 0
9	GII 12 3	4	3	3	3	5	7	7	5	5	5	4 8
10	GII 19 5	3	5	1	1	3	1	1	1	1	1	1 8
11	GIV 18 5	1	1	0	1	0	0	0	0	1	1	0 5
12	M 13 12	1	3	1	3	1						1 8
13	GIII 1 2	3	5	3	5	3	3	5	7	3	3	4 0
14	GIV 4 1	3	3	5	3	5	3	3	3	3	5	3 6
15	GIV 2 5	7	7	9	5	9	9	9	7	7	7	7 6
16	GVI 50	3	5	5	7	3	7	5	5	5	5	5 0
17	GVI 51	3	5	3	3	5	5	5	3	3	7	4 2
18	GVI 54	1	1	1	1	1	1	3	1	1	1	1 2
19	GVI 55	1	1	1	0	0	1	1	0	3	1	0 9
20	GVI 56	1	1	3	1	5	5	1	3	1	3	2 4
21	GVI 59	3	3	3	1	3	1	3	1	3	1	2 2
22	GVI 60	1	1	1	3	3	3	1	1	3	1	1 8
23	GVI-68	3	3	1	1	3	1	1	3	1	1	1 8

belonging to triazole group viz Bayleton (Triadimefon) and Contaf (Hexaconazole) had adverse effect on the height of seedlings Such adverse effect of triazole fungicides like propicanzole hexaconazole and triadimefon on cocoa seedlings was reported by Prior (1985) and Holderness (1990)

4 7 3 1 3 Effect of fungicide as seed treatment on leaf production of cocoa seedlings

The effect of different fungicide as seed treatment on the production of leaves was studied and the data are tabulated (Table 8c)

One month after sowing there was no significant difference among treatments on the number of leaves produced However five months after sowing there was significant difference among the treatments Seedlings from Kitazin treated seeds showed maximum number of leaves and was significantly superior to others All other treatments except control were on par The minimum number of leaves was observed in control

The growth promoting property of Kitazin as pointed out by Nene and Thaphyal (1979) would be the reason for the significant production of leaves in cocoa

4 8 Evaluation of cocoa types for their resistance/tolerance against VSD

This study was carried out to know whether any of the promising cocoa types planted in the seed gardens in the farmers field possessed any resistance/ tolerance against *O theobromae* Observations on disease intensity were recorded from the cocoa types planted at Vazhithala Muttom and Kalaketty and the results are presented below

Table 8c Effect of seed treatment with fungicides on the leaf production of cocoa seedlings

Fungicides	Number of leaves	
	1 month after sowing	5 months after sowing
Kitazin	4 160	24 735 a
Bayleton	4 021	19 532 b
Aureofungin	3 776	19 333 b
Bavistin	4 021	18 277 b
Contaf	4 111	18 432 b
Calixin	4 068	18 140 b
Control	3 809	16 170 c
CD	NS	S

NS Not significant S Significant
CD in Appendix III

4 8 1 Evaluation of cocoa types at Vazhithala

Eight cocoa types planted in the seed garden at Vazhithala were evaluated for their resistance to VSD It was observed that all cocoa types were infected by the pathogen with varying intensities (Table 9a) Minimum average disease score was recorded by the cocoa type M 9 16 and M 16 9 The maximum disease score was noticed in cocoa types GI 15 5 followed by GI 10 3 and GI 4 8 Other cocoa types recorded a disease score between one and two

4 8 2 Evaluation of cocoa types at Muttom

Observation on the intensity of infection of VSD in seed garden at Muttom also showed that all the cocoa types were infected by the disease with varying degrees of intensities (Table 9b) In this seed garden the disease intensity was comparatively less than that of at Vazhathala All the cocoa types recorded a disease score below two The minimum average disease score was recorded in cocoa types M 13 12 and GVI 55 followed by GI 4 8 GVI 54 M 9 16 GI 9 6 GII 20 4 M 16 9 and GIV 18 5 The maximum disease score was noticed in GVI 60 followed by GVI 59 GVI 51 GI 15 5 GIV 2 5 and GVI 56

