COFFEE BERRY DISEASE IN KENYA

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COFFEE BERRY DISEASE IN KENYA

Proefschrift
ter verkrijging van de graad van
doctor in de landbouwwetenschappen,
op gezag van de rector magnificus,
dr. H.C. van der Plas, hoogleraar in de organische scheikunde,
in het openbaar te verdedigen
op woensdag 28 februari 1979 des namiddags te vier uur
in de aula van de Landbouwhogeschool te Wageningen

Ter nagedachtenis van mijn ouders

Thou, who can make mountains move
and rivers flow,
accept our sacrifice
and give us rain.

Excerpt from a Kikuyu-prayer for rain.

nn5201 753

STELLINGEN

1.
Zolang niet overtuigend is vastgesteld, dat er een relatie bestaat tussen *Glomerella cingulata* en *Colletotrichum coffeanum* in Kenia, moet worden aangenomen dat de ascosporen van *G. cingulata* in dat land géén rol spelen in de epidemiologie van de koffiebesziekte.

Dit proefschrift

2.
De *Colletotrichum*-populatie in de bast van koffiebomen is onder invloed van langdurige en intensieve fungicidenbespuitingen in Kenia verschoven in de richting van de pathogene component, *C. coffeanum*, de verwekker van de koffiebesziekte.

Dit proefschrift

3.
Laboratoriumbepalingen van de totale conidiënproduktie van de bast-populaties van *Colletotrichum*-soorten zeggen weinig omtrent de te verwachten oogstverliezen ten gevolge van de koffiebesziekte, noch van de effectiviteit van eerder uitgevoerde bespuitingen met fungiciden.

Dit proefschrift

4.
Op de takken van de koffieboom is het voorkomen van *C. coffeanum* beperkt tot die bastgedeelten waar de vorming van secundaire kurklagen in de cortex nog net niet of juist is begonnen.

Dit proefschrift

5.
In verband met recente waarnemingen over het optreden van flagellaten van het geslacht *Phytomonas* in kokos- en oliepalm is het gewenst de reeds in 1931 door Stahel ontdekte fytopathogene flagellaten ook in Nederland aan een hernieuwd onderzoek te onderwerpen.

Parthasarathy, M.V., Slobbe, W.G. van & Sondant, C. Science 192 (1976).

Slobbe, W.G. van, Parthasarathy, M.V. & Hesen, J.A.J. Principes, 22, 1 (1978).

Stahel, G. Phytopath. Z. 1 (1931), 5 (1932), 4 (1933).

Waters, H. Ann. appl. Biol. 90 (1978).

6.

De algemeen geaccepteerde opvatting, dat *Trypanosoma gambiense* niet meer voorkomt als verwekker van slaapziekte in de Roovallei bij het Victoriameer in Oost Afrika, is waarschijnlijk niet voldoende gefundeerd gezien de parasitologische en biochemische eigenschappen van de trypanosomen, geïsoleerd uit geïnfecteerde tse-tsevliegen uit dit gebied.

Allsop, R. & Baldry, D.A.T. Bull. Wld. Hlth. Org. 47 (1972).

Gibson, W. Trans. Roy. Soc. Trop. Med. & Hyg. (in press) (1979).

Goedbloed, E., Ligthart, G.A. & Minter, D.M. Trans. Roy. Soc. Trop. Med. & Hyg. 65 (1971).

7.

Een zelfstandige ontwikkeling van een pootaardappelproduktie in Indonesië zal alléén mogelijk zijn indien is vastgesteld hoe het optreden van insecten, die de verschillende aardappelvirussen overdragen, op effectieve en economische wijze kan worden tegengegaan, waarbij vooral het gedrag van deze vectoren in aanmerking moet worden genomen.

8.

De door Postgate geuite veronderstelling, dat vrijlevende stikstofbindende bacteriën waarschijnlijk slechts in relatie met de plantewortel hun functie kunnen vervullen, gaat geheel voorbij aan hun betekenis in de fyllosfeer.

Bessems, E.P.M. Nitrogen fixation in the phyllosphere of Gramineae. Agric. Res. Rep. 786 (1973).

Postgate, J.R. Biological nitrogen fixation. In: Companion to microbiology, selected topics for further study (Eds A.T. Bull & P.M. Meadow) Longman, London - New York (1978).

Ruinen, J. Nitrogen fixation by free-living micro-organisms (Ed. W.D.P. Stewart). International Biological Programme Vol. 6 (1975).

9.

Voor het instandhouden en accumuleren van voldoende kennis en ervaring op het gebied van landbouw in de Derde Wereld-landen is een gericht beleid noodzakelijk. Het ontbreken van zo'n beleid in Nederland doet afbreuk aan de kwaliteit van de agrarische ontwikkelingshulp.

10.
De projectduur voor toegepast landbouwkundig onderzoek in door Nederland gefinancierde ontwikkelingshulpprojecten in de Derde Wereld is veelal te kort. Het belang en de moeilijkheidsgraad van dergelijk onderzoek wordt door de beleidsfunctionarissen in Nederland onvoldoende onderkend, hetgeen nadelig is voor het rendement van de ontwikkelingshulp.

11.
Een belangrijk deel van de Nederlandse literatuur over biologisch en agrarisch onderzoek verricht in Indonesië is van blijvende betekenis voor dat land. Door de nog aanwezige kennis van de Nederlandse taal bij de oudere Indonesische vakspecialisten kan deze literatuur nú nog met geringe financiële middelen worden vertaald in het Indonesisch.

12.
Moderne Nederlandse schrijvers verdiepen zich veelvuldig in eigen jeugdtrauma's en -ervaringen. Het is echter te vrezen dat hieraan onvoldoende inspiratie kan worden ontleend voor een blijvend schrijversschap.

13.
De opvattingen in de Nederlandse samenleving met betrekking tot het kolonialisme hebben niet geleid tot een afwijzen van moderne vormen ervan, zoals dit onder meer tot uiting komt in de sociaal-maatschappelijke waardering van de gastarbeider.

14.
Het toelaten tot tentoonstellingen en het verstrekken van prijzen aan honden met gecoupeerde oren op Nederlandse kynologische exposities is in strijd met het sinds 1961 geldende verbod in Nederland op het couperen van oren.

H. Vermeulen,
"Coffee Berry Disease in Kenya",
Wageningen, 28 februari 1979.

CONTENTS

PREFACE

Although many years have passed, I am still grateful to the staff of the Coffee Research Station at Ruiru for their help in the period 1964 - 1966. Furthermore I am most thankful to the Senior Plant Pathologist of the National Agricultural Laboratories, Mr J.J. Ondieki M.Sc. and his staff for their help and hospitality in the years 1967 - 1969. The field observations and collection of coffee material in Embu and Meru were made possible by the cooperation of the field officers of the Ministry of Agriculture in those areas. Finally I wish to acknowledge the opportunity that the Government of the Republic of Kenya and the Kingdom of the Netherlands gave me for this research in cooperation with the then East African Agriculture and Forestry Research Organization under the supervision of the International Agricultural Centre, Wageningen, the Netherlands.

Between my departure from Kenya in September 1969 and the publication of this manuscript in 1979, my secondment to Indonesia from 1970 - 1977 delayed preparation of my CBD research findings *in toto*. After my return to the Netherlands, I was posted to the Laboratory of Phytopathology, Agricultural University, Wageningen, my customary refuge during leaves from overseas. Professor Dr Ir J. Dekker and his staff received me again most kindly, providing all facilities and help.

The idea of preparing a full account of my research in Kenya was reborn in the early part of 1978 and Professor Dekker supported it wholeheartedly. I am most obliged for the encouragement of my old teacher, Professor Dr A.J.P. Oort and for the help I received from Dr Jakoba Ruinen and Ir J.H. van Emden. The continued interest and advice of Professor Dekker were of very great help to me.

I thank Dr Ir P.M. Driessen, Dr Ir R.W. den Outer, Dr Ir W.G. Sombroek and Dr J.C. Zadoks for their advice on particular aspects. Professor Dr Ir Th.M. Wormer (Amsterdam), Dr Ir M. Wessel (Wageningen), Professor Dr J. Kranz (Giessen) and Dr H. Hindorf (Bonn) spent many hours of their time on the manuscript. Their comments about coffee berry disease have undoubtedly increased the value of the report. Parts of the English text were kindly corrected by Mrs Suzanne Beek, Mr J.C. Rigg and Dr Ir P.M. Driessen. Assistance with the illustrative material was provided by Messrs W.C.Th. Middelplaats and G. Eimers and their respective staffs. Mrs Boukje Midden-Krösschell typed the various stages of the draft; I am most thankful for her competent work and efficient help. Mrs Elly Depryck-Steenhaard and her staff bravely shouldered the extra work.

1. INTRODUCTION

Kenya covers an area of 582,646 square kilometers and has a population of more than thirteen million people (ANON., 1976; MAITHA, 1976). Largely as a consequence of differences in altitude and latitude climatic conditions in Kenya vary markedly between areas.

As Kenya lacks sizeable mineral wealth, the economy of the country depends very much on the agricultural sector, which has, in addition to ensuring the major part of local food consumption, provided substantial export earnings and a base for industrial and commercial growth (MAITHA, 1976). The output of approximately 90,000 hectares, planted with arabica coffee, *Coffea arabica* L., is of particular economic importance, as can be seen from the data in Table 1 (after ANON., 1976):

Table 1: Percentages shares of total value of the export of some major commodities, 1970 - 1975 (after ANON., 1976).

	1970	1971	1972	1973	1974	1975
Coffee	21.6	18.2	20.1	22.2	18.2	16.4
Tea	12.3	11.1	13.4	10.5	9.2	10.7
Sisal	1.8	1.4	1.7	3.0	8.0	3.4
Meat and products	2.8	3.5	4.0	2.3	2.1	2.3
Other food and beverages	44.5	37.2	41.6	39.0	34.1	33.3

Before 1963, the year of Kenya's independence, most of the larger coffee plantations, covering approximately 31,000 hectares, were owned by non-African farmers and foreign companies. The coffee on smallholdings was in African hands. Nowadays the coffee industry is to a large extent directly or indirectly owned and managed by Africans (SENGA, 1976).

The coffee industry in Kenya suffered a setback through the economic depression in the 1930's and then during World War II. The occurrence, since 1913, of the destructive leaf rust, caused by *Hemileia vastatrix* Berk. et Br. (RAYNER, 1960) and after 1922 the coffee berry disease ('CBD'), caused by *Colletotrichum*

coffeanum Noack, has adversely affected the progress of the coffee industry (MAINA, 1969). The latter disease was observed for the first time in West Kenya (McDONALD, 1922, 1926) and has spread from there to all coffee growing areas of Kenya, inflicting severe crop losses and even rendering coffee cultivation uneconomic in certain areas marginally suited for coffee (BOCK, 1970; FIRMAN & WALLER, 1977; RAYNER, 1952). Extensive research efforts to find ways and means of controlling CBD have yielded truly effective results only within the last fifteen years.

Some research data on CBD, collected by the author in the period 1964 - 196[illegible] have already been published in scientific and extension papers (VERMEULEN, 1965[illegible] 1966a, b, 1968*, 1970a*, b*, c; VERMEULEN & MEHLICH, 1966; VERMEULEN & PATWA, 1966*; FERNIE & VERMEULEN, 1966; HOCKING et al., 1967*; NUTMAN et al., 1968).

In the present publication unpublished research data of laboratory and field experiments, including mycological and epidemiological studies, are presented together with reprints of some of the earlier papers to give a general view of the progress in research on the coffee berry disease.

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* Reprinted in this publication

2. COFFEE CULTURE IN KENYA

2.1 Biology of *Coffea* spp.

2.1.1 Taxonomy

The taxonomy of the genus *Coffea* (Rubiaceae) is still the subject of scientific discussions (PURSEGLOVE, 1968; WELLMAN, 1961). Most species of this genus originate in Africa and three important commercial species, viz. *Coffea arabica* L. (Arabian or arabica coffee), *Coffea canephora* Pierre ex Fr. (robusta coffee) and *Coffea liberica* Bull. (liberia coffee) are indigenous there (CHEVALIER, 1947).

C. arabica is an allotetraploid believed to be derived from the East African species *Coffea eugenioides* Moore and the West African species *C. canephora*. The distribution of these two species overlaps in Uganda, which is the most likely centre of origin of *C. arabica* (ROBINSON, 1976). The centre of domestication and variation of arabica coffee, however, is in Ethiopia, where no other wild species of *Coffea* occur (FIRMAN & WALLER, 1977).

2.1.2 Varieties and cultivars

C. arabica is autogamous with usually not more than 9 - 11% natural outcrossing (CARVALHO & MONACO, 1969). It has two botanical varieties, respectively *C. arabica* var. *arabica* and *C. arabica* var. *bourbon* (CHEVALIER, 1947; HAARER, 1962).

Selection work at the Scott Laboratories, Nairobi, has led to extensive planting of high yielding and high quality cultivars of *C. arabica*, known as cvs S.L. 28 and S.L. 34.

2.1.3 Morphology

2.1.3.1 General habitat

C. arabica is a relatively small tree or shrub, which prefers tropical highlands (altitude range 1100 - 2500 m) with cool (average daily temperatures around 24°C), but not cold conditions (BROWN & COCHEME, 1969; HAARER, 1962).

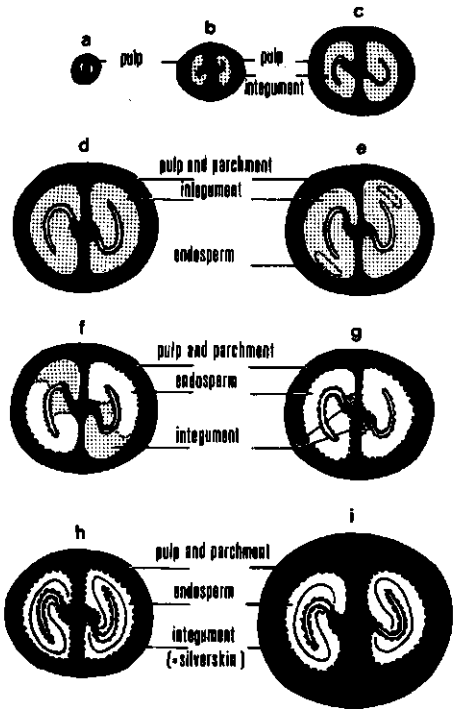

Fig. 2: Stages of berry and bean growth (after WORMER & NJUGUNA, 1966):
a) Pinhead stage (PH); b-c-d) expanding stage (EXS); e-f-g) endosperm growth (ENS); h) endosperm hardening, hard-green berry stage (HGS); i) ripening fruit (RB).

2.2 Arabica coffee in Kenya

2.2.1 Introduction, spread and importance of arabica coffee in Kenya

The crop was introduced to Kenya around 1893 by the early missionaries. According to the available information three different introductions were made, which were known in Kenya as 'French Mission' coffee (JONES, 1956; KIERAN, 1969; POWELL, 1908/1909; TRENCH, 1926). Most of the coffee grown in the mid-1960's, such as cvs S.L. 14, 28 and 34, originated from these introductions.

The economic potential of the coffee industry was realized at an early stage and when white settlers were encouraged to farm in Kenya, large areas were planted with this crop. Before 1933 the cultivation of coffee by Africans was prohibited for economic and phytosanitary reasons (HEYER & WAWERU, 1976). After 1933 permission was granted to plant coffee on a very limited scale in Embu, Meru and

Kisii, but it was not until the end of the 1940's that coffee growing by Africans was allowed in other areas (SMITH, 1976). When they were actively encouraged to participate in the coffee industry (SWYNNERTON, 1954) the role of the African smallholders in the total coffee production increased rapidly, as can be seen from the planted area and production data in Table 3. Nowadays about 270,000 African farmers, organized in cooperative societies, participate in the coffee industry in Kenya (ANON., 1973).

Table 3: Planted area (in thousands of hectares) and production (in thousand metric tons) in coffee estates and smallholdings in the period 1935 - 1974 (after ANON., 1976; KRUG & DE POERCK, 1968; SENGA, 1976).

	Estates		Smallholdings	
	planted area	production	planted area	production
1935	48.0	n.a.	0.4	n.a.
1950	24.0	6.3	0.7	0.2
1955	27.0	12.2	3.1	0.3
1960	28.3	19.1	13.3	4.7
1965	32.5	23.1	50.0	16.2
1970	29.7	27.9	62.6	30.4
1974	28.5	30.8	63.0	39.4

n.a. = not available.

Initially, coffee was grown around Nairobi only, but gradually cultivation spread into areas around Kiambu, Ruiru, Thika, Nyeri, Kitale, Koru, Sotik and eventually even in the Taita Hills, near Voi (ADERO, 1969; HILL, 1956; KRUG & DE POERCK, 1968). The coffee areas in West Kenya, viz. Kisii, Kitale, Koru, Kakamega, etc., are generally referred to as 'West of the Rift' areas, while the regions east of the Rift Valley such as Kiambu, Thika, Ruiru, Embu, Meru, Nyeri, Limuru and Machakos are called 'East of the Rift' areas. The 'Great Rift' is part of the geological system of faults running through Kenya roughly from NNE to SSW, extending from the U.S.S.R. to the Southern part of Africa. The locations of currently producing coffee areas are shown in Fig. 3. For each province the total area planted with coffee is given in Table 4.

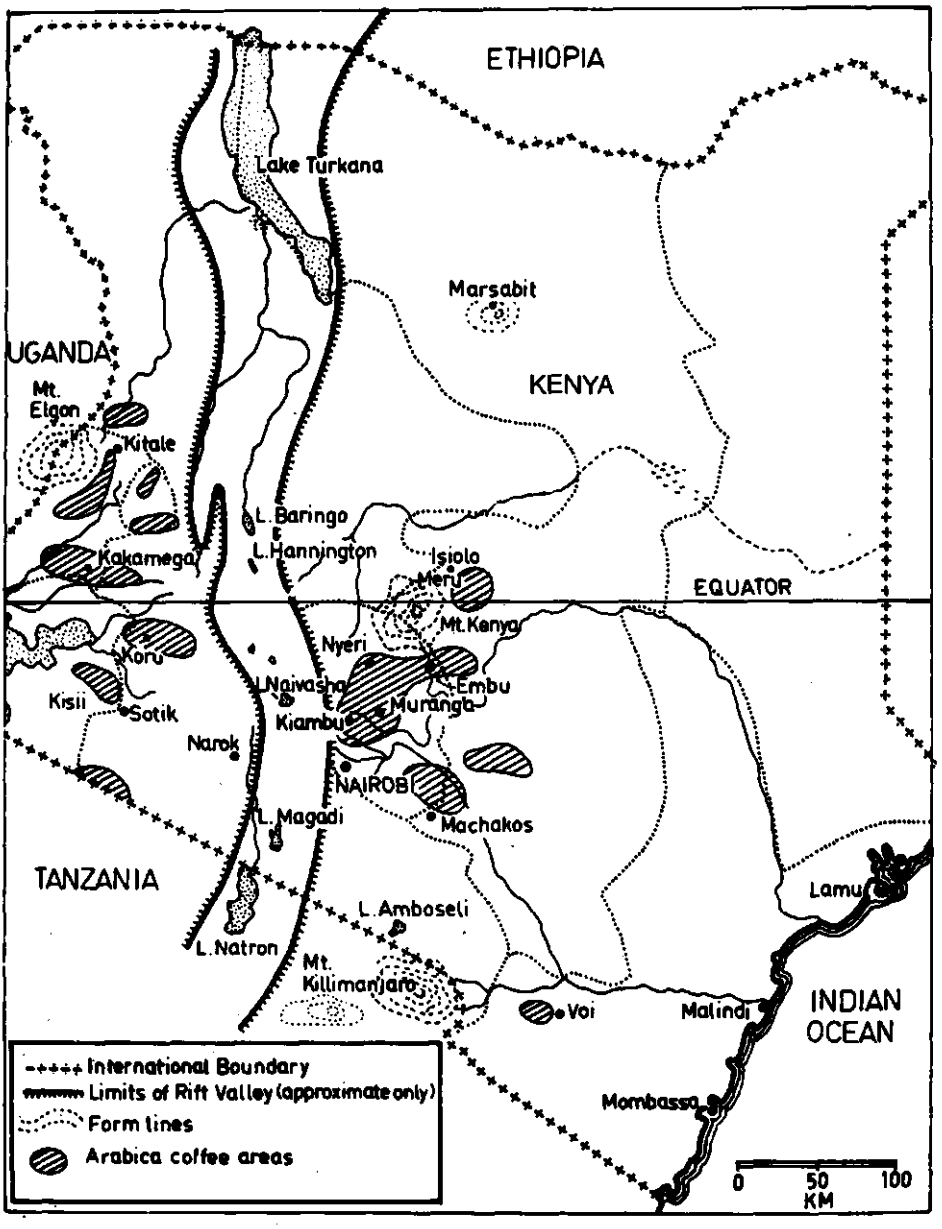

Fig. 3: Main arabica coffee areas in Kenya.

Table 4: Total estate and smallholders coffee areas in thousands of hectares in 1970 by province (after ANON., 1973).

Province	Nyanza	Western	Rift Valley	Central	Nairobi	Eastern	Coast
Estates	-	-	6.1	19.8	1.6	2.2	-
Small-holders	10.4	4.2	0.3	27.0	-	19.1	1.6
Total	10.4	4.2	6.4	46.8	1.6	21.3	1.6

2.2.2 Environmental and cultural aspects of coffee growing in Kenya

2.2.2.1 Environment

a. Climate

C. arabica is grown in Kenya in areas with altitudes ranging from 1200 m up to 2300 m and with annual precipitation figures from 750 mm to more than 2000 mm per annum. Both rainfall and temperature are closely associated with altitude: with increasing altitude temperatures decrease and rainfall increases. According to BROWN & COCHEME (1969) arabica coffee under Kenyan conditions will not thrive in areas where the temperatures regularly rise above 27°C or fall below 10°C at least for many days in succession. In Table 5 climatological data of some locations are presented. The rainfall patterns of the main coffee areas (Fig. 3) are markedly affected by the variations in altitude, exposure and geographical position or characteristics (BROWN & COCHEME, 1969; LUMB, 1966). In most West of the Rift areas, with the exception of Kisii and Sotik in the southern part, there is an absence of a distinct dry season and only one rainy period, the tropical rainfall pattern. In the East of the Rift a distinct bimodal rainfall pattern, the equatorial regime, occurs with the main rainy period in March to May ('long rains') and the slightly less noticeable October to December rain period ('short rains') (BROWN & COCHEME, 1969; HUXLEY et al., 1969). The rainfall patterns are of great importance to coffee, as the onset of the rains triggers the flowering and hence determines the time of the fruiting.