4 8 3 Evaluation of cocoa types of Kalaketty

Data on the intensity of VSD in seed garden at Kalaketty are given in Table 9c The study indicated that all the cocoa types were prone to infection with varying extent Some of the cocoa types exhibited comparitively hygher disease score The cocoa types GIV 18 5 and GVI 55 recorded the minimum disease score followed by GVI 54 GII 19 5 M 13 2 GVI 60 and GVI 68 The cocoa types

Table 9a Evaluation of cocoa types against vascular streak dieback in seed garden at Vazhuthala

Sl No	Cocoa type	Total number of plants	Average disease score
1	GI 15 5	3	4 33
2	GI 10 3	7	4 14
3	GI 5 9	7	1 66
4	GI 4 8	6	2 66
5	GII 20 4	6	1 66
6	GII 19 5	7	1 71
7	M 16 9	7	1 00
8	M 9 16	7	0 85

Table 9b Evaluation of cocoa types against vascular streak dieback in seed garden at Muttom

Sl No	Cocoa type	Total No of plants	Average disease score
1	M 9 16	9	0 55
2	M 16 9	12	0 66
3	M 13 12	27	0 33
4	GI-4 8	2	0 50
5	GI 5 9	3	1 00
6	GI 9 6	21	0 57
7	GI 10 3	2	1 00
8	GI 15 5	9	1 44
9	GI 12 3	9	1 22
10	GII 19 5	7	1 00
11	GII 20 4	7	0 57
12	GIII 1 2	4	1 00
13	GIV-4 1	7	1 00
14	GIV 2 5	9	1 44
15	GIV 18 5	13	0 69
16	GIV 32 5	15	1 13
17	GVI 50	57	1 31
18	GVI 51	7	1 57
19	GVI 54	6	0 50
20	GVI 55	6	0 33
21	GVI 56	5	1 40
22	GVI 59	26	1 58
23	GVI 60	14	1 64
24	GVI 64	4	1 25

GII 1 2 GVI 51 M 9 16 GI 12 3 GVI 50 GI-4 8 GI 10 3 GIV 2 5 and GI 15 5 recorded comparatively higher intensity of the disease The maximum disease score was observed in GI 15 5 GIV 2 5 GI 10 3 and GI 4 8

In recent years resistance to VSD has been included as one of the important criteria in cocoa breeding and selection in many countries (Prior 1978 Keane and Prior 1992) Because of the difficulty in artificial inoculation, resistance screening has often involved exposure of test plants to natural inoculum in the field

Compared to the first locality Vazhithala more number of cocoa types were available for evaluation at other two locations facilitating more scope for selec tion of resistant/tolerant types

Results of the investigation showed that in general the cocoa types GVI 55 GVI 54 M 13 12 and GVI 18 5 recorded comparatively less disease score both at Muttom and Kalaketty thus revealing the ability of these types to resist the severity of the disease Since the pathogen is a systemic one and has a long incuba tion period further observations are essential before finally concluding about the true resistance or susceptibility of the cocoa types However this study indicates that there is possibility of evolving cocoa types resistant/tolerant to VSD

There are reports of resistance or susceptibility of cocoa clones or hybrids against VSD (Chan and Syed 1976 Prior 1978) The occurrence of wide range of field resistance to this disease which is stable and polygenic in nature was well established (Prior 1978 Tan and Tan 1987) Use of such resistant cocoa types which escaped the VSD epidemic has also proved to be effective in combating the disease in Papua New Guinea (Keane and Prior 1992)

4 8 4 Evaluation of germplasm VI of Cadbury KAU Co operative Cocoa Research Project for resistance/tolerance for VSD

Observation on the incidence and severity of VSD on122 collections of cocoa types in germplasm seeds of Cadbury KAU Co operative Cocoa Research Project at Vellanikkara was taken during October 1995 and results are presented in Table 9d

Out of the 122 cocoa types observed 20 cocoa types showed 100 per cent incidence of the disease where as 21 cocoa types remained non infected Observations on the disease score indicated that cocoa types reacted to the disease varyingly Among the cocoa types which were infected 21 types recorded the minimum disease score Comparatively higher disease score of above four was recorded in cocoa types GVI 1 GVI 7 GVI 83 GVI 85 and GVI 89 with the maximum in GVI 83