Due to their bimodal rainfall pattern the coffee regions East of the Rift usually experience two flowering flushes, yielding respectively the 'late crop', harvested in October to November and the 'early crop', harvested in May to June. As a consequence coffee trees in that region carry overlapping crops at various stages of development. Additional flowering flushes are triggered by rain showers, which are not rare during the dry period from August to September.

Table 5: Climatological data of some locations in coffee areas of Kenya (after BROWN & COCHEME, 1969; HINDORF, 1973b).

	Approx. altitude in m.	Approx. annual rainfall in mm	Temperature; mean monthly minima and maxima	General rainfall regime
West of the Rift				
Kitale	1900	1130	$10^{o}/13^{o}$ - $23^{o}/29^{o}$C	Monomodal (Tropical) regime with one rainy period
Kakamega	1554	1922	$14^{o}/18^{o}$ - $26^{o}/30^{o}$C	As Kitale
Koru	1730	1840	$10^{o}/13^{o}$ - $23^{o}/29^{o}$C	As Kitale
Kisii	1981	1300	n.a.	Bimodal (Equatorial). All months humid ($R > E_o$), except July.
Sotik	1646	1000	n.a.	Similar to Kisii, but dry periods more apparent
Rift Valley				
Nakuru	1840	840	$7^{o}/10^{o}$ - $24^{o}/28^{o}$C	Monomodal rain regime; distinct dry period
East of the Rift				
Nyeri	1800	890	n.a.	Bimodal pattern
Machakos (coffee areas)	1800	950	n.a.	Bimodal pattern; distinct dry period
Kiambu (Upper)	1800	830	$7^{o}/11^{o}$ - $26^{o}/30^{o}$C	Bimodal pattern; intermittant rain during dry period
Embu (coffee areas)	1680	1020	n.a.	As Nyeri
Meru	1850	2270	n.a.	As Nyeri; main rainfall in October to December

n.a. = not available.

b. Soils

The soils prevailing in the coffee areas both of the East of the Rift (east and south of Mount Kenya - Nairobi) and the West of the Rift (Mount Elgon, Kisii) are

dark reddish brown to dusky red ('chocolate' coloured), very deep, porous, heavy clays but with a loamy feeling. They are derived from trachyte or phonolite lavas or tuffs, or Tertiary of Early Pleistocene age, often with a recent admixture of volcanic ash. As a group they have been called 'Kikuyu Red Loam'. In the FAO/ Unesco soil legend terminology (1974) they belong to the Nitosols, in the U.S. 'Soil Taxonomy' classification (Soil Survey Staff, 1975) largely to the Rhodic subgroups of the Paleudults or Paleudalfs, or to Palehumults.

Most coffee growing takes place on soils derived from pre-Cambrian crystalline Basement Complex rocks, both in the East Rift (Machakos) and the West Rift (Bungoma, Kakemega, Kapenguria). They are a heterogeneous collection of red and brown soils of various depth, porosity and texture. The majority is classified as Ferric Acrisols or as Orthic Ferralsols (FAO/Unesco, 1974), or as Orthoxic Tropudults or the Tropeptic and Ultic Haplorthox in the U.S. 'Soil Taxonomy' system (SOMBROEK, 1979).

Arabica coffee requires a deep, rich, slightly acid (pH between 5 - 6), free draining soil. When the soil depth is not the limiting factor, the roots of coffee are able to penetrate to 3 - 3.5 m. The cultivation of coffee is confined to flat areas and to hillside slopes, which are only slightly or moderately steep. The steepest slopes with the highest hazards of erosion are carefully protected and the forest vegetation is maintained (BROWN & COCHEME, 1969).

2.2.2.2 Aspects of coffee cultivation

a. Pruning

Two pruning systems are applied in Kenya, viz. the single-stem system (one main vertical stem with bearing horizontal shoots, secondaries and tertiaries, selected during pruning on a framework of permanent branches) and the multiple-stem system (fruits are born on primary and secondary branches, produced laterally from several main vertical stems, which are replaced after five to seven years).

In the 1950's many farmers adopted the multiple-stem system, as it was alleged to give higher yields (HAARER, 1962) and give less pruning problems. The single-stem system was generally recommended for shaded coffee; multiple-stem under conditions without shade (ANON., 1964).

b. Planting distance

The usual planting distance under adequate rainfall conditions, viz. 1000 - 1200 mm per annum, is approximately 2.5 x 2.5 m. Under drier conditions the trees are planted farther apart. In smallholder areas the coffee trees are often planted at 2.0 x 2.0 m. In plantation coffee the interrow spacing is usually not less than 3 m to allow the use of tractor-drawn machinery (WALLIS & WORMER, 1971).

c. Mulch and fertilizers

The application of mulching material has a markedly favourable effect on the coffee yields and quality, while soil erosion and weed growth decrease. As mulch materials banana and maize trash, elephant grass *(Pennisetum purpureum)* and Guinea grass *(Panicum* spp.*)* are applied (ANON., 1964). Extensive research on the fertilizer requirements of coffee has yielded impressive increases in coffee production (ANON., 1964) and especially nitrogen dressings are very rewarding (JONES et al., 1961; PEREIRA & JONES, 1956).

d. Weed control

Weed control in coffee plantations is important, as coffee trees are quickly affected by competing weeds, especially in drier areas. Both hand weeding and chemical control are applied; the former mainly in smallholdings, the latter in plantation coffee (ANON., 1964).

e. Irrigation

In certain stages of the berry growth, viz. the growth of the endosperm *(2.1.3.3)*, an adequate supply of water is required to obtain optimal yields (WORMER, 1964). By irrigation these yields can be realized. On irrigated fields a further increase can be obtained by reducing the plant distances (ANON., 1964; WALLIS & WORMER, 1971).

f. Shade trees

In the higher altitude areas in Kenya coffee is grown under a canopy of shade trees. Usually *Grevillea robusta* A. Cunn. is used, but also species of *Acacia*, *Albizzia* and *Prunus* are planted.

These cultural techniques, when applied in Kenya by skilled farmers, gave considerable yield increases, especially in combination with effective pest and disease control methods *(2.2.4)* and the 'tonic' sprays. The latter increase leaf retention and foliage, thus augmenting coffee yields (RAYNER, 1957; RAYNER & JONES, 1948).

2.2.3 Organization of coffee research

Before 1944 coffee research was carried out by the Department of Agriculture, whose activities were mainly aimed at the large-scale agricultural enterprises. Although hampered by lack of funds, especially during the economic depression in the 1930's, and lack of man-power, the results obtained were considerable, e.g. the high yielding and quality cultivars S.L. 14, 28 and 34 (JONES, 1956; RAYNER, 1952). In 1944 the Kenya Government bought Jacaranda Estate near Ruiru as a centre for coffee research. As such it became fully operational at the end of 1949 (ANON., 1971). Until October 1963 coffee research resorted under the Government, although it received financial support from the Coffee Board of Kenya. Since then it has been run by the Coffee Research Foundation (C.R.F.), which received its finances from the Coffee Board of Kenya and from the Kenya Government (ANON., 1971). The main research station at Ruiru and the substations, located at Meru, Koru and Kisii, are adequately staffed and equipped (ANON., 1973).

2.2.4 General disease and pest situation in Kenya

Arabica coffee suffers, as has been stated before, from two major fungal diseases, viz. leaf rust *H. vastatrix*, and coffee berry disease (CBD), *C. coffeanum*. Leaf rust is effectively controlled by pre-rain sprays of copper containing fungicides (BOCK, 1962a, b; RAYNER, 1962). Although control measures are effectively applied, CBD still constitutes a grave danger (ANON., 1975; FIRMAN & WALLER, 1977).

A list of diseases and pests affecting arabica coffee in Kenya is presented in Table 6.

To reduce losses caused by pests and diseases, a wide range of control measures is recommended and regularly amended to include more effective chemicals or better cultural methods (ANON., 1964, 1970). Thus coffee farmers are provided with up-to-date information prepared by the staff of the Coffee Research Station or through publications in 'Kenya Coffee'.

2.3 Terminology

In this and in most other publications on coffee the coffee fruits are called 'berries', though botanically speaking the fruits are drupes (= stonefruits). As, however, the endocarp of this stonefruit is not very distinctive, the fruit is called 'berry' when still green and 'cherry' when the colour changes from green to red upon ripening.

Table 6: Lost of some major diseases (A) and pests (B) of arabica coffee in Kenya.

Common name	Causal organism	Reference
A.		
Stem Pitting	-	Sheffield, 1963
Warty disease	*Botrytis cinerea* Pers. ex Fr.	Anon., 1970
Root rot	*Armillaria mellea* (Vahl. ex Fr.) Kumm.	Anon., 1970
Elgon die-back	*Pseudomonas syringae* v. Hall	Ramos & Shavdia, 1976
Fusarium bark disease	*Fusarium stilboides* W.	Siddiqi, 1965
Leaf blight and stem dieback	*Phoma tarda* (Stew.) comb. nov. *(basionym Ascochyta tarda)*	Boerema & Bollen, 1975; Firman, 1965; Steward, 1957 (p. 430).
Leaf rust	*Hemileia vastatrix* Berk. et Br.	Rayner, 1960
Coffee berry disease	*Colletotrichum coffeanum* Noack	Firman & Waller, 1977
Berry blotch	*Cercospora coffeicola* Berk. & Cooke	Van der Vossen & Cook, 1975
B.		
Coffee leaf miner	*Leucoptera meyricki* Ghes. and *L. caffeina* Washb.	} Crowe, 1964
Antestia	*Antestiopsis lineaticollis* Stal.	Wheatley, 1962
Thrips	*Diarthrothrips coffeae* Will.	Anon., 1970
Mealy bugs	*Planococcus* spp.	Anon., 1970
Stem borers	*Anthores leuconotus* Pasc.	Tapley, 1960
	Dirphya nigricornis Ol.	Crowe, 1959; Anon., 1970
	Bixadus sierricola White	} Anon., 1970
	Eucosma nereidopa Meyr.	
Lace-wing coffee bug	*Habrochila ghesquierei* Sch.	Anon., 1970
Capsid bugs	*Lamprocapsidea coffeae* Ch.	Anon., 1970
Loopers	*Epigynopteryx strictigramma* Hmps.	Crowe & Leeuwangh, 1965
	Ascotis selenaria reciprocaria Wlk.	Wheatley, 1964
Berry borer	*Hypothenemus hampei* Ferr.	} Anon., 1970
Berry moth	*Phophantis smaragdina* Butl.	
Leaf skeletonizer	*Leucoplema dohertyi* Warren	Crowe, 1960
Brown scale	*Saissetia coffeae* Wlk.	Anon., 1970
Star scale	*Asterolecanium coffeae* Newst.	Crowe, 1962

With regard to the anatomical aspects discussed in this paper, 'bark maturation' will often be mentioned. In young twigs of coffee a phellogen develops in the cortex of green internodes in conjunction with increased radial growth of the bark tissues. This phellogen produces the first periderm or secondary cork. A series of periderms is successively formed beneath the first one as the axis continues to increase in circumference. This process of secondary cork formation and the subsequent colour changes of the surface tissues of the coffee twigs is generally referred to as 'bark maturation'.

In this publication the terms estates and smallholdings are used as follows: a. estates: typical commercial coffee enterprises, usually run and owned by non-Africans and in most cases financially capable of carrying out expensive agronomical and plant protection measures; b. smallholdings: small coffee plantings in a system which is to a smaller or larger extent part of subsistence agriculture, owned by Africans, operating through cooperative societies and often financially restricted to adhere strictly to agronomical and plant protection recommendations, required for the maintenance of the coffee trees.

The following abbreviations are used regularly: a.s.l. = above sea level; CBD = coffee berry disease; CRF = Coffee Research Foundation; CRS = Coffee Research Station; cv(s) = cultivar(s); S.C. = sporulating capacity; S.L. = Scott Laboratories.

3. LITERATURE REVIEW OF COFFEE BERRY DISEASE

3.1 Discovery and spread of coffee berry disease in Kenya

Coffee berry disease (CBD) was detected for the first time in 1922 near Soy and Turbo in Western Kenya close to the border with Uganda (McDONALD, 1922). The disease spread south- and eastwards and by the late 1930's all high altitude coffee areas West of the Rift were affected (RAYNER, 1952).

In 1939 CBD was found on a few coffee plantations near Nyeri on the slopes of Mount Kenya (Fig. 3). The disease appeared in the coffee growing district around Kiambu in 1951, still, however, restricted to altitudes above approximately 1700 m (RAYNER, 1952). In the late 1950's CBD had spread through all high altitude coffee areas East of the Rift (NUTMAN & ROBERTS, 1960a, b).

Coffee areas below 1700 m altitude were found to be affected in 1962 and at the end of the 1960's all coffee growing districts in Kenya (Fig. 3) suffered serious crop losses (GRIFFITHS, 1970).

3.2 Economic losses in Kenya

McDONALD (1926) estimated that CBD, in the first years of its appearance, caused in certain small areas of West Kenya crop losses of approximately 75%. In the early and middle 1930's CBD induced crop losses in the coffee areas West of the Rift, amounting often to 25% of the expected yields (RAYNER, 1952); in some marginal areas coffee growing was abandoned in favour of other cash crops (BOCK, 1970).

The outbreaks of the disease in the main coffee areas of the Central Province resulted after 1951 in some instances in yield reductions, which were estimated by BOCK (1963) at some 75 - 80% of the normal yield. NUTMAN (1966) reckons that CBD reduced the potential crop in 1964 with approximately 19%. MOGK & HINDORF (1975) recorded in their observation sites in the Kiambu area yield losses of up to 80%. The information available (ANON., 1973) suggests that the yields of estate and smallholding coffee dropped from respectively 800 and 1000 kg per hectare to not much more than 400 kg per hectare because of CBD. Since then the estate yields have recovered to pre-1966 levels, but the smallholder yields have remained about 400 to 500 kg per hectare because the costs of CBD control are often prohibitive for the smallholders.

3.3 Symptoms of coffee berry disease

Flowerbuds and flowers are particularly susceptible to CBD. The fruits exhibit an increased susceptibility during certain stages of development (Figs. 2, 6) particularly the expanding stage and part of the endosperm stage of the soft-green berry and the ripening cherry. They can also be affected during the pinhead and hard-berry stages (MOGK, 1970; MOGK & HINDORF, 1975; MULINGE, 1970a).

The first symptoms of attack are usually dark-brown blotches on flowerbuds and flowers or streaks on the white petals. The lesions increase rapidly in size and destruction of the flower is complete within 48 hours. On berries, especially expanding berries, the first symptoms are small dark-brown spots, often on the lateral surface of the berry. These lesions enlarge in area, become slightly sunken and eventually cover the whole berry, while the berry contents are also invaded. Affected berries either fall (berries in the early stages of development) or remain on the tree (hard-berry stage) as blackish, mummified berries. CBD can also cause symptoms on leaves and stems, as shown by HINDORF (1973c). These will not be discussed in this publication.

In the sunken lesions minute, slightly projecting punctuations develop, which are the fruiting bodies (acervuli) of the fungus. Under damp conditions they develop masses of pinkish conidia. If the development of these lesions is halted by the onset of dry weather conditions, they change often in colour from brown-black to ashen-grey, dotted with black acervuli. This type of lesions has been termed 'scab' by McDONALD (1932) and it indicate the inactive presence of CBD on green berries. 'Scab' lesions may change into active (brown-black) lesions again when damp conditions are re-established.

When ripening cherries, which are very susceptible to CBD, are affected yield losses tend to be rather limited, but the processing of these affected berries may give considerable pulping problems. This stage of the disease is often referred to as the 'brown blight' stage, known already in East Africa before the appearance of CBD (McDONALD, 1921; SMALL, 1926) and at that time probably not associated with *C. coffeanum* (McDONALD, 1926).

3.4 Occurrence of coffee berry disease outside Kenya

Except for one recent report on the incidence of CBD in Brazil*, the disease has

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* Dr P. Figueiredo (Inst. Biol. di São Paulo, Brazil), pers. comm. to Dr D. Mulder (Wageningen)

only been reported from arabica growing countries in Africa: Tanzania (TAPLEY, 1964); Ethiopia (FERNIE, 1966; GASSERT, 1976, 1978; MULINGE, 1973); Uganda (BUTT & BUTTERS, 1966); Rwanda (FOUCART & BRION, 1963); Zaïre (HENRARD, 1957); Ivory Coast (BOISSON, 1960; MEIFFREN, 1957); Cameroon (MULLER, 1964, 1970, 1973, 1978; MULLER & GESTIN, 1967) and Angola (DA PONTE, 1966). In the Central African Republic, SACCAS and CHARPENTIER (1969a, b) found *C. coffeanum* also on berries, twigs and leaves of *C. canephora* (robusta coffee) and *Coffea dewevrei* de Wild & Dur. (excelsa coffee).

3.5 *Colletotrichum* spp. on coffee

Colletotrichum spp. have been associated with various symptoms on coffee, viz. the anthracnose symptoms on leaves, branches and fruits and the die-back symptoms of branches, characterized by the desiccation of branch tips (BOISSON, 1960; BUTLER, 1918; HENRARD, 1957; HOCKING, 1966; MUTHAPPA, 1970; SACCAS & CHARPENTIER, 1969a, b; SMALL, 1926). Most of these *Colletotrichum* isolates are classified as *C. coffeanum*, after the isolation described by NOACK in 1901 from coffee in Brazil, and are conidial stages of *Glomerella cingulata* (Stonem.) Sp. & Schr.

SMALL (1926) already pointed out that *Colletotrichum* conidia are so commonly found on coffee, that a large proportion of healthy green twigs and leaves yielded acervuli of *Colletotrichum*, when incubated under moist conditions. RAYNER (1948) postulated that the translucent yellow leaf spots, known as 'weak spots', were caused by a latent infection of *Colletotrichum*. He found that practically out of every piece (up to 1 mm^2) of healthy leaf, green berry stem and pedicel a range of different *Colletotrichum* spp. could be obtained after surface sterilization and subsequent plating on agar. CRITCHETT (1969) presented also evidence for a possible latent phase of CBD in green coffee berries. SHAW (1967) reported a diffuse, yellow leaf spot on arabica coffee in Papua New Guinea, yielding a *Gloeosporium* sp. and a *G. cingulata* and it was suggested that the fungus was present as a latent infection.

The ubiquitous occurrence of *Colletotrichum* spp. particularly on coffee, but also on many other tropical plants, as reported by workers throughout the world (e.g. BOISSON, 1960; BUTLER, 1918; FERNANDEZ, 1961; HENRARD, 1957; HINDORF, 1975; HINDORF & MUTHAPPA, 1974; HOCKING, 1966; MEIFFREN, 1957; MUTHAPPA, 1970; SIMMONDS, 1963; VARGAS & GONZALES, 1972; WELLMAN, 1954) has recently been further documented by the information gathered by MULDER (personnal communication, 1978) in cooperation with research workers in South and Central African coffee growing countries. Numerous *Colletotrichum* isolates from different parts of coffee trees in these

countries were sent to the Netherlands for screening on the presence of *C. coffeanum*.

Hence, it is not surprising that *Colletotrichum* spp. are often isolated from coffee with a range of symptoms or physiological disorders (necrotic spots, moribund tissue, branches with die-back symptoms). An example from Kenya is the Elgon dieback, attributed to *C. coffeanum* (NUTMAN & ROBERTS, 1960a), but caused by *Pseudomonas syringae* v. Hall (RAMOS & SHAVDIA, 1976). On the other hand it is certain that the latent infection of leaves associated with premature leaf fall (RAYNER, 1948) can be controlled with 'tonic' sprays of copper fungicides. This suggests a relationship with the *Colletotrichum* sp. isolated from the leaf stalk (HOCKING, 1967a; HOLLIES, 1967; RAYNER, 1957; RAYNER & JONES, 1948).

3.6 Taxonomy of *Colletotrichum* spp.

The taxonomic classification of *Colletotrichum* species found on coffee is still confused and unsettled. The *C. coffeanum* described by NOACK (1901) was not a pathogen of green berries and the name has been used for practically all *Colletotrichum* isolates obtained from coffee, regardless whether saprophytic or parasitic (FIRMAN & WALLER, 1977; SMALL, 1926).

In East Africa McDONALD (1926) and RAYNER (1941, 1948) already noticed morphological differences in their *Colletotrichum* isolates from coffee and the latter described these differences clearly, proposing the name *C. coffeanum* var. *virulans* for the CBD form isolated from green berries (RAYNER, 1941, 1952). MEIFFREN (1957) in the Ivory Coast recognized three *Colletotrichum* strains based on conidial size and setae. NUTMAN & ROBERTS (1960b) considered the CBD pathogen morphologically indistinguishable from the non-pathogenic forms, as described by SMALL (1926).

In 1969 GIBBS differentiated the isolates of *Colletotrichum* obtained from coffee. He classified four main 'strains' of *Colletotrichum* by colony characteristics, using single spore isolates: CBD (= *C. coffeanum* var. *virulans*, RAYNER, 1941), *ccp* (= *C. coffeanum*, 'pink'), *ccm* (= *C. coffeanum*, mycelial form), and *cca* (= *C. coffeanum*, acervuli form). For the first time a clear differentiation had been made between the *Colletotrichum* species inhabiting coffee bark and hitherto considered to be *C. coffeanum*, the cause of coffee berry disease (NUTMAN & ROBERTS, 1961).

Later, HINDORF (1970, 1972, 1973a, b) made detailed morphological studies of the isolates of *Colletotrichum*, obtained from all parts of the coffee tree. By discriminant analysis of *in vitro* measurements of ranges of morphological

characteristics and relating his findings to VON ARX's work (1957, 1970), he came to the following classification:

Colletotrichum coffeanum Noack (*sensu stricto*) = CBD

C. acutatum Simm. (GIBBS, 1969: *cap*)

C. gloeosporioides Penz. (GIBBS, 1969: *cam*)

C. gloeosporioides (GIBBS, 1969: *cca*)

C. gloeosporioides = greenish mycelial form (VERMEULEN, 1970b)

Glomerella cingulata (Stonem.) Sp. & Schr. = white sterile mycelium, perithecia after 2 - 3 weeks

The three *C. gloeosporioides* isolates are conidial stages of *G. cingulata*. The greenish mycelial form, *C. gloeosporioides*, was also found in Tanzania on affected berries (JOHANNS, cited by HINDORF, 1972).

Although it is realized that the HINDORF classification has distinct taxonomical drawbacks, it is still considered the most suitable system available as it provides an acceptable means for the delimitation of the various components of the *Colletotrichum* complex.

3.7 Other host plants of coffee berry disease

SMALL (1926) was the first to carry out cross inoculation experiments with *Colletotrichum* isolates. These isolates were obtained from coffee and other cultivated plants in Uganda. Under certain conditions the coffee isolates could infect other hosts and *vice versa*. The results were, however, erratic.

HOCKING (1971) carried out infection tests on the fruits of plants of nineteen different families, using a CBD isolate and a *Colletotrichum* isolate from a coffee twig. According to this author he could infect the fruits of three plant species with the CBD isolate. With the twig isolate he was able to induce active lesions on fourteen of the nineteen species. CRITCHETT (1969) reported on the behaviour of *C. coffeanum* on other tropical fruits. He induced once disease symptoms by inoculation of a suspension of *C. coffeanum* conidia on tomato fruits.

SMALL, HOCKING and CRITCHETT tried to infect *in vitro* other plants or fruits of these plants. Their results were on the whole not very conclusive. The isolation of *Colletotrichum* spp. by HINDORF (1974) from 28 host plants in Kenya offered the opportunity to test the pathogenicity of these isolates on coffee berries and to compare the cultural characteristics with the CBD fungus from coffee. None of the isolates could infect green berries or were morphologically similar to *C. coffeanum*. HINDORF (1974) found furthermore, that *C. coffeanum* inoculated on a number of plants could not induce any symptoms, but that saprophytic *C. gloeosporioides* and *G. cingulata* were not host specific. These results

elucidate the findings of SMALL (1926) and HOCKING (1971).

Apart from the alleged incidence of *C. coffeanum* on other *Coffea* spp. (SACCAS & CHARPENTIER, 1969a, b) and the isolation of *C. coffeanum* from the bark of the shade tree *Sesbania sesban* (L.) Merill in Ethiopia (GASSERT, 1978), no further evidence is yet available on the presence of CBD on other host plants.

3.8 CBD research in Kenya

3.8.1 Research between 1922 and 1950

In 1922 CBD was found in West Kenya (McDONALD, 1922). According to some farmers the disease had already been present for some years (RAYNER, 1952) and as it spread through the high altitude areas of West Kenya, research efforts were initially focussed on the nature of the pathogen.

McDONALD (1926) found that a form of *Colletotrichum coffeanum* Noack was always associated with the disease symptoms on green berries. Morphologically this form could be easily distinguished from those known to be associated with blight, die-back of branches and leaf symptoms (BUTLER, 1918; McDONALD, 1921; SMALL, 1926). In series of experiments, McDONALD (1925, 1930, 1931, 1932, 1936) studied such aspects as inoculation, *in vitro* culture, viability of conidia and toxicity of copper and oil sprays to conidia, but the results of his experiments were inconclusive.

Through a number of questionnaires sent to coffee farmers in the affected areas, it could be ascertained that CBD was especially severe in comparatively wet regions with high relative humidities. Furthermore the low temperatures of the high altitude areas, in combination with high relative humidity, were found to coincide with high levels of CBD. One of the most common theories at that time was the 'weakened tree' concept, which blamed the condition of trees in neglected plantations for their high CBD susceptibility. Proper weed control, removal and destruction of dead branches and trees, correct pruning and application of lime or calcium carbide Bordeaux sprays were recommended (McDONALD, 1926; RAYNER, 1952). The theory that only neglected trees were susceptible to CBD could not be substantiated, as extensive field trials with manure and fertilizers carried out throughout West Kenya failed to prove any relationship between low levels of major or minor nutrients and high levels of CBD (RAYNER, 1952).

It was found that fungicide sprays in general exerted a favourable effect on the trees, viz. by extending leaf retention, resulting in denser foliage. This effect was twenty years later attributed especially to copper fungicides (RAYNER, 1957;

RAYNER & JONES, 1948). Extensive fungicide screening trials, carried out in many parts of West Kenya, did not result in any clear recommendation for a specific fungicide: most of the fungicides applied seemed able to some degree of controlling CBD. RAYNER (1952) pointed out that frequent sprays would provide an almost complete fungicide cover on the developing fruits which would result in better CBD control. This observation of RAYNER was ignored in the late 1950's and early 1960's, but was completely vindicated at the end of the 1960's when it was clearly shown that fungicidal sprays applied as protectants during the rains were effective to control CBD (GRIFFITHS et al., 1971; MULLER, 1964, 1968, 1970, 1973, 1978; MULLER & GESTIN, 1967).

In the period 1922 - 1950 the only progress in the field of CBD control was the selection of the disease resistant cultivar Blue Mountain by the Agricultural Department.

At the end of the 1950's RAYNER (1952) summarized the research achievements and listed the following research priorities:

- an investigation of the parasitism of all forms or strains of *Colletotrichum* on coffee and the effects of the tissues, in which these forms develop, on the morphology of *Colletotrichum*;
- the method by which the infection passes from one crop to another;
- the effect of climate on the disease;
- the effect of the physiology of the tree on its susceptibility to the disease;
- the causes of resistance and the subsequent search for resistant high yielding, high quality cultivars.

With this list of priorities RAYNER provided at that time already a clear outline of the problems to be solved.

3.8.2 Research between 1950 and 1966

After 1951, CBD spread through the major coffee areas in the Central Province, but no effective control measures were readily available to reduce the losses caused by these new outbreaks. It was realized that new research should be initiated without delay.

As early as 1956 BOCK provided already valuable information on field and laboratory inoculum techniques, thus enabling a standardization of inoculation experiments. BOCK & RAYNER (1956) achieved effective field control of CBD with monthly sprays of phenyl mercury-acetate during the rainy periods, combined with fortnightly sprays of copper fungicides. Because of the phytotoxic effects of mercury on coffee and the possibility of mercury residues in beans (BOCK et al.,

1958) this practice could not be recommended for application in commercial coffee plantings. The search for more effective fungicides was continued (BOCK, 1959) and eventually it was established that copper fungicides gave the best control of CBD when applied before the onset of the rains (BOCK, 1963; CLARKE & WILLIAMSON, 1963).

In the meantime, NUTMAN & ROBERTS (1960a, b, 1961) studied factors affecting conidial germination and infection by the pathogen and the relationship of these factors to the disease distribution. They found that the fungus capable of infecting green coffee berries inhabited the bark of coffee branches, where it had replaced the saprophytic *Colletotrichum*. The maturing bark thus provided, according to NUTMAN & ROBERTS (1961), a continuous source of conidial inoculum responsible for berry infection and the position of maximum inoculum potential coincided very closely with the region of maximum crop concentrations. Hence, it was concluded that the disease level is apparently a function of the degree to which the maturing bark is infested. This theory became known as the 'inoculum potential' theory. They suggested furthermore that the CBD pathogen may have arisen by mutation from a mildly parasitic strain of *C. coffeanum* at some time prior to 1922.

Both the germination of *C. coffeanum* conidia and the infection of the berries can take place within the temperature range of 17^{o} - 28^{o}C, as was established by NUTMAN and ROBERTS (1960b). A combination of suitable temperatures and favourable high relative humidity for a period of five hours would allow infection of the crop and these sharply defined conditions would explain the restriction of the disease to high altitude areas (NUTMAN & ROBERTS, 1960a, b). They suggested that valuable disease predictions could be derived from meteorological data interpretation (NUTMAN & ROBERTS, 1960b, 1969a).

The 'inoculum potential' theory was generally accepted, especially when BOCK (1963), CLARKE and WILLIAMSON (1963) and NUTMAN and ROBERTS (1962a) could demonstrate the effectiveness of copper fungicides, applied before the onset of the rains. High volume sprays of copper fungicides (NUTMAN & ROBERTS, 1962a) applied at the time of maximum inoculum potential of the pathogen just before or during the onset of the rains, would reduce the inoculum potential to low levels and hence reduce the infection of berries.

The already mentioned 'tonic' effects of copper and the fact that copper fungicides were also effective against leaf rust contributed greatly to the increased application of copper formulations in the late 1950's and the early 1960's.

Thusfar the selection of CBD resistant coffee cultivars did not appear to be very promising. FIRMAN (1964) screened over 100 species and cultivars and found a number of these still to be free from infection after a period of two years. However, his material did not possess the economically interesting characteristics

of high quality and yield. Moreover, as chemical control seemed to be effective, further concentrated research efforts in this field were not pursued at that time.

In the beginning of the 1960's the CBD problem seemed to have been solved and research efforts were directed to other disease problems, e.g. leaf rust. After 1962, when CBD also spread into lower altitude areas (below 1700 m a.s.l.), it became increasingly evident that copper fungicides applied at the recommended time, volume and pressure were still not efficacious. In 1964, however, the losses due to CBD became calamitous. WALLIS and FIRMAN (1965, 1967) found that lower volumes, at the same quantity of copper compound per unit area, gave a similar level of disease control as the recommended high volumes (NUTMAN & ROBERTS, 1962a). WALLIS and FIRMAN (1967) were also the first to find a discrepancy between the inoculum potential data collected routinely in their trials and the actual disease incidence.

FIRMAN (1965) briefly reviewed the achievements after 1950 and listed research approaches deemed urgent:

- the mechanism by which the inoculum potential of the bearing wood is reduced by fungicidal sprays;
- a study of the competitive colonization of the maturing bark by the various forms of *Colletotrichum*;
- the relationship between growth and maturity of the branches and the timing of fungicidal sprays, so that timing can be related to biological criteria instead of calender dates.

3.8.3 Research after 1966

Following reports of mounting losses as a result of the continuous spread of CBD and the apparent inadequacy of the recommended chemical control measures, research efforts were renewed in 1964. Considering the importance of CBD for the whole arabica industry in East Africa, NUTMAN and ROBERTS were posted at the East African Agricultural and Forestry Research Organization (E.A.A.F.R.O.) to investigate - in cooperation with the coffee research stations of the three East African countries (Kenya, Uganda and Tanzania) - the biology and ecology of the pathogen in detail. The Coffee Research Station at Ruiru concentrated before 1966 on the search for more effective fungicides. This research was carried out in cooperation with a number of chemical manufacturers, who - at this stage - provided fungicides only. Later, these manufacturers also actively carried out screening trials of their own (e.g. COLLINS, 1968; FORDYCE & SHAW, 1968; BAYER, cited by VERMEULEN, 1968). After 1966 the number of research workers engaged in CBD research throughout Kenya, increased considerably through the support of the British,

West German and Netherlands governments.

In 1964/1965 it was still believed by many people that the spread of CBD into lower altitude areas was mainly caused by a change of the climate triggered by the floods in 1961/1962 (NUTMAN & ROBERTS, 1962a, 1969a, d). Consequently the established control schedules such as pre-rain sprays, were recommended to be applied even more vigorously (WALLIS, 1964) as no basic changes had been made in the disease control concept. The changed weather concept was later refuted by BROWN and COCHEME (1969) and GRIFFITHS and WALLER (1971). The latter workers analyzed rainfall data from 1951 onwards, when CBD first appeared in the main East Rift districts and they could not find any evidence that the climatic conditions during the 1960's differed from those of the 1950's, neither in the high nor in the low altitude coffee areas.

Intensive screening trials of fungicides were carried out at the Coffee Research Station, based on the concept of pre-rain sprays (BOCK, 1963; CLARKE & WILLIAMSON, 1963). The preliminary results of these trials were presented at the First Specialist Meeting on Coffee Research in East Africa, held in Nairobi in 1966 (VERMEULEN, 1966b). The final outcome of these experiments indicated the disease controlling effects of Ortho Difolatan (captafol) as compared to the standard copper formulations (VERMEULEN, 1968). In these screening trials it was also found that the inoculum potential data, routinely assessed according to the technique described by NUTMAN and ROBERTS (1961), did not coincide with the actual incidence of the disease on berries, especially towards the end of the trials. This information was, however, not considered sufficient to invalidate the inoculum potential theory. Moreover, a compound, viz. Tuzet, was found at that time which reduced the inoculum potential so drastically, that it was believed that the cure to CBD had been found (NUTMAN & ROBERTS, 1969b).

At the aforementioned First Specialist Meeting on Coffee Research at Nairobi, MULLER reported on his work on CBD in the Cameroon. He had achieved efficient control of CBD by repeated applications of copper fungicides during the single rainy season in the Cameroon, thus providing a protection for the expanding berries (MULLER, 1964). MULLER suggested a similar approach for Kenya, but his suggestions were considered inapplicable to Kenya, due to differences in climate and cropping pattern, although VERMEULEN and PATWA (1966) reported favourable results by extended spray applications at Meru and Kitale. In a subsequent visit, MULLER (1968) further detailed his suggestions with special reference to the conditions in Kenya. In the meantime, more timing trials had already been carried out and very soon it was discovered that MULLER's suggestions indeed proved to be effective (GRIFFITHS & GIBBS, 1969; GRIFFITHS et al., 1971). The recommendations

were amended accordingly, using Ortho Difolatan and later for some time also Benlate as the most effective fungicides (VINE et al., 1973a, b).

In the second half of the 1960's considerable advances were made in various fields of CBD research. When GIBBS (1969) managed to differentiate the components of the *Colletotrichum* complex inhabiting the bark of bearing branches, this classification formed the basis for detailed work by HINDORF (1970, 1973a, b, c), MULINGE (1971a, b) and VERMEULEN (1970a, b). The conidial production harvested from bearing branches was found to consist mainly of saprophytic conidia of *Colletotrichum*. These findings led to a completely different evaluation of the inoculum potential theory as drawn up by NUTMAN and ROBERTS (1960a, b, 1961, 1969a) and successfully used by BOCK (1963).

FURTADO (1969, 1970) and VERMEULEN (1970b, c) investigated the effects of copper fungicides on the composition of the *Colletotrichum* complex. They found that in sprayed coffee fields a shift towards *C. coffeanum* incidence occurred within the complex and this resulted in an increased level of the pathogen. This shift in the *Colletotrichum* population had been already the subject of hypothetical considerations by ROBINSON and WALLER (1966) and MULDER and HOCKING (1967), while at a later stage the effects of fungicides not only on CBD, but also on leaf rust, foliation and yield, were further discussed (FIRMAN, 1970; GRIFFITHS, 1971; HINDORF, 1973d; MULINGE & GRIFFITHS, 1974a; NUTMAN & ROBERTS, 1969c). The relation between susceptibility to CBD and crop development stages (MOGK, 1970; MOGK & HINDORF, 1975; MULINGE, 1970a), the dispersion of conidia of *C. coffeanum* in trees (MULINGE, 1970b, 1971a; WALLER, 1972a), the effects of altitude on *C. coffeanum* distribution (HINDORF, 1972, 1974; MULINGE, 1971b), the temperature and rain conditions necessary for CBD infection and fungicidal effects on disease development (MOGK, 1973; MULINGE & GRIFFITHS, 1974b; STEINER, 1973a, b, c, 1974) were among the other research findings, which contributed greatly to the knowledge of CBD.

Although the concerted efforts aimed at the causal organism of CBD and its control met with considerable success, it was realized that in the long run this approach would offer at best only a partial disease control and certainly be of a temporary nature, considering the target of the disease, viz. the expanding berries and the physical location of the pathogen in the maturing bark. Therefore an extensive coffee breeding programme was initiated, financed by the Governments of Kenya and the Netherlands. This breeding programme still continues and the results regarding high quality and yielding cultivars with CBD resistant characteristics are most encouraging, involving also international cooperation between coffee producing countries (VAN DER VOSSEN, 1973, 1974; VAN DER VOSSEN et al., 1976).

At present all aforementioned expatriate research staff have been withdrawn,

with the exception of the plant breeder still residing at the Coffee Research Station at Ruiru. Current research on CBD is largely geared to the screening of fungicides with special reference to the increased fungal resistance to systemic fungicides (BAKER, 1972, 1973; COOK, 1975; COOK & PEREIRA, 1977; OKIOGA, 1976; OKIOGA & MULINGE, 1974).

FIRMAN and WALLER (1977) have recently reviewed in detail all research endeavours since 1922.

4. MATERIALS AND METHODS

4.1 Coffee cultivars

In laboratory experiments detached, green, expanding berries (MOGK, 1970; MOGK & HINDORF, 1975; MULINGE, 1970a) of the CBD susceptible cultivars S.L. 28 and 34 (JONES, 1956) were used for the assessment of pathogenicity and for conidial germination trials.

For details on cultivars used in fungicide screening and spray timing trials reference should be made to the reprinted papers by VERMEULEN, 1968; p. 80) and VERMEULEN and PATWA (1966; p. 66). The same applies for information on the cultivars used in the anatomical and mycological studies (VERMEULEN, 1970a, b; p. 36, p. 44).

The field observations on aspects of CBD on African smallholding sites were carried out on the cultivars S.L. 34, French Mission and Kent's selection K7.

4.2 Fungi

For the laboratory screening of fungicides, conidia of the CBD pathogen, *C. coffeanum* were at first harvested from CBD lesions on cherries of cv. S.L. 34 as detailed in the reprinted paper (VERMEULEN, 1968; p. 80). When it was found that on ripe cherries other saprophytic *Colletotrichum* spp. were also present (GIBBS, 1969), only green berries with marked CBD lesions were used, but otherwise the routine remained the same.

In the pathogenicity and germination tests conidial suspensions of the various *Colletotrichum* spp. and of *G. cingulata* obtained from mono-spore cultures (TUITE, 1969) are listed in the reprinted paper (VERMEULEN, 1970a; p. 36).

4.3 Fungicides

A list of chemicals and fungicides tested in laboratory and field experiments is presented in Table 7.

Cadmium containing chemicals were included as standard chemicals in the laboratory tests, because cadmium is positioned near mercury in the periodic system and might possess the same strong CBD controlling characteristics as mercury without, however, the phytotoxic and residual side-effects. Moreover, cadmium

Table 7: Chemical and trade names, code numbers and manufacturers of the compounds screened for CBD controlling characteristics.

Chemical name or code number	Trade name	Manufacturer	Remarks
cadmium chloride crystals	-	-	Pure chemicals used as standard
cadmium sulphide	-	-	
cadmium sulphate	-	-	
mercury bichloride	-	-	
stannous citrate			
cuprous oxide	Perenox	I.C.I.	
Shell-75	-	Shell	Code number
cuprammonium carbonate	Fungex	Murphy Chem.	
cupric oxychloride	Coprantol	I.C.I.	
copper oxychloride 20%	Colloidox	Agricola	
cupric sulphate	Cupravit-Blue	Bayer	
fentinhydroxide	Du-Ter	Philips-Duphar	
fentinacetate	Brestan	Hoechst	
anilazine	Dyrene	Chemagro Co.	
glyodin	Crag Fruit Fungicide 341	Union Carbide	
cycloheximide	Actidione	Upjohn Co.	
blasticidin-S	Blasticidin	Shell	
ethylenebis (tetra-hydrodimethyl thiadiazine-thione)	Fungicide 328	Du Pont	Experimental fungicide
captan	Orthocide	Chevron Co.	
EL-211	-	Eli Lilly	Code number
lead arsenate	Lead arsenate	-	
zineb + nickel ethylene bisdithiocarbamate	Sabithane-M	Rohm & Haas Co.	
mancozeb	Dithane M 45	Rohm & Haas Co.	
dodine acetate	Cuprex	Am. Cyanamid	
zineb + ferbam + maneb	Tricarbamix	Vondelingenplaat	
folpet	(Ortho) Phaltan	Chevron Co.	
captafol	(Ortho) Difolatan	Chevron Co.	
chlorothalonil (DAC 2787)	Daconil	Diamond Co.	
Olin-1763	-	Olin Co.	Code numbers
Olin-1562	-	Olin Co.	
dichlorophen	Halophen (Turbicide)	Sindar	
venturicidin 10%	Venturicidin	Murphy Chem.	
dichloran	Allisan	Boots Pure Drug Co.	
phenyl mercury-acetate	Verdasan	I.C.I.	
maneb	Manzate-D	Du Pont	
ziram	Zerlate	Rohm & Haas Co.	

fungicides are already commercially available. Stannous citrate was included to provide a standard for the tin compounds included in the list of fungicides.

4.4 Isolation of fungi

The methods, used for isolation of fungi from plant material, are described in the reprinted papers (VERMEULEN, 1970a, b; p. 36, p. 44).

4.5 Inoculation methods

The assessment of conidial pathogenicity of isolates or mixed suspensions of *Colletotrichum* spp. (4.2) was carried out on expanding, green berries (4.1), following the inoculation technique of BOCK (1956). To further standardize this technique, droplets of 0.025 ml of conidial suspensions of known concentrations and composition were pipetted with a micropipet onto the berry surfaces.

The boxes, each containing 100 berries and wet blotting paper, were kept closed for 15 - 20 days. Starting from the 8th day after inoculation all infected berries, viz. with CBD lesions originating from the inoculation droplet, were removed and counted every second day. The percentage of affected berries after 18 - 20 days was adopted as a measure of the pathogenicity of the inoculated conidial suspension.

Inoculation tests were also carried out with mixed conidial suspensions of *C. coffeanum* and each of the three saprophytic *Colletotrichum* spp. (HINDORF, 1970, 1973a) at concentration levels varying from 10 to 10^6 conidia per ml. For each concentration level four mixture combinations of pathogen : saprophyte were tested: 4 : 1, 3 : 2, 2 : 3 and 1 : 4.

Mixed conidial suspensions, similarly obtained from the incubated and subsequently washed branches (NUTMAN & ROBERTS, 1961) were inoculated on berries as described under 4.7.