The collection of vegetatively propagated cocoa types in germplasm VI of the Cadbury KAU Co-operative Cocoa Research Project has been established since 1983 and consists of many introduced ones with wide range of variability In germplasm VI incidence of VSD has been noticed since 1993 The evaluation of cocoa types in germplasm VI against VSD indicated that some of them were not at all infected while others showed varying intensities of the disease which point out the possibility of resistant/tolerant ones in this large collection Since VSD made its appearance only recently and also of the fact that its development being slow which is typical of a systemic disease in perennial plants further observations are needed for longer period for the proper selection of resistant/tolerant ones against the disease

Table 9d Evaluation of cocoa types in germplasm VI of Cadbury KAU Co operative Cocoa Research Project against vascular streak dieback

Sl No	Accession number	Total number of plant	Number of plants infected	Percentage incidence score	Average disease
1	2	3	4	5	6
1	GVI 1	3	3	100	5 0
2	GVI 2	5	3	60	0 6
3	GVI 3	5	5	100	2 3
4	GVI-4	5	4	80	1 2
5	GVI 5	4	2	50	0 5
6	GVI 6	3	1	33	0 3
7	GVI 7	5	5	100	6 4
8	GVI 8	5	3	60	0 6
9	GVI 9	2	1	20	0 2
10	GVI 10	5	5	100	1 0
11	GVI 11	4	3	60	0 6
12	GVI 13	5	1	20	0 2
13	GVI 14	5	4	80	1 6
14	GVI 16	4	2	40	0 4
15	GVI 17	5	3	60	0 6
16	GVI 19	5	4	80	1 2
17	GVI 20	5	2	40	0 4
18	GVI 21	5	5	100	1 4
19	GVI 23	5	2	40	0 4
20	GVI 24	4	2	50	0 5
21	GVI 25	4	2	40	0 4
22	GVI 26	5	1	20	0 2
23	GVI 27	5	2	20	0 2
24	GVI 28	5	2	20	0 2
25	GVI 29	5	4	80	0 8
26	GVI 30	5	3	60	0 8
27	GVI 31	5	3	60	0 6
28	GVI 32	4	3	75	1 3
29	GVI 33	5	2	40	1 6
30	GVI 34	5	2	50	0 5
31	GVI 35	5	4	80	0 8
32	GVI 36	5	2	40	0 4
33	GVI 37	5	5	100	1 4
34	GVI 38	5	4	80	1 6
35	GVI 39	4	3	75	0 7
36	GVI-40	5	2	40	0 4
37	GVI 41	5	5	100	1 4
38	GVI-42	5	3	60	0 6

Contd

Table 9d Continued

1	2	3	4	5	6
39	GVI-43	4	2	50	1 0
40	GVI-44	5	4	80	1 2
41	GVI-45	5	4	80	0 8
42	GVI-46	5	1	20	0 2
43	GVI-48	5	3	60	0 6
44	GVI-49	4	2	50	0 5
45	GVI 50	5	5	100	2 2
46	GVI 51	5	4	80	0 8
47	GVI 52	5	5	100	1 4
48	GVI 53	5	3	60	0 6
49	GVI 54	5	1	20	0 2
50	GVI 55	4	1	25	0 2
51	GVI 56	5	1	20	0 2
52	GVI 57	5	1	20	0 2
53	GVI 59	5	3	60	0 6
54	GVI 60	5	2	40	0 4
55	GVI 61	5	1	20	0 2
56	GVI-64	5	2	40	0 4
57	GVI 67	5	3	60	0 6
58	GVI-68	5	1	20	0 2
59	GVI 71	5	4	80	1 2
60	GVI 73	4	4	100	1 5
61	GVI 74	3	1	33	1 0
62	GVI 75	5	2	40	0 4
63	GVI 76	4	4	100	2 5
64	GVI 77	5	4	80	1 6
65	GVI 79	5	2	40	0 4
66	GVI 80	4	2	50	0 5
67	GVI 82	5	5	100	2 2
68	GVI 83	4	4	100	6 5
69	GVI 84	5	2	40	0 4
70	GVI 85	3	3	100	5 0
71	GVI 86	5	5	100	1 8
72	GVI 87	4	4	100	3 0
73	GVI 88	4	4	100	1 5
74	GVI 89	5	5	100	4 2
75	GVI 94	5	5	100	3 4
76	GVI 100	5	0	0	0 0
77	GVI 102	5	0	0	0 0
78	GVI 104	5	0	0	0 0
79	GVI 106	3	0	0	0 0
80	GVI 108	5	1	20	0 2