4.6 Assessment of conidial germination and pathogenicity

The experiments included germination tests for a range of conidial concentrations of *C. coffeanum*, the saprophytic *Colletotrichum* spp. and mixtures of conidia of *Colletotrichum* spp. (3.6, 4.5) in hanging drops of sterile water in Van Tieghem cells, on slides with water agar (Fig. 4) and berry surfaces. Furthermore the effects were investigated of conidial exudates on the germination and pathogenicity of *C. coffeanum* on agar and on berries respectively, while also the pathogenicity

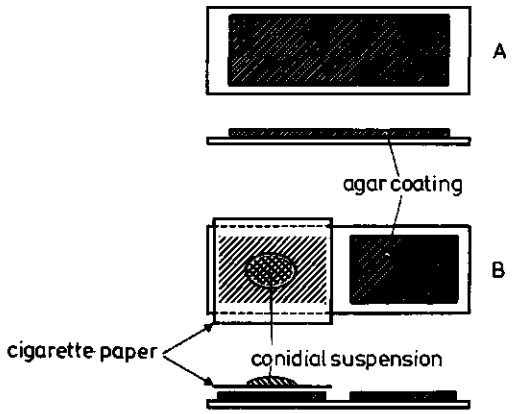

Fig. 4: Slides coated with water agar used for germination tests (A) and to establish effects of exudates (B) of *Colletotrichum* spp.

of *C. coffeanum* alone and in mixtures of conidial suspensions with the saprophytic *Colletotrichum* spp. on berries was assessed. Conidia of the *Colletotrichum* spp. were obtained as described under 4.2.

Germination rates were observed after 8 - 10 hours incubation at a constant temperature of 22°C (NUTMAN & ROBERTS, 1960b); germination was considered positive when the germination tube was at least 2½ x the length of the conidium, or when an appressorium had been formed.

The effect of conidial exudates on germination of *C. coffeanum* on agar was assessed by seeding suspensions (0.1 ml droplets) of conidia (10^5 per ml) of a saprophytic *Colletotrichum* sp. on cigarette paper placed on one half of a divided block of agar (Fig. 4). The paper with the suspension on top of it was removed the next morning, taking care not to spill the suspension. Subsequently on both halves of the agar blocks a suspension of *C. coffeanum* conidia was then pipetted (0.1 ml droplets of 10^5 conidia/ml). After 8 - 10 hours cottonblue-lactophenol was added to the agar blocks and the germination assessed under the microscope.

The effect of the exudates of the saprophytic *Colletotrichum* spp. on the germination of and the infection by *C. coffeanum* of berries was investigated by seeding droplets (0.025 ml) of conidial suspensions (10^5 conidia/ml) on cigarette paper disks (1½ cm diameter) placed on top of green berries. The next morning

these disks and the suspensions on them were removed and the berries immediately inoculated with droplets (0.025 ml) of a CBD fungus conidia suspension (5×10^4 conidia/ml). The infection rate was assessed in the same way as described by BOCK (1956).

When on berries only the germination was assessed the boxes with the berries were opened the morning after inoculation; the inoculation droplets were rapidly dried under a fan and a thin coating of colourless nail-varnish applied to the dried-up droplet. After some minutes the coating could be stripped off easily and mounted in cottonblue-lactophenol. Clear casts of the berry surface with the germinating conidia were thus obtained and counts were be made under the microscope. For the higher concentrations ($10^3 - 10^7$ conidia/ml) the germination of 100 conidia in every droplet was scored, in the order in which they came into view.

Germination and subsequent infection on the surface of berries of green CBD susceptible cultivars was assessed by inoculating expanding berries with a range of concentrations of the CBD fungus and also conidia of *C. coffeanum* in the four mixture combinations and concentration levels (4.5) with the conidia of the saprophytic *Colletotrichum* spp.

4.7 Production of conidia on the bark and their pathogenicity

For the fungicide screening trials, the sporulating capacity of the *Colletotrichum* population in coffee bark (GIBBS, 1969; GRIFFITHS & GIBBS, 1969) was assessed according to the NUTMAN and ROBERTS' technique (1961, 1969a). Slight modifications of this technique were used for the observation in the African smallholding areas.

Random samples of 25 branches per site were collected at three-weekly and later at five-weekly intervals. At the distal end of each branch the green internodes (Fig. 5) were discarded, the cut being made one internode above that which showed the obvious first signs of bark maturation. The surface area of the internodes with little or no signs of bark maturation was still smooth and uncracked by cork formation. The basal portions of each branch (Fig. 5) were also discarded, leaving an eight-internode length of twig (NUTMAN & ROBERTS, 1961). All these lengths were divided into single internodes with cuts at the nodes and subsequently placed into screw-topped jars labelled 7 - 14, the first containing all the 25 distal internodes of the branches from one site and the last those which were fully mature (Fig. 5). The sub-samples were then washed in two changes of water by shaking them in a mechanical shaker for five minutes. After draining, the bottles were incubated for 24 hours at 22-24°C. The newly produced conidia on the branches were then recovered from the internodes by gently rinsing the material in

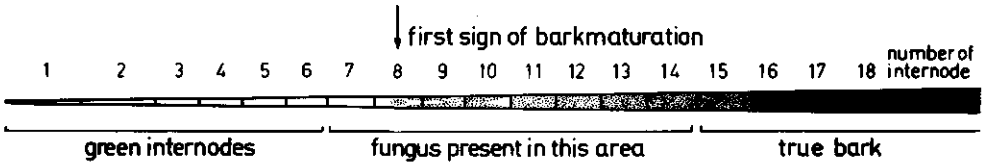

Fig. 5: Longitudinal schematic presentation of a coffee branch and the various stages of bark maturation. The internodes are numbered from the apex to the basal part of the branch. Bark maturation usually starts at internode 8, but this may be different from one branch to another.

two separate changes of water (10 ml each) which were subsequently combined to one sample of 20 ml. Half of this 20 ml suspension was then centrifuged; the conidia in the pellets were killed and stained by addition of two droplets of acid fuchsin in lacto-phenol, and the volumes brought up to 1 ml. The conidia in these samples were counted in duplicate on a haemocytometer. The surface area of the internodes was determined from measurements of their length and water displacement. The number of conidia produced from each twenty-five internode batch was expressed as the conidia production per cm^2 per hour.

However, in order to obtain a necessary reduction in the volume of the work, from April 1968 onwards the internodes 7 + 8, 9 + 10, 11 + 12 and 13 + 14 had to be taken together. Otherwise the procedure remained the same.

The other half of the 20 ml conidial suspension was used for pathogenicity tests on green berries (4.5). Care was taken to adjust the suspensions of all samples by diluting or centrifuging to a concentration of 2×10^4 conidia/ml in order to compare the infectivity of all suspensions with each other and with the infectivity of a 2×10^4 conidia/ml suspension of fresh *C. coffeanum* conidia. These inoculation tests were carried out from March 1967 to June 1969.

4.8 Assessment of CBD levels in field experiments

As it proved difficult to get reliable yield data and thus to obtain information on the effects of CBD on yield from the owners of smallholding sites, visual assessments on the actual CBD incidence were made regularly for each site at Embu and Meru. Ten branches per site were selected at random and marked. The following scoring system was applied, using only the numbers of visually affected flowers and fruits, excluding the intensity of the infection:

no CBD present	: 0
1 - 20% CBD	: 1
21 - 40% CBD	: 2
41 - 60% CBD	: 3
61 - 80% CBD	: 4
81 - 100% CBD	: 5
absence of flowers, pinheads and/or berries	: *

Considering the varying levels of susceptibility to CBD (MOGK, 1970; MOGK & HINDORF, 1975; MULINGE, 1970a) in the natural sequence (Table 2, Fig. 6) of flowers (FL) - pinheads (PH) - soft-green berries (EXS + ENS) - hard-green berries (HGS) - ripe berries (RB), a score of *-*-2-1-0 would mean, that approximately 21 - 40% of the soft-green berries and 1 - 20% of the hard-green berries had been affected by CBD and that no flowers and pinheads had been present. A score of 1-*-*-1-2 would mean, that 1 - 20% of the flowers, 1 - 20% of the hard-green berries and 21 - 40% of the ripe berries had been affected by CBD; pinheads and soft-green berries had been absent.

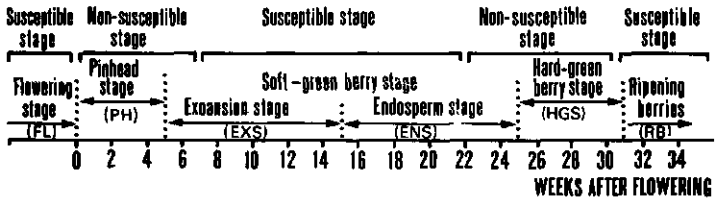

Fig. 6: Natural sequence of flowering and cropping stages in conjunction with susceptibility to CBD (after MOGK, 1970; WORMER & NJUGUNA, 1966).

4.9 Screening of fungicides

The laboratory and field screening methods, used for screening of fungicides, are listed in the reprinted paper on fungicide screening (VERMEULEN, 1968; p. 80).

5. ANATOMICAL AND MYCOLOGICAL ASPECTS

5.1 Coffee berry disease in Kenya. I. Colletotrichum spp. colonizing the bark of Coffea arabica

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Abstract

Several species of *Colletotrichum* occur in maturing bark of *Coffea arabica* branches in Kenya. The *Colletotrichum* population inhabits the bark tissue external to the developing phellogens in the cortex. The *Colletotrichum* species are unable to invade green bark tissue, where the phellogen has not yet been differentiated, while colonization ceases on the phelloderm of the true bark.

Only one of the *Colletotrichum* species discussed in this paper, *C. coffeanum*, can infect green coffee berries. Shortly after the initiation of the first phellogen in the cortex the parasite is in a small area in the bark. It cannot be found in bark tissues where more phellogens have been formed and where the colour of the bark has changed from yellow-green to brown or black.

From the bark of *C. liberica* trees, grown in Kenya, and *C. arabica* cv. 'Bourbon', grown in greenhouses in the Netherlands one of the saprophytic components of the *Colletotrichum* population could be isolated.

It was impossible to induce die-back symptoms or mere infection by inoculation of green internodes even after wounding of live branches of *C. arabica* with any of the *Colletotrichum* components colonizing the bark. It is suggested that die-back systems of coffee in Kenya are primarily caused by unfavourable growing conditions.

Introduction

Colletotrichum coffeanum Noack occurs on *Coffea arabica* L. sometimes as a pathogen but mainly as a saprophyte in all coffee-growing areas of the world (Noack, 1901; Butler, 1918; Small, 1926; Gutierrez, 1954 and Meiffren, 1957). Recently Saccas and Charpentier (1969) reported the occurrence of *C. coffeanum* on *C. canephora* Pierre ex Froehner (Robusta coffee) and *C. dewevrei* d. W. & Dur. (Excelsa coffee) in the Central African Republic.

In 1922 the first definite report of a fungal attack on green coffee berries appeared on the files of the Department of Agriculture in Kenya (Rayner, 1952). Macdonald (1926) described the causal agent as a pathogenic form of *C. coffeanum*. The losses due to berry infection and berry drop were serious and the disease was subsequently named coffee berry disease (CBD).

With CBD gradually spreading through all high-altitude coffee-growing areas (above 1800 m.) of Kenya, research efforts were concentrated on the control of the disease (Macdonald, 1937; Rayner, 1952). These efforts, however, met with little success. Around 1951 CBD had established itself in all high-altitude areas in Kenya. A separate CBD research-unit was founded in 1955 to investigate all aspects of the disease.

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As a result of the work of the CBD research-unit, Nutman and Roberts (1960 and 1961) reported that *C. coffeanum* habitually colonizes the maturing bark external to the developing phellogens in the cortex. They suggested (1961) that the pathogenic form, which probably arose as a mutant before 1922, had displaced the saprophytic form of *C. coffeanum* in the cortical tissues of bearing wood in heavily affected CBD areas. These workers showed that the sporulation of the pathogen on the bearing wood supplied inoculum for both flowers and fruits, while under certain weather conditions the pathogen in the bark would be able to induce 'Elgon die-back' symptoms (Nutman and Roberts, 1960). Reduction of this 'inoculum potential' (Nutman and Roberts 1961) by applying fungicidal sprays before the onset of the rains would give a better and more economic control, than protecting developing berries. Bock (1963) reported favourable results with pre-rain copper sprays.

Shortly after the CBD research-unit had been dissolved and after the very wet 1961–1962 period losses due to the disease increased alarmingly when the low- altitude coffee areas (below 1800 m), previously free of the disease, became infested with CBD. New research efforts were initiated in 1964. At that time it became apparent that the recommended spray programme of pre-rain copper applications was partially or totally ineffective (Wallis and Firman, 1965). A reassessment of earlier work was considered necessary, also because of information obtained from other African countries where CBD occurs (Muller, 1964; Mendes da Ponte, 1966; Butt and Butters, 1966).

Recently Gibbs (1969) and Hindorf (1970) reported in detail on the various components of the *Colletotrichum* population, isolated from *C. arabica* in Kenya. In view of the confused state of the taxonomy of *Colletotrichum* the former author used the generic name alone for the four groups of isolates by him on coffee branches. Hindorf made the following classification for his isolates, based on their cultural characteristics:

1. *C. coffeanum* Noack (Rayner, 1952: *C. coffeanum* var. *virulans*; Gibbs, 1969; CBD),
2. *C. acutatum* Simmonds (Gibbs, 1969: *ccp*),
3. *C. gloeosporioides* Penzy (Gibbs, 1969: *ccm*),
4. *C. gloeosporioides* (Vermeulen, 1970),
5. *C. gloeosporioides* (Gibbs, 1969: *cca*) and
6. *Glomerella cingulata* (Stonem.) Spauld. & v. Schr.

In this classification isolate 1 is the causal agent of the coffee berry disease while isolates 2, 3, 5 and 6 are saprophytes. The mildly parasitic nature of isolate 4 will be discussed at a later stage (Vermeulen, 1970).

This paper will cover the anatomical aspects of bark maturation in relation to the colonization of cortical tissues by the various isolates of *Colletotrichum* (Rayner, 1948; Mulder and Hocking, 1967) and the sporulating capacity of bearing wood of coffee branches (Nutman and Roberts, 1961). The classification given by Hindorf (1970) will be used in this paper.

Materials and methods

To investigate the invasion of the fungus in the cortical tissues in relation to bark maturation (Nutman and Roberts, 1961) branches of three coffee varieties ('Harar', 'S.L. 34' 'Mocha'), susceptible to *C. coffeanum*, were cut off from trees at the National

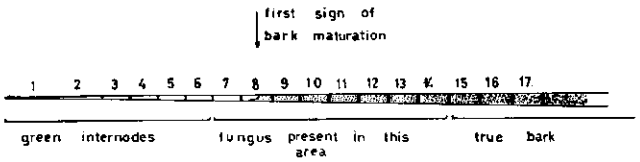


Fig. 1. Longitudinal diagram of a coffee branch. The internodes are numbered from the apex to the basal part of the branch. The position of internodes showing the first signs of bark maturation can differ from one branch to another. *Colletotrichum* spp. are only present in internodes 7–14, not in green internodes or in true (fully mature) bark.

Fig. 1. Lengtediagram van een koffietak. De internodiën zijn genummerd vanaf de top naar het oudere gedeelte van de tak. De plaats van internodiën met het eerste teken van bastrijpheid kan van tak tot tak verschillend zijn. Colletotrichum soorten zijn alleen te vinden in internodiën 7–14 en niet in de groene internodiën of in de internodiën waar de bastrijpheid volkomen is.

Agriculture Laboratories, Nairobi, Kenya, altitude 1700 m. Three or four branches per variety were divided into internodes and placed serially into screw-top jars, each with a number indicating the position of the specific internodes on the branch and the degree of bark maturation (Fig.1), The internodes in the bottles were washed for three minutes in 0.1 % $HgCl_2$ and rinsed twice with sterile water.

Pieces of about 1 mm^2 were cut aseptically from the bark of successive internodes and plated out on water or malt agar. From each internode a series of at least seven or eight pieces of bark was taken along its length, progressing from the distal part towards the basal part of the internode. Any fungus growth originating from the bark pieces on the agar plates was transferred to malt agar after three to four days. The cultures were identified after two to three weeks The conidia of *C. coffeanum*-like cultures were used for inoculation on green berries in order to test each isolate for pathogenicity (Bock, 1956).

Material treated as described above was also taken from *C. liberica* Bull. ex Hiern. and *C. arabica* cv. 'Bourbon' trees. The former trees were grown at the National Agriculture Laboratories, Nairobi, Kenya and the latter in a greenhouse of the Department of Tropical Crop Husbandry of the Agricultural University, Wageningen, The Netherlands.

Inoculation experiments were carried out with the *Colletotrichum* isolates 1, 2, 3 and 5 obtained from maturing bark to assess their capability to invade green internodes of live branches of Arabica trees (cv. 'S.L. 34') and induce symptoms similar to die-back symptoms (Thorold, 1945; Nutman and Roberts, 1960). Agar discs from pure cultures of each of the strains were placed on internodes, which had first been surface sterilized with 0.1 % $HgCl_2$ and washed with water. The inoculum was kept moist with wet cotton-wool and fixed with a piece of polythene sheet around the internodes. After applying the inoculum around the internodes, half of the treated internodes was wounded by needle-pricks.

Of all internodes freehand, freeze microtome and paraffin cross sections were made to study the anatomical changes in the cortex of the maturing bark in the course of the invasion by the components of the *Colletotrichum* population. For the freehand and freeze microtome sections cotton blue-lactophenol or Sudan IV stains were used. The paraffin sections were stained with thionin-light green – orange G, according to Margolena's method (Gray, 1954, p.499).

Results

Nine extensive sets of experiments were carried out from March 1969 to June 1969. In all experiments attention was paid to the colour of the pieces cut from the bark material. It was found that the bark maturation progress often differed from one branch to another on the same tree. Sometimes, also, internodes were already yellow-brown or brown on top, while they were still green on the lower side of the same internode. Therefore, it was decided to use the colour of the bark as a criterion, rather than the actual position of the internode on the branch (Fig.1). In Fig.2 the pieces of bark taken from the internodes are grouped according to colour of the bark. From each group 200 pieces of bark were taken, giving a total of 1200 pieces of bark plated out. It can be seen that out of the six *Colletotrichum* isolates defined by Hindorf (1970,) isolate 3 was found most frequently in the groups green II, yellow, yellow/brown I and brown II, followed by isolate 2 and 5. *C. coffeanum*, the causal agent of CBD, could be found exclusively in groups green II, yellow and yellow/brown, though it occurred even there at low frequency, In the cortex of these tissues the first phellogen had just been initiated (Fig. 3) near the stonecell-layer, and the exterior cortical tissue had been cut off very recently from the cambial tissue by the phellogen. As soon as more phellogens had been formed (Fig. 4) and the colour of the bark had changed from yellow-green to brown no *C. coffeanum* isolates could be obtained from the bark tissue. No growth of any *Colletotrichum* has been detected in green bark tissues which did not show signs of phellogen formation (Fig. 5). On the true bark, where two or three phellogens had been formed, no *Colletotrichum* isolates could be obtained either.

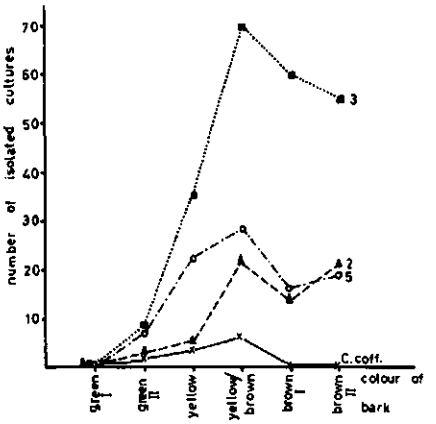
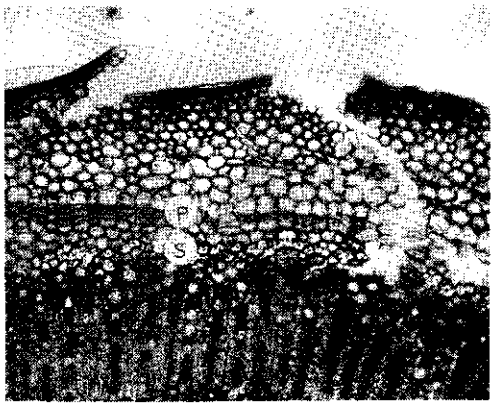


Fig. 2. Total of all cultures of four *Colletotrichum* isolates obtained in nine tests from 1200 pieces of bark cut from the area where *Colletotrichum* is present and plated out on agar. These pieces of bark material are not grouped according to the actual internode position, but according to the colour of the bark; from each group 200 pieces of bark were taken:
green I: completely green bark tissue, no phellogen in cortex;
green II (bordering yellow bark); yellow and yellow/brown: one phellogen in cortex; brown I (bordering yellow/brown bark) and brown II: brown bark tissue, two phellogens in cortex.
C. coff. = *C. coffeanum* = CBD; 2 = *C. acutatum*; 3 = *C. gloeosporioides*; 5 = *C. gloeosporioides.*

Fig. 2. Totaal van alle cultures van vier Colletotrichum isolaten verkregen in negen proeven uit 1200 stukjes bast van het gebied waar Colletotrichum aanwezig is en uitgelegd op agarvoedingsbodem. Deze stukjes bast zijn niet ingedeeld volgens de positie van de internode op de tak, maar ingedeeld volgens de kleur van de bast; van iedere groep waren 200 stukjes bast genomen: groen I: volkomen groen bastweefsel, geen fellogeen in de cortex; groen II (liggend naast de gele bast), geel en geel/bruin: één fellogeen in de cortex; bruin I (liggend naast geel/bruine bast) en bruin II: bruin bastweefsel, twee fellogeenlagen in de cortex.
C. coff. = C. coffeanum = CBD; 2 = C. acutatum; 3 = C. gloeosporioides; 5 = C. gloeosporioides.

Fig. 3. Cross section through green-yellow bark of a coffee branch. The first phellogen (P) has been formed just outside the stonecell layer (S). Magnification about × 130.

Fig. 3. Dwarsdoorsnede van groen-gele bast van een koffietak. Het eerste fellogeen (P) is gevormd juist buiten de steencellaag (S). Vergroting ongeveer 130 ×.



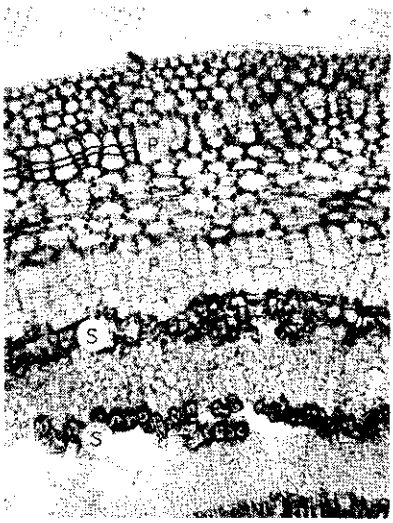


Fig. 4. Cross section through brown bark of a coffee branch. Two phellogens (P) and two stone-cell layers (S) are now present in the bark. Magnification about × 130.

Fig. 4. Dwarsdoorsnede van bruine bast van een koffietak. Twee fellogeenlagen (P) en twee steencellagen (S) zijn nu aanwezig in de bast. Vergroting ongeveer 130 ×.

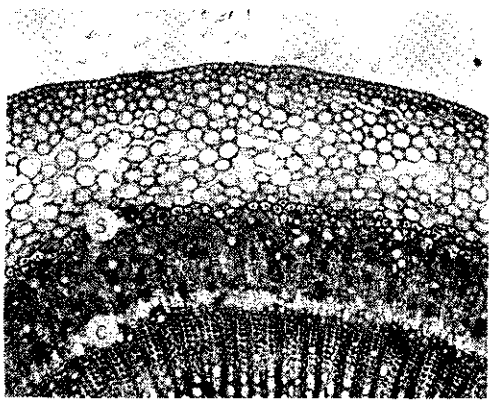


Fig. 5. Cross section through green bark of coffee branch. Outside the cambial tissue (C) only one stonecell layer (S) has been formed. Magnification about × 130.

Fig. 5. Dwarsdoorsnede van groene bast van een koffietak. Buiten de cambiale laag (C) is slechts één steencellaag gevormd. Vergroting ongeveer 130 ×.

The results of the inoculation experiments with *Colletotrichum* isolates 1, 2, 3 and 5 on live green internodes always yielded negative results and no symptoms of die-back could be induced in any of the trials even after wounding the green tissue with needle pricks. This agrees with the conclusion from the results mentioned above, that the *Colletotrichum* isolates are not able to invade tissue where no phellogen has been formed.

The isolates made from *C. liberica* and *C. arabica* cv. 'Bourbon' yielded in all cases isolate 3. The 'Bourbon' material had been maintained in greenhouses for at least twelve years and it seems likely that the fungus was already present in the coffee cuttings imported into the Netherlands.

Discussion

From the data presented in this paper it becomes evident that *C. coffeanum* could only be isolated infrequently from green II, yellow and yellow-brown bark tissues (Fig. 2), where the first phellogen had been formed. The other, saprophytic isolates are present more frequently in the same tissues (respectively approximately ten and twenty times higher than *C. coffeanum*), and continue to be present in the brown bark (Fig. 2, brown I and II respectively). This means that on a bearing branch the role of *C. coffeanum* in the *Colletotrichum* population is usually relatively unimportant and the presence of the parasite is virtually restricted to a small area just apical of the zone with brown colouration. The observations of Nicholls (1969) indicate, however, that there can be appreciable increase in the amount of yellow bark in the early part of the Long Rains (March-April) on certain types of branches. Considering the data presented in this paper the increase of yellow bark surface would mean a higher incidence of *C. coffeanum* and this might explain the CBD-outbreaks during or shortly after the rains. Data presented by Gibbs (1969) and Hindorf (1970), similarly show the relatively low proportion of *C. coffeanum* in the *Colletotrichum* population. The fact that *C. coffeanum* cannot occupy brown cortical tissue has most likely nutritional reasons. No explanation can as yet be offered for the fact that the saprophytic isolates of *Colletotrichum* are also capable of occupying green II and yellow bark. The hypothesis put forward by Mulder and Hocking (1967), that the saprophytes and the parasite would occupy cell-layers at different depths in the cortical tissues might apply to the green-yellow tissues.

Furthermore it becomes clear that the conidia produced on the eight internodes specified by Nutman and Roberts (1961) will be mainly conidia of the saprophytic isolates. Vermeulen (1968) proposed the term 'potential conidial production', covering all *Colletotrichum* conidia produced on the branch. More recently the term 'sporulating capacity' (s.c.) has been introduced (Gibbs, 1969; Griffiths and Gibbs, 1969; Nutman and Roberts, 1969). With our present knowledge of the ratio saprophytic-parasitic conidia produced on the bark it is easy to understand why Wallis and Firman (1965) and Vermeulen (1968) had difficulties to relate their s.c. findings with actual disease incidence.

It should be kept in mind that the data presented in this paper apply to an altitude of approximately 1700 m. Hindorf (personal communication) has observed variations in the composition of the *Colletotrichum* population with the altitude. The role of *C. coffeanum* in the whole population remains, however, relatively small at all altitudes.

The failure to induce die-back symptoms in green internodes of healthy coffee branches with the *Colletotrichum* isolates 1, 2, 3 and 5, even after wounding the bark tissues, suggests that die-back symptoms of branches are indeed most likely to be caused primarily by unfavourable growing conditions (Thorold, 1945). Under adverse conditions the ubiquitous *Colletotrichum* may invade the green bark tissues and even attack the cambial layer, thus causing die-back symptoms. No experimental evidence could be obtained that *C. coffeanum* is able to invade green internodes of live branches (Nutman and Roberts, 1960).

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Samenvatting

Koffiebesziekte in Kenia. I. Colletotrichum spp. die de bast van Coffea arabica koloniseren.

Een aantal soorten van *Colletotrichum* komt voor in de rijpende bast van *Coffea arabica* takken (Fig. 1 en 2) in Kenya. De schimmelpopulatie koloniseert het bastweefsel, dat zich in de cortex buiten de ontwikkelende fellogeenlagen (Fig. 3 en 4) bevindt. Deze schimmels zijn echter niet in staat groen bastweefsel te koloniseren, waar nog geen fellogeen (Fig. 5) is gevormd. In het deel van de tak waar echte schors voorkomt, met in de cortex twee tot drie fellogeenlagen, kon geen *Colletotrichum* worden geïsoleerd. De *Colletotrichum* soort, die groene koffiebessen kan infecteren, *C. coffeanum*, is slechts sporadisch te vinden in een klein gebied van de bast kort na de vorming van het eerste fellogeen (Fig. 3) in de cortex. In bastweefsel waar meer fellogeenlagen zijn aangelegd en de kleur van de bast van groen-geel tot bruin of zwart is veranderd, komt *C. coffeanum* niet meer voor, maar wel andere *Colletotrichum* soorten (Fig. 4).

Met geen van de *Colletotrichum* soorten – beschreven in dit artikel – konden instervings-symptomen worden geinduceerd na kunstmatige infectie van groene internodiën op levende takken. Het was zelfs niet mogelijk na verwonding van de bast infectie te verkrijgen van het bastweefsel, waarin nog geen fellogeen was gevormd. Het lijkt daarom waarschijnlijk dat ongunstige groeiomstandigheden de primaire oorzaak zijn van instervings-symptomen, waargenomen bij koffie in Kenya.

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5.2 Coffee berry disease in Kenya. II. The role of Glomerella cingulata in the Colletotrichum population, colonizing the bark of Coffea arabica

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Abstract

On the maturing bark of cut branches of *Coffea arabica* previously sprayed with copper fungicides perithecia of *Glomerella cingulata* were easily found after two to ten days of incubation. Without fungicides the number of perithecia was decidedly lower. On prunings left on the ground under the coffee trees for 6 to 24 weeks the perithecia could also be found, but the numbers declined rapidly with time.

Perithecia of *G. cingulata* could forcibly discharge ascospores under laboratory conditions. Monospore cultures obtained by catching ascospores on agar, invariably belonged to three *Colletotrichum* types. It was rarely possible to isolate a *Colletotrichum* able to infect green coffee berries. Growth-rate, colour of the mycelium, number of conidia produced *in vitro* and infectivity on green coffee berries, however, differed substantially from *C. coffeanum*, the cause of coffee berry disease.

In Kenya no evidence has been obtained that ascospores from perithecia on bark could infect wounded or unwounded green coffee berries. Neither has any infection been obtained with ascospores from perithecia grown *in vitro*. Possible explanations for the difference with previous findings are offered. Based on the data presented in this paper, it is concluded that *G. cingulata* is not likely to play a role in the epidemiology of the coffee berry disease in Kenya.

Introduction

Macdonald (1926) and Small (1926) described the incidence of *Glomerella cingulata* (Stonem.) Spaud. & V. Schrenk in relation to certain saprophytic isolates of *Colletotrichum coffeanum* Noack obtained from *Coffea arabica* L. in East Africa. In West Africa, Meiffren (1957) and Boisson (1960) published data on the occurrence of *G. cingulata* also on Arabica coffee. No information on the incidence of *G. cingulata* under field conditions was available from East Africa until Nutman and Roberts reported at the First Coffee Specialist Conference at Nairobi, Kenya in 1966 that they had found perithecia of this ascomycete on cut branches of coffee. At that time it was still uncertain which role *G. cingulata* played in the epidemiology of the coffee berry disease (CBD), the most serious coffee problem in East Africa, caused by a parasitic *Colletotrichum* species, now specifically named *C. coffeanum* Noack (Hindorf, 1970).

Hocking *et al.* (1967) reported on findings in Tanzania with *G. cingulata*, while Nutman and Roberts (1969) provided information on the effects of some fungicides on *G. cingulata* in relation to the control of CBD. No further details were, however,

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available on the role of *G. cingulata* in the *Colletotrichum* population colonizing, coffee bark.

This paper reports on the following aspects of *G. cingulata*:

a. the occurrence on sprayed and unsprayed coffee trees,
b. the discharge and the distance of discharge of ascospores;
c. the various forms of *Colletotrichum*, which could be obtained after passing through the *G. cingulata* stage;
d. the pathogenicity of the *Colletotrichum* isolates;
e. the pathogenicity of the ascospores of *G. cingulata*.

Materials and methods

Unsprayed branches from a farm near Nairobi, at 1800 m, or those sprayed with copper from the National Agriculture Laboratories, Nairobi, at 1700 m of 'S.L. 34', 'French Mission', 'Harar' or 'Mocha' varieties of *C. arabica* were cut off; the distal portion of each branch was discarded, the cut being made one internode above the one showing obvious signs of bark maturation. The basal portions were also discarded, leaving an eight-internode long twig (Nutman and Roberts, 1961, 1969). These were divided serially in screw-topped jars. The first bottle contained the internodes 1 + 2, the second 3 + 4, the third 5 + 6, and the fourth 7 + 8. The internodes were washed either with water containing 10 ppm 'Teepol' and afterwards washed in three changes of water or with 0.1% $HgCl_2$ for three minutes and afterwards rinsed in three changes of water.

Prunings left on the ground under coffee trees for periods ranging from 6 to 24 weeks were also collected and treated in almost the same way, although it was very difficult to distinguish any previous signs of the progress of bark maturation on the then moribund tissue. Therefore the three distal internodes were usually discarded and the remaining part of the branch divided serially as described above.

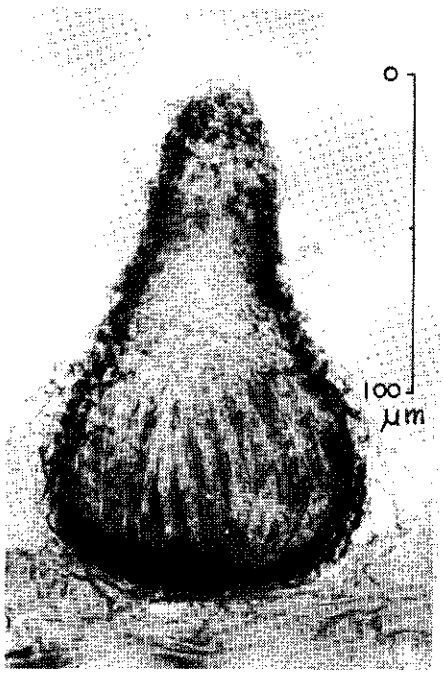
The identification of perithecia of *G. cingulata* was relatively easy as the translucent rostra of the ripe perithecia were typical of this fungus (Fig. 1). None of the other fungi colonizing the bark produced fruiting bodies similar to those of *G. cingulata*.

The discharge of ascospores from perithecia on the bark of cut branches was observed by putting small pieces of the bark material, on which perithecia could be found after two to ten days, facing upwards in the lids of inverted Petri dishes. Vertically discharged ascospores could be found on the agar inverted above the bark material. The horizontal discharge was observed by keeping the Petri dishes with the bark stuck to the lids stored sideways. The distance between bark material and agar could be changed by using different amounts of agar in the Petri dishes. During all experiments the temperatures varied between 22°–24°C.

When the ascospores were found on the agar above the bark material, mono-spore isolations were made under the low-power magnification of the microscope by cutting with a fine, sterile needle small blocks of agar containing one ascospore and transferring these blocks to malt agar plates. The isolates grown from these transfers were after ten to fifteen days classified according to Hindorf's system (1970): 1. *C. coffeanum* (Gibbs, 1969: *C. coffeanum* '*var. virulans*'), 2. *C. acutatum* Simmonds (Gibbs, 1969: *ccp*). 3. *C. gloeosporioides* Penzy (Gibbs, 1969: *ccm*), 4. *C. gloeosporioides*, 5. *C. gloeosporioides* (Gibbs, 1969: *cca*) and 6. *G. cingulata*. This classification and nomen-

Fig. 1. Section through a perithecium of *G. cingulata*, growing on the bark of *C. arabica*.

Fig. 1. Doorsnede van een perithecium van G. cingulata op de bast van C. arabica.



clature have been used throughout this paper. In case of cultural resemblance with *C. coffeanum*, infection tests on green coffee berries (cv. 'S.L. 34') were carried out to establish the pathogenicity of the isolate (Bock, 1956).

To establish the pathogenicity of ascospores, mature perithecia on bark, previously treated for three minutes with 0.1 % $HgCl_2$ and rinsed with sterile water were selected under the binocular microscope. The contents of the fruiting bodies, showing clearly the translucent rostra of the ripe *C. cingulata*-perithecia, were transferred with a fine needle to a bottle with water. After transferring the contents of 100–150 perithecia in this way, the suspension was centrifuged. The supernatant water was then decanted, so that a pellet of ascospores and asci remained in the centrifuge tube. This pellet was suspended in one or two ml water and the concentration assessed on a haemocytometer. Green berries were then inoculated with this suspension; the berries were divided into wounded (needle-prick after inoculation in the centre of the inoculation-droplet) and unwounded berries. Ripe perithecia of *G. cingulata*, grown *in vitro*, were treated in the same way. For all these tests the berries of the CBD-susceptible 'S.L.-34' variety were used, while also in all tests concurrently a batch of berries was inoculated with a fresh conidial suspension of *C. coffeanum* of the same density of inoculum as the ascospore suspension.

Tests were also carried out to assess the effect of sterile tap-water on the germination of ascospores. The Petri dishes were partly filled with sterile tap-water. Pieces of coffee bark with ripe perithecia were stuck to the lids of those dishes. Discharged ascospores were observed floating in the water.

Results

After four to seven days many ripe perithecia were found on the maturing bark of cut branches of coffee, previously sprayed with copper fungicides. Sometimes mature perithecia occurred even after two to three days incubation, only the numbers were much lower than after four to seven days. It was also possible to find new ripe perithecia after eight, nine or ten days. Then numbers of maturing fruiting bodies decreased rapidly. The number of perithecia on coffee branches, which had never been sprayed before with any fungicide was 60–90% lower than on sprayed branches. It was still possible, however, to find fruiting bodies of G. *cingulata* on unsprayed branches. A few perithecia were observed on prunings left for 6 to 24 weeks, but it became increasingly difficult to detect perithecia in the course of this period because of the growth of secondary fungi.

Selected at the proper time, the perithecia in the inverted Petri dishes, started to discharge ascospores after approximately sixteen hours at 22°–24°C. About 72 hours after the bark material had been put in the Petri dishes, discharge was no longer observed. Discharge of ascospores also occurred after surface sterilization, although the numbers of spores caught were 20–40% lower than in the case of bark material only washed with water. The ascospores caught on the agar above the bark material were clearly distinguisable (Fig. 2). When water agar was used the ascospores very often produced not only a germination tube and an appressorium. but also a conidiophore bearing a *Colletotrichum* conidium (Fig. 3). This was also observed when the ascospores germinated in sterile tap-water.

Observations were made on the distance covered by discharged ascospores both vertically and horizontally. A classification was made on how this discharge occurred: the number of spores per discharged projectile in relation to the distance covered (Fig. 4). The data presented in Fig. 4 apply to the vertical discharge range from 0.1–1.2 cm. The horizontal discharge pattern is very similar to that of vertical discharge and the data are therefore not presented in Fig. 4. Projectiles containing four to eight

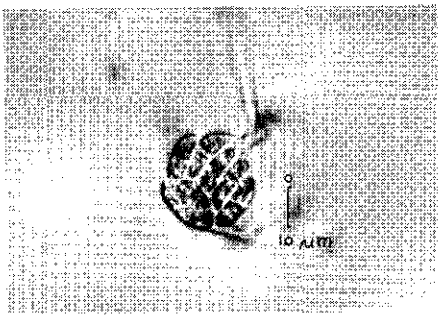


Fig. 2. A group of ascospores of *G. cingulata* caught on agar as one projectile. Germination tubes visible in the agar.

Fig. 2. Een groep van ascosporen van G. cingulata gevangen op agar als één projectiel. Kiembuizen zichtbaar in de agar.

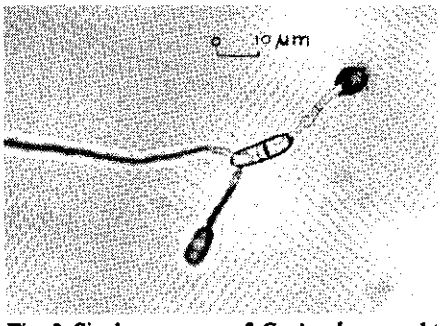
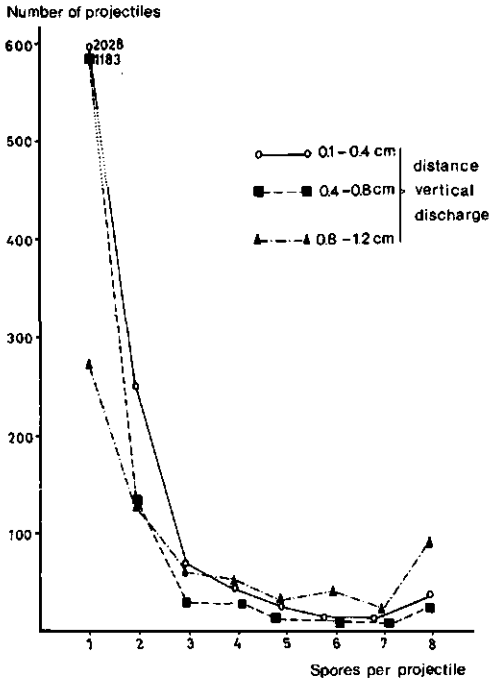


Fig. 3. Single ascospore of *G. cingulata* caught on agar. Germination tube, appressorium, and conidiophore bearing conidium are clearly distinguishable.

Fig. 3. Enkele ascosporen van G. cingulata gevangen op agar. Kiembuis, appressorium en conidiophoor met een conidium zijn duidelijk zichtbaar.

Fig. 4. Diagram of vertically discharged ascospores of *G. cingulata* in discharge distance and number of spores per discharged projectile.

Fig. 4. Grafiek van verticaal uitgeschoten ascosporen van G. cingulata in relatie tot afstand van uitschieten en het aantal sporen per projectiel.



spores were occasionally found at distances of 1.5 cm at most for the vertical discharge and 2.0 cm for the horizontal discharge. These observations are also not given in Fig. 4 because of the very low numbers. Mono-spore isolates from perithecia previously sprayed with copper fungicides yielded in about 90% of the transfers isolate 3, in about 5% isolate 5 and 2–3% isolate 6, the saprophytes classified by Hindorf (1970) as *C. gloeosporioides* (3 and 5) and *G. cingulata* (6). In four cases -during the rain periods of April-May 1967, March 1968, October 1968 and March-April 1969- an isolate mildly parasitic on green berries was isolated which was classified as *C. gloeosporioides*, isolate 4 (Hindorf, 1970). This type was always obtained from material of internodes 1 + 2 and 3 + 4, the younger internodes where the bark maturation had just started. The growth-rate of the greenish mycelium exceeded that of *C. coffeanum*, while relatively few conidia were produced *in vitro*. The pathogenicity of the conidia of isolate 4 on green berries at approximately 10^3 con./ml was about 1/4 of the pathogenicity of *C. coffeanum* at the same density of inoculum. As there were much less perithecia on non-sprayed bark the findings on the distribution of the four types are less reliable. Inoculations with ascospore suspensions, derived from perithecia on bark or perithecia grown *in vitro*, at concentrations ranging from 10^4–10^6 spores/ml. on green coffee berries did not result in obvious lesions on the berries, even after wounding. The *C. coffeanum* inoculations carried out concurrently at the same concentration range gave infections varying from 50–90%.

Discussion

Kaiser and Lukezic (1966) described the discharge of ascospores of *G. cingulata* on bananas and Hocking *et al.* reported the violent discharge of ascospores of the same fungus on coffee in Tanzania. Nutman and Roberts (1969) in Kenya, however, could not catch any airborne ascospores when they left coffee branches carrying large numbers of ripe perithecia suspended vertically above a spore trap in a polythene-covered chamber equipped with a humidifier. Therefore they concluded that *G. cingulata* on coffee lacked the ability to liberate ascospores directly into the air.

The experiments described in this paper and carried out from March 1967–July 1969 show that the ascospores of *G. cingulata* are discharged forcibly into the air under the conditions provided by the inverted Petri dish technique in the laboratory. Discharge also occurs after surface sterilization of the bark containing perithecia. This eliminates any possibility of contamination by *Colletotrichum* conidia sticking to the outside of perithecial tissues. The ascospores caught on the agar above the bark material had the typical form of ascospores of *G. cingulata*. When an ascospore germinated on water agar or in water, it very often produced a conidiophore bearing a typical *Colletotrichum* conidium. The mono-ascospore isolates always gave rise to *Colletotrichum* cultures.

It is difficult to offer an explanation for the discrepancy between the conclusion of Nutman and Roberts (1969) that ascospores cannot be discharged violently and the findings presented in this paper. It may well be that the difference in the findings are only caused by differences in laboratory techniques. Kaiser and Lukezic (1966) found that moisture was the most important environmental factor affecting ascospore discharge. Discharge only occurred when the perithecia were wet or had been kept at 100% R.H. for fifteen hours. This agrees with the findings described in this paper when discharge started after about sixteen hours in the inverted Petri dishes.