Contd

Table 9d Continued

1	2	3	4	5	6
81	GVI 109	5	0	0	0 0
82	GVI 110	5	1	20	0 2
83	GVI 111	5	0	0	0 0
84	GVI 112	5	3	60	0 6
85	GVI 113	5	0	0	0 0
86	GVI 114	5	2	40	0 4
87	GVI 115	5	0	0	0 0
88	GVI 116	5	2	40	0 8
89	GVI 117	5	0	0	0 0
90	GVI 118	5	3	60	0 6
91	GVI 122	5	1	20	0 2
92	GVI 124	3	0	0	0 0
93	GVI 125	5	0	0	0 0
94	GVI 127	3	1	33	0 3
95	GVI 128	5	2	40	0 4
96	GVI 129	5	3	60	0 6
97	GVI 130	4	1	25	0 2
98	GVI 131	5	2	40	1 2
99	GVI 132	5	3	60	1 0
100	GVI 133	4	1	25	0 2
101	GVI 134	5	5	100	1 8
102	GVI 135	5	4	80	1 2
103	GVI 136	3	1	33	0 3
104	GVI 137	2	1	50	0 5
105	GVI 138	1	0	0	0 0
106	GVI 139	5	1	20	0 2
107	GVI 140	5	0	0	0 0
108	GVI 141	5	0	0	0 0
109	GVI 142	5	1	20	0 2
110	GVI 143	5	1	20	0 2
111	GVI 144	5	3	60	0 6
112	GVI 145	3	0	0	0 0
113	GVI 146	5	2	40	0 4
114	GVI 147	5	2	40	0 4
115	GVI 148	5	1	20	0 2
116	GVI 149	5	0	0	0 0
117	GVI 150	4	0	0	0 0
118	GVI 151	5	0	0	0 0
119	GVI 152	5	0	0	0 0
120	GVI 153	4	0	0	0 0
121	GVI 154	5	2	40	0 4
122	GVI 155	5	0	0	0 0

4 9 Effect of fungicides in preventing the incidence of VSD

Experiment was carried out to find out the effect of different fungicides in preventing the incidence of VSD The results are presented in Table 10a and 10b

Statistical analysis of the cumulative data on incidence of disease during March 1996 revealed significant difference among the treatments in preventing the incidence of disease (Table 10a and 10b) Calixin sprayed plants recorded minimum incidence of disease and was significantly superior to all other treatments Maximum disease incidence was recorded in Bavistin sprayed plant

Observation on the incidence of VSD at different intervals is given in Table 10c It is observed that occurrence of VSD on seedlings varied with treatments at different intervals Incidence of VSD was first noticed in the middle of October 1995 in all treatments except in calixin In calixin treated plants the incidence of disease was seen only during the end of October and November and middle of December 1995 There after none of the plants in this treatment showed any symptoms of the disease Thus the study indicated the effectiveness of Calyxin a systemic fungicide in preventing the incidence of VSD in young seedlings

There are many reports of fungicidal control of VSD especially in seedlings with triazole group of fungicides (Holderness 1990) Prior (1980) stated that protective fungicides are ineffective in controlling VSD in Papua New Guinea However according to Abraham and Ravi (1991) Bordeaux mixture and Kitazin were effective in checking the severity of VSD to a certain extent in older plants

Table 10a Effect of fungicides in preventing the incidence of vascular streak dieback of cocoa

Fungicides	Number of infected seedlings	Number of non infected seedlings
Bordeaux mixture	17(65 4)	9(34 6)
Indofil M-45	18(69 2)	8(30 8)
Calixin	6(23 1)	20(76 9)
Kitazin	15(57 7)	11(42 3)
Bavistin	21(80 8)	5(19 2)
Control	17(65 4)	9(34 6)

X_5^2 21 0926552**

Value in the parenthesis indicate values in percentage

** Significant at 1% level

Table 10b Effect of calyxin over other treatments in preventing the incidence of vascular streak dieback of cocoa

	Calyxin	Other treatments
Number of infected seedlings	6(6 4)	88(93 6)
Number of non infected seedlings	20(32 3)	42(67 7)

X_1^2 18 0090618**

Value in parenthesis indicates values in terms of percentage

** Significant at 1% level

Table 10c Effect of fungicides on the incidence of vascular streak dieback in seedlings at different intervals

Fungicides	Percentage of VSD incidence									
	Oct 15	Oct 30	Nov 15	Nov 30	Dec 15	Dec 30	Jan 15	Jan 30	Feb 15	Feb 28
Bordeaux mixture	23 1	10 0	16 7	13 3	23 1	0	7 7	0	0	0
Mancozeb	11 5	17 4	21 0	6 7	14 3	0	8 3	0	18 2	11 1
Calyxin	0	7 7	0	8 3	9 1	0	0	0	0	0
Kitazin	19 2	0	28 6	0	6 7	0	7 1	0	15 4	0
Bavistin	34 6	35 3	27 3	12 5	0	0	0	0	28 6	0
Control	39 0	16 0	19 0	23 5	15 4	0	0	9 1	0	10 0

Possible usefulness of systemic fungicides on the control of the disease was suggested by Keane and Prior (1992) Hence the fungicide calixin could be used effectively in preventing the incidence of disease in young cocoa seedlings This will help in the production of disease free planting materials in disease prone areas to a great extent

Summary

SUMMARY

Vascular streak dieback (VSD) is a serious disease of cocoa causing considerable damage to the crop Thus studies were undertaken on the pathogen symptomatology histopathology transmission of the disease evaluation of cocoa types for host resistance and fungicidal protection against the disease

Studies on the standardisation of the isolation technique reveal that Corticium culture medium Water agar and Potato dextrose agar were good for isola tion of the fungus Among the different plant parts tried for isolation maximum success was observed with petiole and midrib of leaves Lot of contaminants like *Fusarium* sp and *Colletotrichum gloeosporioides* were noticed during the isolation especially when hard stem pieces were used Use of bark removed hard stem avoided contamination to large extent Cocoa callus not ideal for isolation of the pathogen

Of the three media used for the growth of the pathogen Potato dextrose agar recorded the maximum growth followed by Corticium culture media and Water agar

The morphological characters of the pathogen from naturally occurring sporophores were studied Observations on the moniloid hyphae basidium sterigmata and basidiospores were taken Moniloid hyphae in the sporophores collected from the natural field condition measured 6 12 μm (mean 10 2 μm) breadth and 30-120 μm (mean 69 4 μm) length and were comparatively larger then that seen in the culture where it measured 6 9 μm (mean 7 5 μm) breadth and

24-48 μm (mean 31 2 μm) length Basidia holobasidate capitate clavate and measured 3 6 μm (mean 4 5 μm) basal width and 7 10 μm (mean 7 9 μm) apical width Sterigmata four conical straight or curved of 6-12 μm (mean 8 μm) length and less than 3 μm in width Basiodiospores hyaline ellipsoid with one side flattened and 9 15 26 x 3 6-9 μm in size Based on these characters the causal organism of VSD was identified as *Oncobasidium theobromae* Talbot and Keane

Symptoms of the disease on seedlings and older trees were similar Some variation in the symptom expression of VSD was observed in this study as those reported elsewhere Initial symptoms developed as pale green areas with intermingled normal portions starting from the proximal end of the leaves Such leaves later turned to yellow with green islets and fall off Three brown marks were observed on the scars on the fallen leaves In certain cases the infected leaves may not fall off but remained on the plant with yellow area turning to dark brown and green islets as such Lenticel enlargement was not observed always Profuse axillary buds sprouts were noticed on the infected twigs Young flushes of infected plants showed typical calcium deficiency symptom Cambium of infected twig turned rusty brown faster than healthy ones when bark was peeled off Characteristic vascular streaking noticed when the bark was peeled off are stem split open Fructification of the fungus developed on the scars of the infected fallen leaves Finally the infected twigs die off