The detailed observations by Nutman and Roberts (1969) on the marked effect of spray treatments on perithecial production of *G. cingulata* could be confirmed. The unsprayed branches in the experiments described in this paper showed, however, a decidedly lower production of perithecia than the control treatments of Nutman and Roberts. This is probably due to the fact that the control material used by Nutman and Roberts may have had a spray history, while the unsprayed material used here had never been sprayed with fungicides in the past. This observation supports the suggestion made by Nutman and Roberts (1969) that fungicidal sprays can influence the relative abundance of certain components of the microflora of the maturing bark and in particular the *Colletotrichum* spp. with a *G. cingulata*-stage. Only types 3, 4, 5, and 6 could be grown from mono-ascospore isolates of *G. cingulata* and this agrees with the findings of Hindorf (1970). Isolate 3 being the most common *Colletotrichum* component in the maturing bark at this altitude, it is possible to explain its high level of incidence (Vermeulen, 1970), while the ability of this type to produce perithecia after a relatively short time (Hindorf, 1970) should also be taken into account. *C. coffeanum* and *C. acutatum*, were never obtained after passage through *G. cingulata*. The possibility that *C. coffeanum* would be a danger to the Kenya coffee industry through the transport of ascospores by air seems therefore to be negligible. The mildly parasitic nature of isolate 4, its low level of occurrence and the apparent low productivity of conidia *in vitro* do not seem to indicate that this particular strain will be a

threat to a country like Kenya with an already high level of CBD. This type might be of interest in countries with little or no CBD. Although isolate 4 occurred during the same time of the year and in the same internodes as *C. coffeanum*, this isolate could not be obtained directly from bark (Vermeulen, 1970). This would suggest an even lower level of incidence of isolate 4 than *C. coffeanum*. Research should be continued.

The results described above for the yields of the *Colletotrichum* isolates (approximately 98% saprophytic), obtained through *G. cingulata*, agree with those of direct inoculations of green berries with suspensions of ascospores from perithecia either produced on branches or *in vitro*. Even after wounding no infections could be induced. The ascospore germination was not affected by the tap-water used, as the spores germinated easily in sterile tap-water. These results do not agree with the previous findings of Hocking, Johanns and Vermeulen (1967) who reported successful infections on berries, using ascospores from perithecia of *G. cingulata* on green coffee berries. Several possible explanations for the difference in findings may be offered:

1. The inoculation experiments reported by Hocking *et al.* from Tanzania were carried out with ascospores from perithecia on green berries and not from perithecia on branches or perithecia grown *in vitro*.
2. *C. coffeanum*, introduced only recently in the main coffee area of Tanzania, may not yet have lost its capacity to produce perithecia in that country[1].
3. *C. gloeosporioides*, isolate 4 (Hindorf, 1970) is more prevalent in Tanzania and has been mistaken for *C. coffeanum*, because of its capacity to cause infection on green berries.
4. The level of saprophytic components of the *Colletotrichum*-population is so high in Kenya, that the rare perfect stage of *C. coffeanum*, because of the low incidence of the parasite, has been overlooked.

The observations made on the discharge of ascospores in relation to discharge distance agree with the findings of Ingold (1965, p. 52–74). The discharge distance is amply sufficient to make the spores airborne. As it was hardly likely that *G. cingulata* plays a role in the epidemiology of *C. coffeanum* in Kenya, no further investigations were carried out to assess the liberation of ascospores under field conditions.

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Samenvatting

Koffiebesziekte in Kenia. II. *De rol van Glomerella cingulata in de Colletotrichum populatie, die de bast van Coffea arabica koloniseert.*

Op afgesneden takken van met fungiciden bespoten *Coffea arabica* konden de peritheciën van *Glomerella cingulata* (Fig. 1) in grote hoeveelheden worden gevonden op de

[1] J. C. Hudson has found recently again perithecia of *G. cingulata* on CBD-affected berries in Tanzania (pers. comm.).

rijpende bast na twee tot tien dagen incubatie. Op takken, die nog nooit met fungiciden waren bespoten, lagen de aantallen peritheciën aanzienlijk lager. Ook werd *G. cingulata* gevonden op snoeihout onder de koffiebomen, maar de aantallen peritheciën verminderden snel na het tijdstip van snoeien.

Het bleek mogelijk te zijn om onder laboratoriumomstandigheden peritheciën van *G. cingulata* ascosporen in de lucht te laten schieten (Fig. 2). Ook nadat de takken uitwendig waren ontsmet met sublimaat werden ascoporen uitgestoten. De opgevangen sporen vormden vaak op water agar en in water niet alleen een appressorium en een kiembuis, maar soms ook een conidiophoor met een *Colletotrichum* conidium (Fig. 3). De maximale afstand afgelegd door uitgeschoten ascosporen bedroeg verticaal ongeveer 1.5 cm, horizontaal ongeveer 2.0 cm (Fig. 4).

Een monosporenisolatie van een uitgeschoten en op agar opgevangen ascospore gaf altijd één van vier typen die tot de *Colletotrichum* populatie behoren. Eén van deze vier bleek een *C. gloeosporioides* te zijn, die in staat was groene koffiebessen te infecteren. Groeisnelheid, kleur van het mycelium, het aantal gevormde conidiën *in vitro* en de pathogeniteit op groene koffiebessen bleken echter aanzienlijk te verschillen van *C. coffeanum*, de veroorzaker van de koffiebesziekte. Er konden geen bewijzen worden gevonden, dat ascosporen afkomstig van peritheciën op de koffiebast in staat waren gewonde of niet-gewonde groene koffiebessen te infecteren. Ook ascosporen afkomstig van peritheciën van *G. cingulata in vitro* konden geen groene koffiebessen aantasten. Verschillende mogelijkheden werden gesuggereerd om het verschil te verklaren tussen deze waarnemingen en eerder gepubliceerde gegevens, waaruit bleek dat ascosporesuspensies verkregen van peritheciën op groene bessen wel in staat waren geweest infectie op koffiebessen te veroorzaken.

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5.3 Ascospore Production, Discharge and Infection by *Glomerella cingulata* causing Coffee Berry Disease

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The anthracnose of green coffee berries known in East Africa as 'coffee berry disease' (CBD) causes severe losses in African countries which grow coffee. In Kenya the economic loss last season was estimated at £ 3,000,000 or some 20 per cent of the overall crop. To this must be added another £ 250,000, the cost of control measures[1].

There has been considerable research on the disease and its causal fungus, a virulent form of *Colletotrichum coffeanum* Noack[2-8] (a conidial state of *Glomerella cingulata* (Stonem.) Spauld. and Schenk), as well as on the universally occurring 'brown blight', a related disease restricted in coffee to ripe coffee berries and caused by less virulent forms of the same fungus[9,10]. The virulent form is distinguished from other forms only by its ability to infect green berries and cannot be distinguished on a consistent morphological basis[2,11].

CBD is predominantly dispersed locally by rain splash of conidia produced by saprophytic infections in the bark of the bearing twigs[12,13]. Dispersal over long distances can and does occur through the movement of infected seedlings. This is shown by the presence in non-endemic areas of isolated trees or small groups of trees on which every branch bears infected berries. This does not, however, explain the occurrence of new outbreaks to which no movement of seedlings can be traced, and in which only a very few infected berries are found on isolated single branches on trees otherwise free from disease. Long distance transmission of disease by conidial dispersal through human or other agencies is unlikely because of the susceptibility of conidia to desiccation[14]. The obvious propagule for investigations is the ascospore, which plays an important part in the transmission of many other plant diseases.

Perithecia with ascospores of brown blight forms of *G. cingulata* have been found on twigs[9,15] and on leaves[16] of arabica coffee, but earlier workers have failed to find the perfect state of the form causing CBD[2,3,5-8]. Perithecia have been found in agar cultures said to be of the virulent form[6,8,16,17], but no tests of pathogenicity either of the culture or of the ascospores have been reported.

During our inoculation experiments with isolates of the virulent form from a number of sites in Kenya, fertile perithecia were formed on occasional green berries after prolonged incubation. After this finding, large numbers of infected green berries were collected in the field from various sites and incubated in moisture chambers for 5 weeks at ambient laboratory conditions. 13 per cent of them bore abundant fertile perithecia. Further searches in areas in which CBD is

endemic revealed an occasional berry, stil on the tree, bearing perithecia. A total of eight such berries was found.

At the same time we looked for perithecia on other tissues. Twig prunings and fallen leaves were incubated and invariably bore perithecia in the laboratory. Prunings with abundant perithecia were found occasionally in the field, particularly in wet seasons and areas. The dimensions of asci and ascospores from all sources are shown in Table 1.

Table 1: Dimensions of asci and ascospores from all sources.

	Mean	Range
Asci	61 x 10μ	53-76 x 8-12μ
Ascospores	16 x 4μ	12-23 x 3- 5μ

The ascospores were unicellular, slightly curved and slightly pointed towards the ends, with a distinct central refractive area; they conformed in every way to *Glomerella cingulata*[18].

Ascospores from twigs, leaves and berries germinated readily *in vitro* or on the surfaces of coffee berries, becoming generally one-septate and forming appressoria. Cultures on agar of single ascospores from twigs were infective to green berries in 15 per cent of trials. Cultures of single ascospores from green berries invariably produced infective conidia which in the first generation were 80 per cent as virulent as the same density of inoculum collected from lesions on berries in the field.

By repeatedly washing large numbers of perithecia from green berries until the washings were free from conidia, pure suspensions of ascospores (10^4 spore/ml) were prepared. These were directly infective to detached unwounded green berries (7 per cent) and to detached unwounded internodal regions of green twigs (5 per cent). The latter have previously been found to resist direct invasion by conidial inoculation[6].

By placing mature perithecia on the lids of inverted Petri dishes and finding ascospores stuck to glass slides suspended above, and by observing the growth of cultures on agar inverted above, it was shown that the ascospores were discharged violently. The maximum height of vertical projection was 2.2 cm. This agrees with the results for *G. cingulata* from other hosts[19] and is ample for such spores to become airborne.

Green berries inoculated with ascospores or with conidia from monoascospore cultures usually but not invariably gave rise to further perithecia. This supports the contention that certain strains have an inherent capacity to form perithecia.

The greater frequency of perithecia on twigs and leaves than on berries may be explained by the observation that such tissues are more likely to be invaded by a greater genetical variety of strains. Considering the complexity of the genetics of sexuality in *G. cingulata*[20], it is not surprising that isolated strains on berries are perithecial only infrequently.

Although it remains to be seen whether or not these ascospores are more resistant to adverse conditions than are conidia, this is usually the case with similar fungi, one of the principal functions of the sexual stage being survival. Thus our discovery of the occurrence and infectivity of ascospores, particularly to green twigs, implies that these may be an important source of new outbreaks of coffee berry disease.

A second important function of sexual reproduction in fungi is genetic segregation and recombination. The ability to infect green coffee berries, however, appears to be controlled by a single gene locus and is not a quantitative factor[11]. The discovery of the operation of sexual reproduction in the form of *G. cingulata* which caused CBD does not therefore promote the possibility of a new, yet more virulent, form arising through recombination. On the other hand, it does not exclude the occurrence of further mutation, the probable origin of the present virulent form.

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5.4 Conidial germination and infection

5.4.1 Introduction

NUTMAN and ROBERTS (1960a, b, 1961, 1969a) assumed that the CBD pathogen had completely replaced the saprophytic *Colletotrichum* spp. in the maturing bark of coffee. When it was found, however, that the bark is colonized by a number of *Colletotrichum* spp. (GIBBS, 1969; HINDORF, 1970, 1972, 1973a; VERMEULEN, 1970a, b) it became apparent that the conidia produced on the bark in the past usually referred to as 'inoculum potential' (NUTMAN & ROBERTS, 1961), would be a mixture of conidia of the *Colletotrichum* spp., inhabiting bark tissues.

Experiments were carried out to investigate whether or not the conidia of the different *Colletotrichum* species would affect each other directly or indirectly during germination, thus influencing berry infection by *C. coffeanum*. These included the following aspects: a) the germination of conidia, either in a suspension from a single isolate or a conidial mixture of *Colletotrichum* spp. in water, on agar and on berry surfaces; b) the effects of conidial exudates of saprophytic *Colletotrichum* spp. on the germination of and subsequent infection by *C. coffeanum*

conidia respectively on agar and berry surfaces; and c) the effects on berry infection of conidial mixtures of saprophytic *Colletotrichum* spp. and *C. coffeanum*.

In Table 8 the calculated numbers of *C. coffeanum* conidia present in the germination or inoculation droplets (0.1 and 0.025 ml respectively), at various concentration levels ($10 - 10^7$ conidia/ml) and in mixtures with saprophytic *Colletotrichum* spp. are given.

Table 8: Calculated numbers of *C. coffeanum* conidia present in various droplets and mixtures with saprophytic *Colletotrichum* spp. at a range of concentration levels (see 4.5, 4.6).

Droplet	Conidia concentration per ml	Numbers of conidia per droplet	Numbers of *C. coffeanum* conidia in *Colletotrichum* mixtures (pathogen : saprophyte)			
			path./ sapr. 1 : 4	path./ sapr. 2 : 3	path./ sapr. 3 : 2	path./ sapr. 4 : 1
0.025 ml*	10	0.25	0.05	0.1	0.15	0.2
	10^2	2.5	0.5	1	1.5	2
	10^3	25	5	10	15	20
	10^4	250	50	100	150	200
	10^5	2.500	500	1.000	1.500	2.000
	10^6	25.000	5.000	10.000	15.000	20.000
	10^7	250.000	50.000	100.000	150.000	200.000
0.1 ml**	10	1	0.2	0.4	0.6	0.8
	10^2	10	2	4	6	8
	10^3	100	20	40	60	80
	10^4	1.000	200	400	600	800
	10^5	10.000	2.000	4.000	6.000	8.000
	10^6	100.000	20.000	40.000	60.000	80.000
	10^7	1.000.000	200.000	400.000	600.000	800.000

* Droplets used in infection tests on green berries.

** Droplets used in germination tests on agar.

5.4.2 Results of germination and infection experiments

The germination of the conidia of all *Colletotrichum* spp. proved to be slightly lower on water agar and in hanging drops of sterile water, than the germination on

berry surfaces. On berries the conidia of the saprophytic *Colletotrichum* spp. often formed appressoria without, however, causing infection of the berries. Only the combined results of six series of germination tests on water agar are presented in Fig. 7.

The germination rates of each of the *Colletotrichum* spp. separately did not differ significantly at any of the concentrations tested. At very low concentrations single conidia often did not germinate. Above approximately 10^5 conidia per ml the germination rate of the conidia of all *Colletotrichum* spp. declined slightly. The germination of conidia harvested from pure cultures appeared to be lower than the germination of the CBD fungus conidia from berries.

Conidial mixtures of CBD with conidia of each of the other *Colletotrichum* spp. did not yield germination rates on agar and on berry surfaces different from those observed separately.

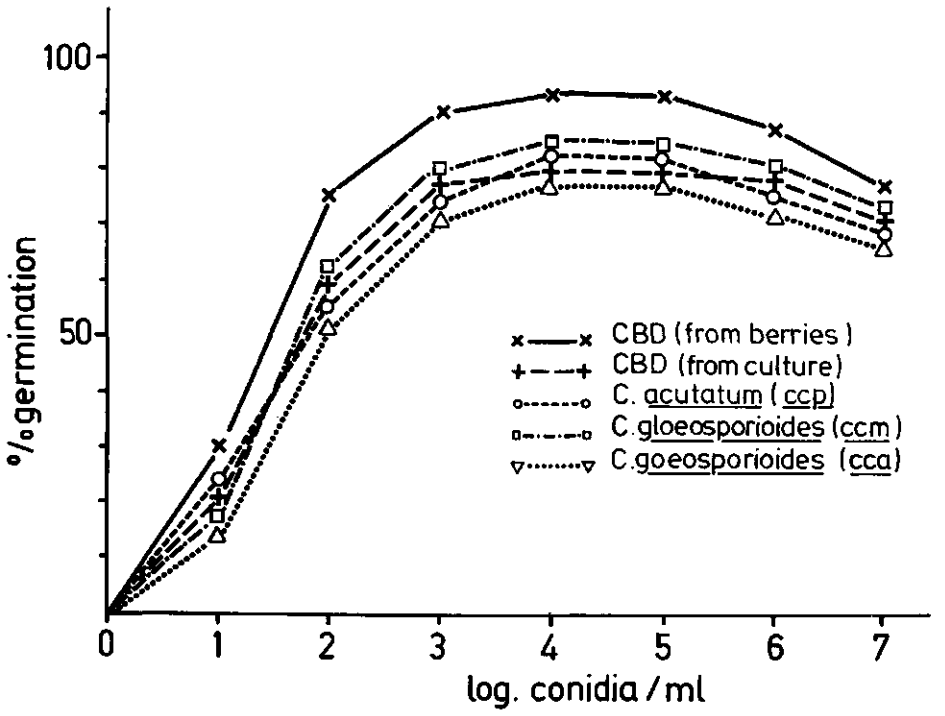

Fig. 7: Conidial germination of *C. coffeanum* (from affected berries and pure cultures) and saprophytic *Colletotrichum* spp. (from pure cultures) on water agar after 8 - 10 hours at 22°C.

Ten series of tests on agar slides and six series of tests on berries were carried out to assess the effects of conidial exudates of each of the *Colletotrichum* spp., separately or mixed. Neither on the agar slides nor on the green berries significant effects could be detected of any of the exudates of saprophytic *Colletotrichum* spp. on the germination of and the subsequent infection by *C. coffeanum* conidia.

The results of nine experiments, in which the infection rate on green berries was studied as a function of the conidial concentration of *C. coffeanum* and the ratio between parasitic and saprophytic *Colletotrichum* spp., are presented in Fig. 8 and Table 9. They show that:

- the rate of infection increases with increasing concentrations of conidia up to a concentration of 10^5 conidia per ml;
- above a concentration of 10^5 conidia per ml the infection rate slightly decreases;
- in mixtures of *C. coffeanum* and the saprophytic *Colletotrichum* species, the infection rate is positively correlated with the percentage of *C. coffeanum* in the mixtures at each conidial concentration level;
- under no conditions an infection rate higher than 75% has been achieved in these trials.

Table 9: Infection percentages on green berries (a) of a range of conidial mixtures of *C. coffeanum* with saprophytic *Colletotrichum* spp. in relation to the calculated number of *C. coffeanum* conidia present in an inoculation droplet of 0.025 ml (b).

Concentration of conidia per ml	Percentage of *C. coffeanum* in conidial mixtures (0.025 ml)									
	20		40		60		80		100	
	a	b	a	b	a	b	a	b	a	b
10^7	12	50.000	25	100.000	40	150.000	55	200.000	70	250.000
10^6	15	5.000	28	10.000	45	15.000	60	20.000	75	25.000
10^5	20	500	32	1.000	50	1.500	62	2.000	70	2.500
10^4	10	50	15	100	22	150	30	200	32	250
10^3	2	5	4	10	6	15	12	20	15	25

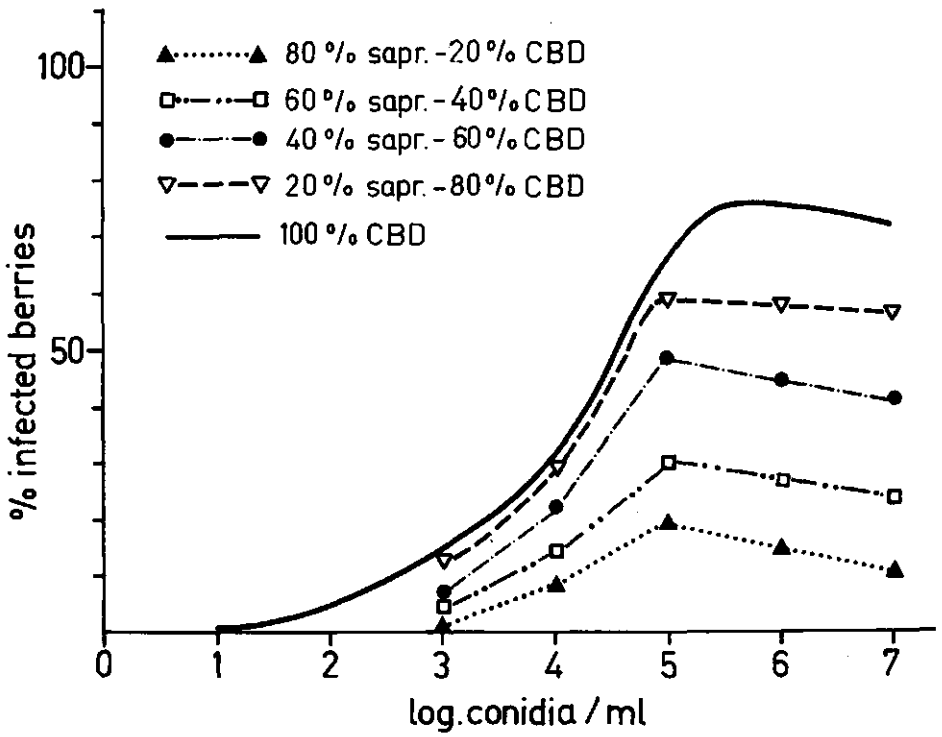

Fig. 8: Infection rate on green, expanding berries inoculated with *C. coffeanum* conidia and with a range of mixtures of conidia of *C. coffeanum* and saprophytic *Colletotrichum* spp. at different concentration levels.

5.5 Discussion

In the tissues of the older internodes, viz. internodes 9 - 14, the saprophytic *Colletotrichum* spp. could be found in considerable numbers, initially increasing with the advance of the bark maturation, but declining again in the oldest two internodes (13 - 14) to a low level (VERMEULEN, 1970a). The presence of *C. coffeanum* was found to be restricted to those internodes with the very first signs of bark maturation, i.e. in cortical tissues where only one phellogen was being formed or had just been formed outside the stone-cell layer. These internodes still have a smooth surface, which is of importance in relation to the tenacity of fungicides during the rainy season (6.6, 7.2). Consequently the parasite is restricted to a small bark area just apical of the zone with advancing stages of bark maturation. As has been shown by NICHOLLS (1969) these areas with the first signs of bark

maturation can extend appreciably in growth flushes during the early parts of the rainy season. The level of *C. coffeanum* in the *Colletotrichum* population will therefore most likely increase during the rains, because of the aforementioned enlargement of *C. coffeanum* containing tissues. In sections 6.3 and 6.4 this increase in *C. coffeanum* level will be further discussed in conjunction with the actual pathogenicity of the bark inoculum.