Histopathological study revealed the presence of fungal hyphae and deposition of phenolic materials in the xylem vessels of infected stem

Transmission of the disease through grafting and budding showed no graft or buds establishment when they were taken from the diseased twigs However

there was vascular streaking on the rootstock indicating transmission of the disease especially in October month The efficacy of fungicides given as a prophylatic measure on the cocoa seeds against possible transmission of VSD could not be evaluated as the disease was not found to be transmitted through seeds However none of the fungicides has significant influence of the germination of the seeds Seedlings from Kitazin and Bavistin treated seeds showed significant increase in height of plants Kitazin had an effect on the production of leaves also

Evaluation of cocoa types planted as seed garden at Vazhuthala Muttom and Kalaketty revealed that types reacted varyingly to the disease In general cocoa types GVI 55 GVI 54,GIV 18 5 and M 13 4 showed comparatively less disease score in Muttom and Kalaketty, thus indicating the ability of these types to resist the severity of the disease

Evaluation of 122 cocoa types in germplasm VI of Cadbury KAU Co-operative Research Project indicated that some of them were not at all infected while other showed some varying intensity of the disease

Among the five fungicides tried Calixin gave better effect in preventing the incidence of VSD in seedlings

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* Originals not seen

Appendices

APPENDIX 1
Media composition

Sweet potato sucrose agar

Sweet potato	250 g
Sucrose	20 g
Agar	20 g
Distil water	1000 ml

Czapek Dox agar

Sodium nitrate	2 g
Potassium dihydrogen phosphate	1 g
Magnesium sulphate	0 5 g
Potassium chloride	0 5 g
Ferrous sulphate	0 01 g
Sucrose	30 g
Agar	20 g
Distil water	1000 ml

Nutrient agar

Beef extract	1 g
Yeast extract	2 g
Peptone	5 g
Sodium chloride	5 g
Agar	15 g
Distil water	1000 ml

Potato peptone agar

Potato	200 g
Peptone	15 g
Agar	15 g
Distil water	1000 ml

Richards agar

Potassium nitrate	10 g
Potassium dihydrogen phosphate	5 g
Magnesium sulphate	2 5 g
Ferric chloride	0 02 g
Sucrose	500 g
Agar	15 g
Distil water	1000 ml

Coticium culture medium

Potassium dihydrogen phosphate	1 25 g
Calcium nitrate	2 36 g
Magnesium sulphate	0 59 g
Maltose	6 25 g
Agar	20 g
Distil water	1000 ml

Potato dextrose agar

Potato	200 g
Dextrose	20 g
Agar	20 g
Distil water	1000 ml

Water agar

Agar	15 g
Tap water	1000 ml

Oat meal agar

Rolled oats	50 g
Agar	20 g
Distil water	1000 ml

Host (leaf) extract dextrose agar

Cocoa leaves	200 g
Agar	20 g
Distil water	1000 ml

Woody plant medium

Ammonium nitrate	400 mg
Boric acid	6 2 mg
Calcium chloride 2 hydrate	96 mg
Calcium nitrate 4 hydrate	556 mg
Copper sulphate	0 25 mg
Ferrous sulphate	27 8 mg
Magnesium sulphate 1 hydrate	22 3 mg
Magnesium sulphate 7 hydrate	37 mg
Potassium sulphate	990 mg

Potassium dihydrogen phosphate	170 mg
Sodium molybdate	0 25 mg
Zinc sulphate	8 6 mg
Inositol	100 mg
Nicotinic acid	0 5 mg
Thiamine	1 mg
Pyridoxine	0 5 mg
Glycine	2 mg
Sucrose	3%
Agar	0 8%
Water	1000 ml

MS medium

Ammomum nitrate	1650 mg
Boric acid	6 2 mg
Calcium chloride 2 hydrate	440 mg
Cobalt chloride	0 025 mg
Copper sulphate	0 025 mg
Ferrous sulphate	27 8 mg
Magnesium sulphate 1 hydrate	28 3 mg
Magnesium sulphate 7 hydrate	370 mg
Potassium iodide	0 83 mg
Potassium dihydrogen phosphate	170 mg
Sodium molybdate	0 25 mg
Zinc sulphate	8 6 mg
Inositol	100 mg
Nicotinic acid	0 5 mg
Thiamine	0 1 mg
Pyridoxine	0 5 mg
Glycin	2 mg
Sucrose	3%
Agar	0 8%
Distil water	1000 ml

APPENDIX II

Analysis of variance table Effect of fungicide seed treatment on height of cocoa seedling

Source	1 month after sowing		5 months after sowing	
	df	Mean square	df	Mean square
Treatments	6	172 915**	6	193 865 NS
Error	322	6 219	315	113 185
Total	328		321	

** Significant at 1 per cent level
NS Not significant

CD value for different treatment combinations

Treatment combination	CD value	Treatment combination	CD value
(1 2)	0 993	(3 5)	1 000
(1 3)	0 983	(3 5)	1 000
(1 4)	0 993	(3 6)	1 015
(1 5)	1 004	(3 7)	1 000
(1 6)	1 01	(4 5)	1 019
(1 7)	0 993	(4 6)	1 025
(2 3)	1 000	(4 7)	1 008
(2 4)	1 008	(5 6)	1 036
(2 5)	1 019	(5 7)	1 019
(2 6)	1 025	(6 7)	1 025
(2 7)	1 008		

APPENDIX III

Analysis of variance table Effect of fungicides as seed treatment on leaf production of cocoa seedlings

Source	1 month after sowing		5 months after sowing	
	df	Mean square	df	Mean square
Treatment	6	1 044 NS	6	341 743**
Error	322	0 521	315	22 537
Total	328		321	

** Significant at 1 per cent level
NS Not significant

CD values for different treatment combinations

Treatment combinations	CD value	Treatment combinstions	CD value
(1 2)	1 9	(3 4)	1 94
(1 3)	1 92	(3 5)	1 97
(1 4)	1 9	(3 6)	1 98
(1 5)	1 933	(3 7)	1 97
(1 6)	2 00	(4 5)	1 95
(1 7)	1 9	(4 6)	1 96
(2 3)	1 94	(4 7)	1 919
(2 4)	1 919	(5 6)	1 99
(2 5)	1 95	(5 7)	1 95
(2 6)	1 964	(6 7)	1 96
(2 7)	1 919		

VASCULAR STREAK DIEBACK OF COCOA AND ITS MANAGEMENT

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ABSTRACT OF A THESIS

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requirement for the degree

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ABSTRACT

Vascular streak dieback (VSD) is a destructive disease of cocoa Corticium culture medium Water agar Potato dextrose agar gave promising results in isolation of the pathogen Petiole and midrib gave maximum success in isolation Potato dextrose agar and Corticium culture media supported the growth of the fungus *Fusarium* sp and *Colletotrichum gloeosporioides* were the major contaminants interfering in the isolation of VSD

The morphological characters of the pathogen were studied from the sporophores occurring on naturally infected cocoa plants Based on these characters the pathogen causing VSD was identified as *Oncobasidium theobromae* Talbot and Keane

The disease produced various typical symptoms on leaves and stems of infected plants like pale green colour of leaves and subsequent yellowing with green islets defoliation brown marks on the scars of fallen leaves axillary bud growth of the infected stem rusty discolouration of cambium vascular streak whitish sporophores on the leaf scar of fallen infected leaves and finally the death of the infected twig

Histopathological studies showed the presence of fungal mycelium in the xylem vessels

Transmission studies by grafting and budding revealed no establishment of buds or grafts But there was vascular streaking No seed transmission was

observed In general Kıtazın and Bavıstın as a seed treatment had an effect on the heıght of plant and leaf productıon

Evaluatıon of cocoa types planted at three seed gradens mdıcated that some of them possess resıstance/tolerance agaınst VSD Varıatıon ın dısease ıncıdence and ıntensıty of VSD was noted ın germplasm VI

Calıxın sprayıng had an effect ın preventıng the ıncıdence of the dısease ın seedlıngs