The fact, that *C. coffeanum* cannot occupy brown cortical tissues was tentatively attributed by VERMEULEN (1970a) to the nutritional requirements of the parasite. Although the nutritional preference cannot be excluded completely, other still unknown factors are probably involved, permitting the pathogen not only to colonize habitually well-defined areas in bark tissues, but also to attack the coffee at the various stages. The inability of *C. coffeanum* to invade younger, green internodal tissues (internodes 1 - 6, Fig. 5) and thus to induce Elgon dieback symptoms, as was alleged by NUTMAN and ROBERTS (1960a), was already demonstrated by THOROLD (1945), HOCKING (1966) and again confirmed by VERMEULEN (1970a). Recently RAMOS and SHAVDIA (1976) showed that Elgon dieback is caused by *Pseudomonas syringae* v. Hall.

The isolations made by VERMEULEN (1970a) from bark material yielded, as was established later, a composition of the *Colletotrichum* population typical for coffee branches at that altitude (approximately 1700 m a.s.l.) and subjected to a copper spraying regime. HINDORF (1973c, 1974) and MULINGE (1971b) found marked variations in the *Colletotrichum* composition, depending on differences in altitude. Considerable information is now also available on the effects of fungicides on the composition of the *Colletotrichum* complex, inhabiting the coffee bark (FURTADO, 1969, 1970; GIBBS, 1969, 1972; GRIFFITHS, 1972; HINDORF, 1970, 1972; VERMEULEN, 1970a, b). With these findings on distribution and composition of the *Colletotrichum* bark population, the inoculum potential theory (BOCK, 1963; NUTMAN & ROBERTS, 1960a, b, 1961) had to be reconsidered and re-evaluated. The consequences of the reconsideration of the inoculum potential theory for disease control are discussed in section 7.2.

The production of mixed populations of *Colletotrichum* conidia may have - after dissemination throughout the coffee tree (WALLER, 1972a) - an influence on the infection of berries. Therefore, the germination of conidia in mixed populations and the subsequent infection of berries by these conidia were studied experimentally.

The germination of *C. coffeanum* conidia on water agar was not affected by the presence of other *Colletotrichum* spp., although both in pure and mixed conidial suspensions the germination rate declined above approximately 10^5 conidia per ml. This decline - also noticed by NUTMAN and ROBERTS (1970) - might be attributed to a 'lumping effect', i.e. conidia remain clustered in concentrated suspensions and

hence the germination rate declines. In very diluted suspensions (10 to 10^2 conidia per ml) single conidia often did not germinate for reasons still unknown. Also STEINER (1973a) noticed both the 'lumping effect' and the absence of germination of single spores.

No effects of the exudates of the saprophytic *Colletotrichum* conidia were observed in the series of experiments, either on the germination of *C. coffeanum* conidia on agar or the pathogenicity of *C. coffeanum* conidia on berries. More conclusive evidence whether or not exudates of *Colletotrichum* conidia would at any time exert effects on the germination of and infection by the conidia of CBD is still required and more research should be carried out to ascertain the observations presented in this publication.

The results of the laboratory infection tests with *Colletotrichum* conidial mixtures on berries also show like the germination rate a decline of the infection rate above approximately 10^5 conidia per ml for both pure CBD suspensions and the mixtures. This decline may again be attributed to the 'lumping effect' as mentioned before. Apart from the physical obstruction exerted by the lumped conidia on each other, it is also possible that the clusters of conidia will not receive the germination impulses from the berry surface, as reported by STEINER (1972b) for the berries of CBD susceptible coffee cultivars.

The decline in the infection rate, at concentrations above 10^5 conidia per ml, is, however, of academic interest only, as similar high conidial concentrations do not occur under field conditions even in the periods conductive for infection, i.e. the rainy season (WALLER, 1971, 1972).

For concentrations below 10^3 conidia per ml, as stated before, either a threshold effect or a reduction of the infection probability might have caused the decline of infection. From Table 9 it appears that at a given concentration of the conidial suspension the percentage of infection is proportional to the increasing number of conidia of *C. coffeanum*, not depending on the numbers of conidia of the saprophytic *Colletotrichum* components of the inoculation droplets. (FURTADO, 1969) and GRIFFITHS and FURTADO (1972) were also unable to find any distinct antagonistic effects exerted by the saprophytic *Colletotrichum* spp. on *C. coffeanum*. Antagonistic effects may, however, be excerted by other micro-organisms persisting on coffee branches (WALLER, 1972a).

By spraying fungicides the total sporulating capacity of the *Colletotrichum* fungi inhabiting the bark is reduced, as was shown by BOCK (1963), NUTMAN and ROBERTS (1961) and was confirmed by the findings presented in Chapter 6, viz. the sporulating capacity data from Meru. GIBBS (1972) established that the

sporulating capacity of *C. coffeanum* soon rose after the termination of spray applications (in the case of pre-rain sprays). In combination with the aforementioned enlargement of *C. coffeanum* containing tissues during the rains and thus the expected higher conidial production of *C. coffeanum*, this would lead to a higher level of *C. coffeanum* conidia during the rains. Hence, in droplets with conidial suspensions deposited on berries, a shift from 20% to 80% CBD at the normal concentration level of 10^3 conidia per ml (WALLER, 1972a) would increase the infection probability from 2% to 12% (Table 9), thus enhancing the importance of bark inoculum of *C. coffeanum*, available for berry infection, considerably.

When *G. cingulata* was found on copper sprayed trees (NUTMAN & ROBERTS, 1969c), most attention was then given to the role this perfect stage would play in the epidemiology of CBD. NUTMAN and ROBERTS were unable in repeated laboratory experiments to catch air-borne ascospores on spore traps used in these trials. However, the results presented by VERMEULEN (1970b) and reprinted here (p. 44), show that ascospores are ejected forcibly over distances of about 1.2 cm vertically, a distance sufficient to make the spores air-borne. From subsequent numerous mono-ascospores isolations only two forms of saprophytic *C. gloeosporioides*, a mildy parasitic *Colletotrichum* sp. and *G. cingulata* could be obtained. Neither *C. coffeanum* nor *C. acutatum* were ever obtained from *G. cingulata*. Despite many attempts, there are no records of the presence of the perfect stage of *C. coffeanum*, having been found in Kenya. As ascospores of *G. cingulata* were found to be incapable to cause infection on green berries, VERMEULEN (1970b) concluded that *G. cingulata* did not play a part in the epidemiology of CBD in Kenya.

In Tanzania, however, HOCKING et al. (1967) found perithecia of *G. cingulata* on green coffee berries after inoculating those berries with a CBD suspension. HINDORF (1972) cited another instance of the presence of *G. cingulata* on berries in Tanzania (JOHANNS, personal communication), giving rise to a *C. gloeosporioides*, capable to cause a low level of berry infection. VERMEULEN (1970b) suggested a number of possibilities to explain the differences between the Kenya and Tanzania findings, e.g. that *C. gloeosporioides* had been mistaken for *C. coffeanum*, because of its cultural similarities and that *C. coffeanum*, newly reported in Tanzania, had not yet lost its capacity to produce perithecia. FIRMAN and WALLER (1977) considered the first suggestion unlikely and pointed out that - with regard to the latter explanation - the CBD in Tanzania was most likely introduced from Kenya, where the fungus was not known to produce perithecia.

As the matter of the disease epidemiology has not yet been settled and the suggestion of transport of CBD with planting material (seedlings), forwarded by

FIRMAN and WALLER (1977), is still without sufficient research evidence, the discrepancy between the results obtained with *G. cingulata* in both Kenya and Tanzania remains unresolved.

6. SPORULATION AND PROBABILITY OF INFECTION IN RELATION TO RAINFALL AND TIMING OF FUNGICIDE APPLICATIONS

6.1 Introduction

For efficient disease control, it was considered important to obtain information about the seasonal variations in sporulating capacity of and infection probability by *C. coffeanum*, inhabiting coffee branches, in relation to rainfall and timing of fungicide application. For this purpose two areas in Kenya were selected, namely in the Embu district on the south-eastern slopes and in the Meru district on the north-eastern slopes of Mount Kenya (Fig. 3). In each district four sites of African smallholders were selected with different levels of CBD and spraying histories. The details of all sites are listed in Table 10. In addition to the information in this table, the following comments should be made:

- In the Embu area the four sites were all situated in Runyenjes, a village about 25 km from Embu-town. The rainfall in the observation area was recorded in the village. In the Meru area the four sites were all near the substation of the Coffee Research Foundation, where the rainfall was recorded.
- The Embu district has climatic conditions almost similar to that of the coffee growing areas around Kiambu. The climatic conditions of Meru differ from the rest of the East of the Rift in this respect that the main rainy season usually occurs in September to November (HUXLEY et al., 1969).
- The spray programme applied on some of the selected sites (Table 10) could be modified to a certain extent in 1968 and 1969 in order to include not only the pre-rain sprays, but also sprays applied during the rains. These modifications made it possible to carry out observations on the favourable effects of the protective action of fungicides, as already indicated by the results of the spray timing trials at Kitale and Meru (VERMEULEN & PATWA, 1966; reprinted on p. 66-71) and also effectively demonstrated by MULLER (1964) in Cameroon.

The data collected in the period March 1967 to July 1969 covered rainfall, sporulating capacity (4.7), pathogenicity on green berries of conidial suspensions obtained from branches (4.7) and three-monthly visual scorings of CBD incidence in the field on labelled branches (4.8). It is clear that a selection of coffee sites in regions more thoroughly researched, would have yielded more standardized information on CBD.

Table 10: Details of selected smallholding sites in the Embu and Meru areas of Kenya.

Site code	Location	Altitude in m	Coffee cultivars	Spray history and schedule	Spraying code
A	Embu	1500	French Mission, S.L. 34, Kent	- before 1969: 2 - 3 copper sprays before the rains - 1969: 2 copper sprays before the rains 2 - 4 copper sprays during and after the rains	Cu-3
	Meru	2000	S.L. 34	- before 1968: 2 - 3 copper sprays before the rains - 1968/1969: 2 copper sprays before the rains - 1969: captafol sprays during the rains	Cu-3
B	Embu	1650	French Mission, S.L. 34, Kent	- before 1968: 2 - 3 copper sprays before the rains - 1968/1969: no application of fungicides	Cu-2
	Meru	2000	Kent	- before 1968: occasional copper sprays before the rains - 1968/1969: copper sprays before and during the rains - 1969: captafol sprays during the rains	Cu-3
C	Embu	1600	French Mission, S.L. 34	- before 1964: pre-rain copper sprays - after 1964: no fungicides applied	Cu-1
	Meru	2000	S.L. 34	- before 1964: no fungicides applied - 1967: 2 copper sprays before the rains - 1968: 2 copper sprays before and during the Febr. to June rains - 1969: 2 copper sprays and 2 captafol sprays during the rains	Cu-3
D	Embu	1600	French Mission, S.L. 34, Kent	no application of fungicides ever	Cu-0
	Meru*	2000	S.L. 34, Kent	no application of fungicides ever	Cu-0

Legend: CU-3 = recommended programme of 4 - 6 copper sprays per annum.

2 = programme of copper sprays either just commenced or terminated during observations.

1 = history of copper applications.

0 = no copper or other fungicide applications ever.

* Observations started in January 1968.

6.2 A short note on some coffee spray timing trials against coffee berry disease

by
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(reprinted from Kenya Coffee, 31, 368, 343-345, 1966)

Introduction

The main problem of the coffee industry of Kenya is coffee berry disease (CBD) caused by the fungus *Colletotrichum coffeanum* Noack. BOCK (1963) found that fungicides based on copper were more efficient than other fungicides in controlling CBD. He also established a spray timing schedule for both East and West Rift areas, having concluded that there was for both regions a critical period in February - April, while for West Rift areas a second period of spraying during June - October was also advisable.

As CBD has now spread into virtually all coffee-growing areas of Kenya and the losses caused by the diseases have increased steadily during the last four to five years, it has been necessary to obtain more detailed information on spray timing for those areas where previously CBD was of little importance.

This paper summarises the results of two copper spray timing trials carried out in the Kitale and Meru Districts.

Spray Timing Trial - Kitale

This trial was started at the beginning of 1965. It had to be discontinued at the end of that year because of the prevalence of suberised berries which interfered with the assessment of the incidence of CBD.

The following treatment were applied in a randomised block design with eight replications:

T_1 September - October, two sprays, at two-weekly intervals.
T_2 February - March, four sprays at two-weekly intervals.
$T_1 + T_2$ Two sprays in September - October and four sprays in February - March.
C Control-unsprayed.

A cuprous oxide fungicide ('Perenox') was applied at a rate per acre of 10 lb for the first, 7 lb for the second and third and 10 lb for the fourth spray in the T_2 treatment and at 10 lb per acre for the T_1 treatment. All sprays were applied with a Cooper Pegler No. 9 bucket pump provided with two lances.

The incidence of CBD in this trial was assessed by weighing separately the healthy and affected ripe berries and by counting the number of healthy and affected green berries on three labelled branches per tree from three trees per plot. Because the occurrence of suberised berries increased greatly about six to eight months after the trial began, it became more and more difficult to assess the incidence of CBD. However, some results of interest were obtained.

The data from the green berry counts are presented in Table 1, while the yields are given in Table 2. Although the data cover only about four months, they suggest that spraying in February - March was more effective than spraying only in September - October, and that a combination of these two treatments was most effective. However, it should be noted that even the $T_1 + T_2$ treatment gave a poor control of CBD since there was about a 40 per cent loss of crop. Whether or not this can be attributed in part to the occurrence of suberised berries is still unknown.

Spray Timing Trials, Meru

These trials were started in 1962 in this locality because Meru district has a climatic pattern different from the rest of the East Rift. It was also hoped that these trials would provide information on the effectiveness of copper spraying on a smallholding scale. The trials were concluded at the beginning of 1965 after adequate information had been obtained.

Four different experimental sites were selected and on these sites the trials were laid out as randomised blocks with the following treatments:

T_1 September - October, four sprays at two-weekly intervals.

T_2 February - April, four sprays at two-weekly intervals.

$T_1 + T_2$ Four sprays in September - October plus four sprays in February - April.

C Control-unsprayed.

Cuprous oxide ('Perenox') was applied with 'Saval' hydraulic knapsack sprayers at a rate of 10 lb per 100 gallons per acre. The yield of each of the plots on all sites was recorded by weighing all ripe healthy and affected berries. Furthermore, healthy and affected green berry counts were carried out at fortnightly intervals on branches on untreated plots adjacent to the four trials.

The information obtained after two and a half years was difficult to interpret as too many different factors had been included. In Table 3 the results are presented. The analysis of the yield results at four sites was carried out on the combined two seasons in both 1963 and 1964. The second crop of 1962 gives in effect the total yield of that year as, due to the floods in that year, there had been no first-season crop.

In the three analyses carried out on the three years yield results from four different sites the treatments between sites showed no differences. In all the three years at all four sites the $T_1 + T_2$ treatment gave the highest yield of clean coffee followed by the T_1 treatment (Table 3). Spraying only in February - April (T_2) gave a less effective control of CBD (Table 3). These results show that the main time for spraying against CBD in the Meru district is the September - October period, with a very beneficial effect of the additional February - April sprays. The spray timing schedule in the Meru district therefore differs by six months from the East Rift regions, where the best control against CBD is achieved in February - April with an additional effect of September - October sprays (BOCK, 1963).

Summary

1. In the Kitale district the main spraying period should be February - April, but additional sprays in June - October are advisable.

2. In the Meru district the main spraying period should be September - October, with an additional CBD-controlling effect of February - April sprays.

Acknowledgments

The Kitale trial was carried out by Mr. R.A. Reynolds, Mr. J.K. Ammonds and the field staff of the Kitale Coffee Research Sub-station. Mr. J.B. Jacob, Kitale, kindly offered the opportunity to carry out this trial on his estate.

The Meru trials were started by Mr. B.F. Hanger and Mr. I.D. Firman and carried out by Mr. J.H.G. Waithaka, Mr. J.W.E. Njagi, Mr. S.E. Ngatia and the field staff of the Meru Coffee Research Sub-station. Four private farmers were kind enough to allow us to carry out the trials on their plots.

Reference

BOCK, K.B. (1963). 'The control of Coffee Berry Disease in Kenya'. *Emp. J. exp. Agric.* 31, 122, 97-107.

Table 1 *(reprinted paper Kenya Coffee, 31, 368, 343-345, 1966)*

KITALE CBD SPRAY TIMING TRIAL

Results of the green berry counts (figures in the table are means of 72 branches)

Overall percentage number diseased berries					
Counts	Dates in 1965	T_1	T_2	$T_1 + T_2$	Control (unsprayed)
1	5 June	0.88	0.20	0.10	0.30
2	21 June	5.77	1.87	1.11	2.25
3	5 July	5.81	1.57	1.18	1.39
4	19 July	5.94	2.51	1.65	2.56
5	4 August	7.71	3.21	1.61	5.11
6	20 August	9.05	3.35	1.28	7.41
7	3 September	10.37	4.63	1.78	8.87
8	17 September	9.33	4.32	1.29	7.99
9	30 September	9.29	4.11	1.54	6.96
Total		62.15	25.79	11.52	42.84
Mean		6.91	2.86	1.28	4.76

T_1 = Sept. - Oct., 2 sprays
T_2 = Febr. - March, 4 sprays
$T_1 + T_2$ = Sept. - Oct., 2 sprays + Febr. - March, 4 sprays
C = unsprayed control

Table 2 *(reprinted paper Kenya Coffee, 31, 368, 343-345, 1966)*

KITALE CBD SPRAY TIMING TRIAL 1965

Table showing percentage (weight) of diseased crop and the individual yield (kg) per treatment

Picking date in 1965	11 October		13 October		25 October		1 November		8 November		15 November		Average percentage diseased	Total yield (kg)
Treatments	Percentage diseased cherry (Mean)	Yield (kg)	Percentage diseased cherry (Mean)	Yield (kg)	Percentage diseased cherry (Mean)	Yield (kg)	Percentage diseased cherry (Mean)	Yield (kg)	Percentage diseased cherry (Mean)	Yield (kg)	Percentage diseased cherry (Mean)	Yield (kg)		
T_1	68	9.98	71	3.13	72	3.49	39	6.06	60	15.24	39	6.95	58	44.85
T_2	39	16.63	57	4.73	57	7.49	34	7.56	66	12.43	59	5.85	52	54.69
$T_1 + T_2$	46	14.66	51	3.62	51	7.74	30	7.99	46	13.13	34	7.71	43	54.85
Control	64	11.49	56	4.10	53	6.54	34	7.29	62	16.96	48	6.88	53	53.26
Standard Error of a Mean	± 8.68	–	± 9.12	–	± 9.71	–	± 6.52	–	± 7.68	–	± 7.68	–	± 4.98	
	$T_2 < C$ (= 0.01)		N.S.		N.S.		N.S.		$T_1 + T_2 < T_2$ ($p = 0.05$)		$T_1 + T_2 < T_2$ ($p = 0.01$)		$T_1 + T_2 < T_1$ ($p = 0.05$)	

T_1 = Sept. – Oct., 2 sprays T_2 = Febr. – March, 4 sprays $T_1 + T_2$ = Sept. – Oct., 2 sprays + Febr. – March, 4 sprays

C = Unsprayed control

Table 3 MERU CBD SPRAY TIMING TRIALS *(reprinted paper Kenya Coffee, 31, 368, 343-345, 1966)*

(a) 1962 yield

Mean yield over four sites of each treatment

Treatments	Average yield in kg/plot	Clean coffee in cwt./acre
T_1	90.80	7.99
T_2	76.30	6.74
$T_1 + T_2$	93.60	9.49
Control	66.90	5.90

A two-way table showing the least significant difference (kg cherry per plot)

Control	T_1	difference
66.90	90.80	+ 23.90
T_2	$T_1 + T_2$	
76.30	93.60	+ 17.30
difference + 9.40		

S.E. = ± 4.87
L.S.D. = ± 9.69 p = 0.05
± 12.84 p = 0.01

(b) 1963 yield (the two cropping seasons combined)

Mean yield over four sites of each treatment

Treatments	Average yield in kg/plot	Clean coffee in cwt./acre
T_1	54.35	4.80
T_2	52.53	4.64
$T_1 + T_2$	61.20	5.40
Control	39.64	2.61

A two-way table showing the least significant difference (kg cherry per plot)

Control	T_1	difference
39.64	54.35	+ 14.71
T_2	$T_1 + T_2$	
52.53	61.20	+ 8.67
difference + 12.89	+ 6.85	

S.E. = ± 4.27
L.S.D. = ± 8.50 p = 0.5
± 11.26 p = 0.1

(c) 1964 yield (the two cropping seasons combined)

Mean yield over four sites of each treatment

Treatments	Average yield in kg/plot	Clean coffee in cwt./acre
T_1	53.42	4.71
T_2	51.69	4.55
$T_1 + T_2$	74.38	6.56
Control	43.84	3.86

A two-way table showing the least significant difference (kg cherry per plot)

Control	T_1	difference
43.84	53.42	+ 9.58
T_2	$T_1 + T_2$	
51.69	74.38	+ 22.69
difference + 7.86	+ 20.96	

S.E. = ± 3.26
L.S.D. = ± 6.49 p = 0.05
± 8.60 p = 0.01

N.B. The average yield in kg/plot is converted to clean coffee weight expressed in cwt./acre.

6.3 Sporulating capacity in relation to rainfall and fungicide applications

The data obtained of the total S.C. of the branches of the sites at Embu and Meru are depicted in Figs 9 and 10, respectively, together with the rainfall figures of each area and the copper spray schedule applied. The seasonal fluctuations of the total S.C. are similar to those previously reported for the Kiambu area by NUTMAN and ROBERTS (1961, 1969a), i.e. the S.C. produced peaks in conidial production before the onset of and during the rains. The various S.C. levels of the individual sites showed, however, marked differences caused by the different spray regimes. Comparing the S.C. data from Embu and Meru it should be noted that the Embu figures show a gradual increase towards the end of the observation period, viz. the beginning of 1969, while at Meru the levels of S.C. remain lower and more regular. In both locations the highest S.C. figures are those for the unsprayed control treatments.

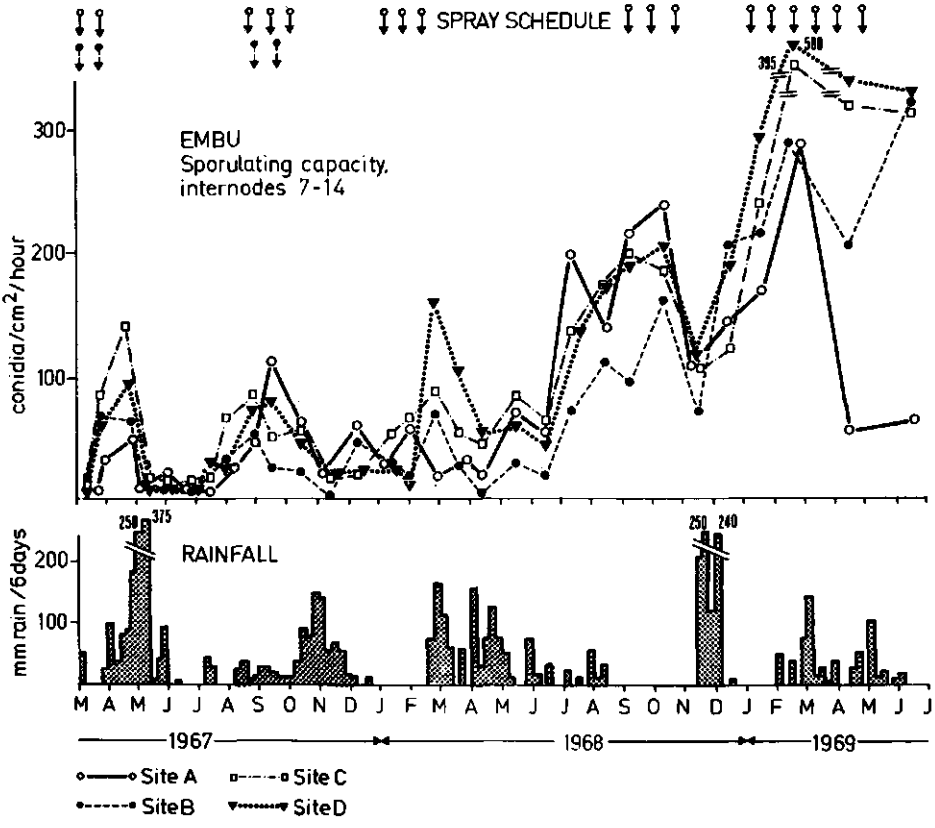

Fig. 9: Sporulating capacity data (*sensu* NUTMAN & ROBERTS), rainfall figures and spraying schedule for copper fungicides (indicated by vertical arrows) in the Embu observation area.

The S.C. data of the individual internodes, viz. internodes 7 - 14 of each site are not given separately, as the presentation of all these data would have been too detailed. They all show the same seasonal rhythm with the highest numbers of conidia produced per cm^2/h for the internodes 10, 11 and 12, as reported by NUTMAN and ROBERTS (1969a) for material obtained in the Kiambu area.

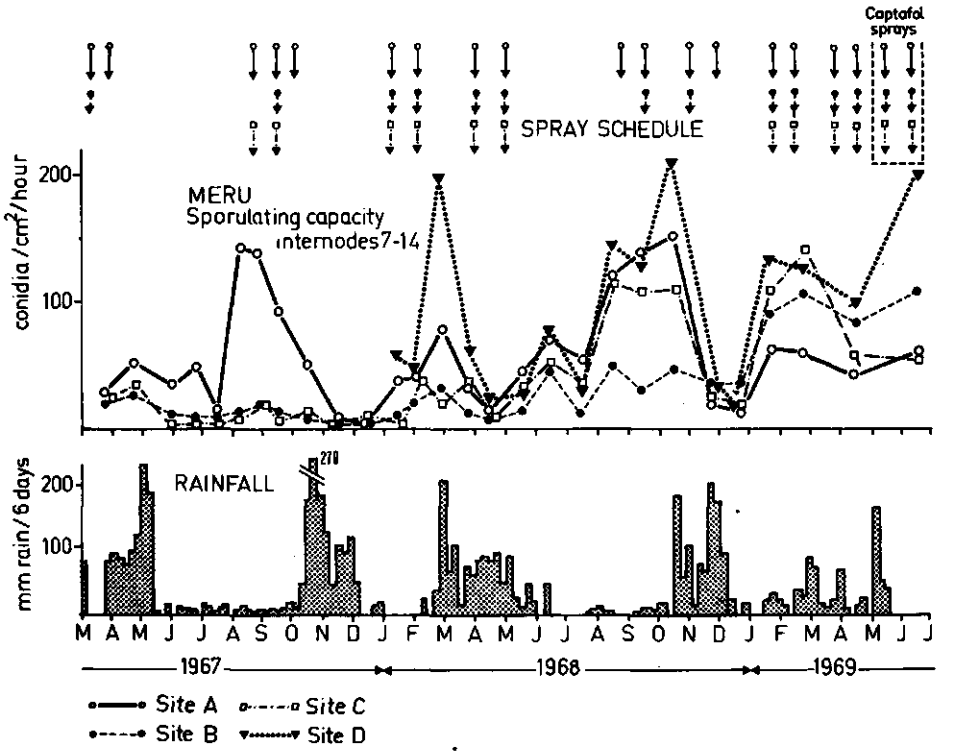

Fig. 10: Sporulating capacity data (*sensu* NUTMAN & ROBERTS), rainfall and spraying schedule for copper and captafol fungicides (indicated by vertical arrows) in the Meru observation data.

6.4 Pathogenicity of conidia produced on branches in relation to sporulating capacity

The pathogenicity of conidia washed off branches was assessed for each of the eight internodes separately. No infections on green berries of susceptible coffee varieties could be obtained with the conidia suspensions of internodes 9 - 14. From the sprayed sites the conidial suspensions of internodes 7 and 8 only gave marked infections on green berries usually during or shortly after the rains. In Figs 11

and 12 these findings are represented for Embu and Meru respectively, combining the cropping cycles on the trees and the S.C. data of the internodes 7 and 8 with their pathogenicity on berries.

The pathogenicity of conidial suspensions obtained from internodes 7 and 8 shows fluctuations related to rainfall and fungicide applications. The effect of the latter on the pathogenicity is very marked on the sites subjected to copper sprays, viz. Embu, site A and Meru, sites A and B (see also Table 10). The implications of these findings will be discussed in detail in 6.6.

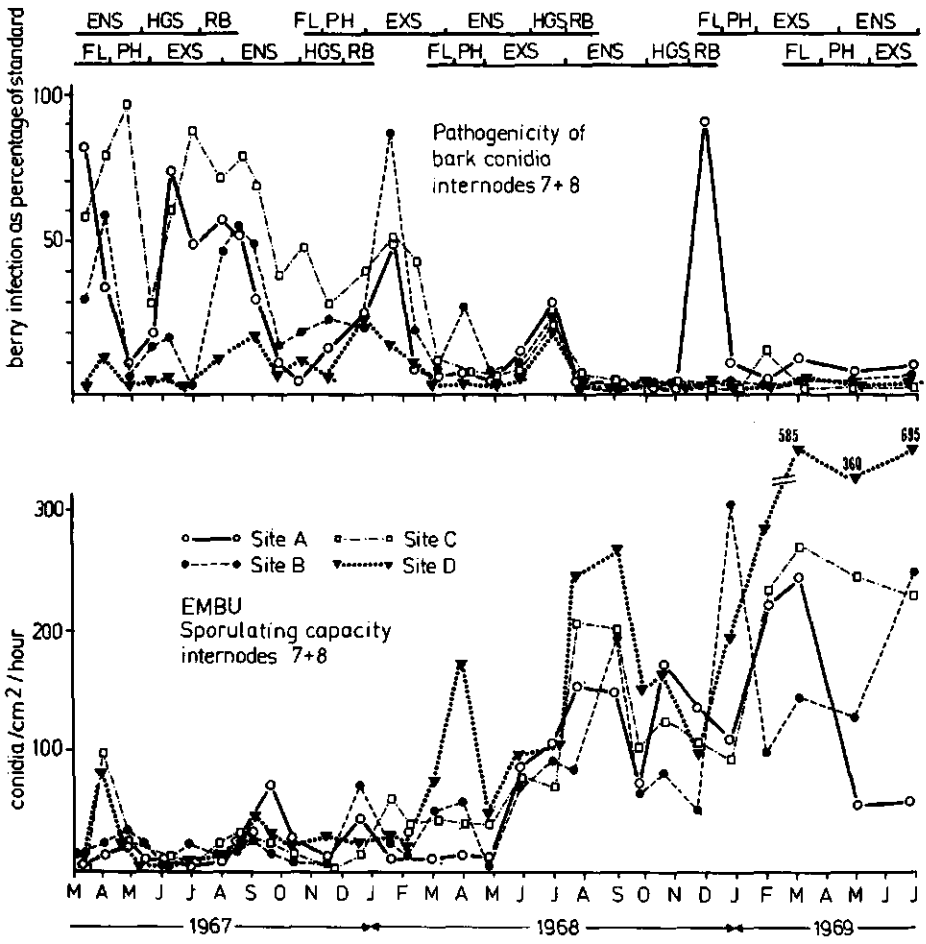

Fig. 11: Sporulating capacity data of internodes 7 + 8, pathogenicity of the conidia of internodes 7 + 8 and the sequence of cropping cycles in the Embu coffee area (see text 2.1.3.3, 4.7, 4.8).

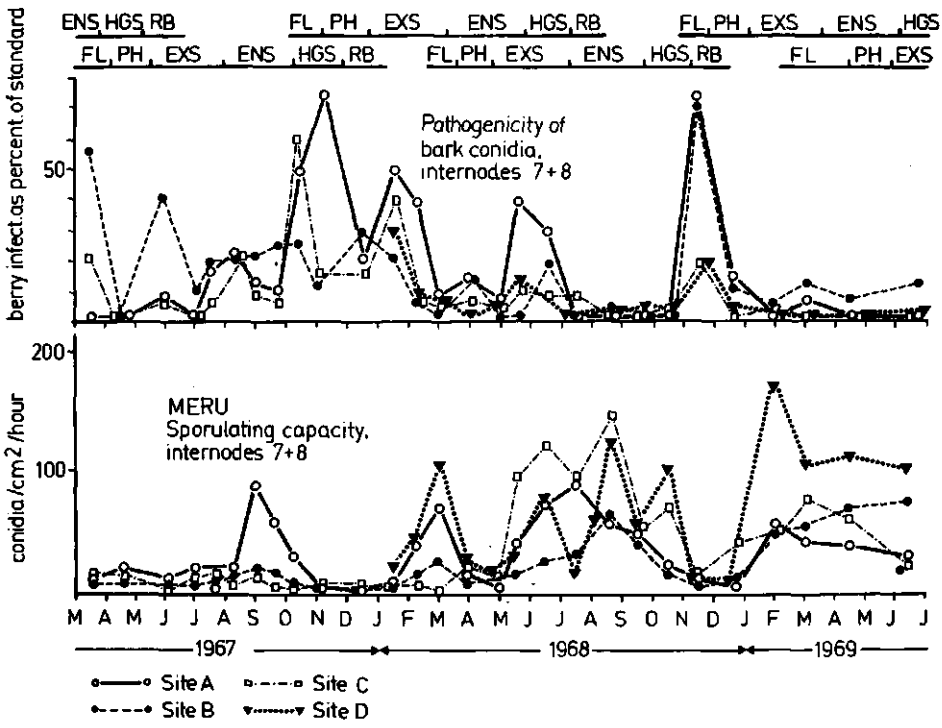

Fig. 12: Sporulating capacity data of internodes 7 + 8, pathogenicity of the conidia of internodes 7 + 8 and the sequence of cropping cycles in the Meru coffee area (see text 2.1.3.3, 4.7, 4.8).

6.5 CBD incidence in the field on labelled branches

The data on the three-monthly, visual CBD scorings on marked branches provided useful information on the incidence of the disease in the smallholding sites. In Table 11 these results are presented.

The main crop, picked in October to November, in Embu is triggered by the March to May rains and is followed by a smaller crop, picked in February to March. In Meru the reverse occurred. In general the potential crop, viz. the crop potentially present on the trees shortly after the termination of the flowering and subsequent fruit set, in 1967 on sites subjected to a spraying programme (Embu A, B and Meru A, B or C) or with a recent history of spray application (Embu C) was higher than on the unsprayed site, viz. Embu D. The high incidence of CBD during parts of the cropping cycle resulted in considerable losses and ultimately

Table 11: Results of three-monthly, visual CBD scorings made on flowers and crop of ten marked branches per site in the period March 1967 to June 1969 (2.1.3.3, 4.8 and Table 10).

Date	Embu				Meru			
	A (Cu-3)	B (Cu-2)	C (Cu-1)	D (Cu-0)	A (Cu-3)	B (Cu-3)	C (Cu-2)	D (Cu-0)
09-03-1967	1-0-1-*-*	1-0-1-*-*	0-0-0-*-*	0-0-0-*-*	1-0-2-*-*	1-0-2-*-*	0-0-1-*-*	-
10-06-1967	*-*-3-2-0	*-*-2-2-0	*-*-2-1-0	*-*-1-0-0	*-*-2-0-2	*-*-3-0-2	*-*-0-0-1	-
28-09-1967	*-*-*-3-2	0-0-0-2-1	*-*-*-1-0	*-*-*-1-0	1-0-2-*-*	1-0-2-*-*	1-0-1-*-*	-
23-12-1967	*-1-0-0-3	*-1-0-0-2	*-0-0-0-1	*-0-0-0-1	*-*-2-0-3 >>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>	*-*-3-0-2	*-*-1-0-1	-
06-03-1968	2-0-2-*-*	1-0-1-*-*	0-0-1-*-*	0-0-1-*-*	2-0-2-*-*	2-0-3-*-*	1-0-2-*-*	0-0-1-*-*
28-06-1968	*-*-3-2-0	*-*-2-2-0	*-*-1-1-0	*-*-1-0-0	*-*-2-0-1	*-*-3-0-1	*-*-1-0-1	*-*-1-0-0
30-09-1968	*-*-*-2-1	*-*-*-1-2	*-*-*-1-1	*-*-*-0-0	*-*-0-1-2	*-*-0-0-1	*-*-0-0-1	*-*-0-0-0
29-12-1968	2-1-0-*-* >>>>>>>>>	1-1-0-*-*	1-1-0-*-*	1-0-0-*-*	0-0-*-*-1	0-0-*-*-1	0-0-*-*-1	0-0-*-*-0
11-03-1969	2-0-2-0-*	1-0-1-0-*	0-0-1-0-*	0-0-1-0-*	1-0-1-*-*	0-0-1-*-*	0-0-1-*-*	0-0-0-*-*
27-06-1969	*-*-2-1-0	*-*-1-1-0	*-*-1-0-0	*-*-1-0-0	*-*-1-0-0[1]	*-*-1-0-0[1]	*-*-0-0-0[1]	*-*-0-0-0[1]

Legend: 0 = no CBD present

1 = 1 - 20% CBD

2 = 21 - 40% CBD

3 = 41 - 60% CBD

* = absence of flowers or/and crop

>>>>> = indicates switch from pre-rain spray programme to continuous spray schedule (Table 10)

[1] = scoring after application of Ortho Difolatan

the crop harvested from the various sprayed sites did not differ from the yield on the unsprayed plot.

The same could be observed in 1968 and 1969: higher potential crops on sprayed sites, but serious losses by CBD reduced the yield. In those cases where farmers, viz. in 1968 Meru A, B and C, Embu B and in 1969 also Embu A, switched to spray applications during the rains, a marked decrease of CBD incidence (Table 11) could be observed especially in the susceptible, soft-green berry stage. The application of two Ortho Difolatan sprays at the end of the observation period in Meru furthermore improved the CBD controlling effects of the copper sprays applied during the rains.

6.6 Discussion

The results of the spray timing trials in Kitale and Meru provided at that time (1966) already indications that copper fungicides acted as protectants on berries (MULLER, 1964). The most effective regime in the two areas proved to be the combination of pre and post-rain sprays. Considering the persistence and redistribution of copper fungicides (Chapter 7) and the different levels of susceptibility of the growth stages of crop to *C. coffeanum*, the following explanation seems valid: the pre-rain copper sprays provided protection to the susceptible flowering stage and exerted most likely still some disease controlling effects on the expanding berries six to eight weeks after application; the post-rain sprays protected the last part of the soft-green berry stage and the beginning of the hard-green and ripening berry stages (Table 2; Fig. 6). Each treatment separately, viz. either pre or post-rain sprays, could not protect more than a part of the crop cycle, respectively the flowers and the beginning of the expanding green berries or the endosperm stage and the onset of the berry ripening (Fig. 6). Although the results of these timing trials in fact already implied the protectant action of the fungicides (Chapter 7), the general recommendations remained in those years aimed at the eradication of bark inoculum of *C. coffeanum*.

The climatic conditions in Embu and Meru only differ to the extent that the major flowering occurs respectively in Embu in March to April and in Meru in October to November. In both areas usually a second flowering occurs respectively in October to November and March to April and the presence of preceding crops, as shown in Figs 11 and 12, seriously affected the newly initiated crop, because the smallholders were not willing to sacrifice the smaller crop by stripping it off to prevent re-infection of the main crop (MULLER, 1964). The overlap of the two crops therefore caused considerable losses, especially in the susceptible stages, viz.

flowers, soft-green berries and to a lesser extent the ripe berries (MOGK, 1970). Although affected ripe cherries could still be picked, they were, while on the trees, important disease transmitters (GIBBS, 1969). GIBBS (1971) found that in estate coffee the role of overlapping crops in transmitting CBD is frequently less important, when adequate spray applications protect both the old and the new crop. Under the smallholding conditions, however, sprays are often applied less frequently and efficiently and under these conditions crop overlap often results in serious crop losses.

In the Embu area the S.C. figures of the sites with little or no copper spraying showed a gradual increase during the observation period as compared to the S.C. of the copper sprayed plot (A). In Meru the S.C. figures were generally lower than in the Embu area, due possibly also to the altitude effect (NUTMAN & ROBERTS, 1969a), while the continued application of fungicidal sprays (A, B, C) kept the total level of sporulation suppressed.

The S.C. findings of individual internodes of each site showed the same seasonal rhythm as noted by NUTMAN and ROBERTS (1969a). The internodes 7 and 8 gave much lower conidial counts than the internodes 9, 10, 11 and 12, while internodes 13 and 14 gave also lower figures than the internodes 9 - 12. As no infection could be induced with conidial suspensions derived from the internodes 9, 10, 11, 12, 13 and 14, it is certain, also considering the anatomical evidence (VERMEULEN, 1970a), that *C. coffeanum* occupies only branch sections with early signs of bark maturation and is hardly or not present in the older internodes, viz. the internodes 9 - 14 (FURTADO, 1970; VERMEULEN, 1970a). The internodes, containing *C. coffeanum*, are still relatively smooth and without any surface cracks and crevices (2.3, 4.7) and therefore it is likely that pesticides cannot remain attached to this surface for a long period of time, especially during the rains. Hence, this suggests that fungicides would be washed off more rapidly from these smooth surfaces than from the cracked and rough surfaces of older internodes, permitting the colonizing *C. coffeanum* in the young internodes to produce on the branch surface conidia able to infect crop more readily that on other internodes.

The pathogenicity of the conidial suspensions obtained from the internodes 7 and 8 on detached berries, as depicted for Embu in Fig. 11 and for Meru in Fig. 12, showed for certain sprayed or recently sprayed sites definite peaks in pathogenicity generally just before or after increases of the S.C. on the branch internodes 7 and 8. The pathogenicity of the conidia obtained from sites B and C at Embu gradually decreased in conjunction with the decreased number of spray applications, while the S.C. figures of these sites increased in the observation period. On the Meru sites the pathogenicity of conidia from site A (copper sprays),

B (copper sprays) and C (copper applications started in 1967) was in most cases higher than the pathogenicity of conidia from the unsprayed control in 1968 and 1969, especially for the two first sites (Fig. 12). It should, however, be kept in mind that the recorded pathogenicity was based on laboratory findings under ideal conditions for the pathogen. In the field a more continuous infectivity at a lower level - due to weather conditions (WOODHEAD, 1968) - seems likely to occur but it may, however, be assumed that in the field the increased release by the bark of *C. coffeanum* conidia also takes place during and shortly after the rains.

In Embu the effects of the inoculum of *C. coffeanum* from bark were most marked in 1967 and the first three months of 1968 for sites A, B and C (Fig. 11). It seems certain that susceptible crop present at the peaks of pathogen production in the bark, was infected at that time, especially when rainfall was light and provided the opportunity for bark washings to be deposited for periods sufficiently long for infection on berries (WALLER, 1972a; WOODHEAD, 1968). In 1968 only one more, very marked potential infection peak in the *C. coffeanum* bark production occurred on site A (continuous copper spraying). This source of inoculum most likely affected at that time the new flush of flowers. In the Meru area the pathogenicity of *C. coffeanum* in the bark could be established during or just after the rains on those sites sprayed with fungicides. Again it may be assumed that crop infection in the field occurred under conditions favourable for dissemination of *C. coffeanum* bark conidia to neighbouring crop.

The effect of applications of fungicides during the rains in 1968 (Meru A, C) and in 1969 (Embu A; Meru A, B and C) could be gauged from the CBD scoring data in Table 11 where a gradual decrease in numbers of affected green berries was observed. The actual action of berry protection by the fungicide, as shown by MULLER (1964, 1968) in Cameroon and later by GRIFFITHS et al. (1971) in Kenya was thus also found in the Embu and Meru areas.

7. FUNGICIDE SCREENING TRIALS

7.1 SCREENING FUNGICIDES FOR CONTROL OF COFFEE BERRY DISEASE IN KENYA

SUMMARY

Fourteen out of 43 fungicides were selected, after laboratory screening, for field trials against coffee berry disease. Each fungicide was compared with a commercial cuprous oxide formulation and an unsprayed control, by assessing conidial production of bearing coffee branches and by recording the percentage of healthy ripe berries picked. In assessments of fungicidal efficiency, healthy ripe berry counts gave more consistent results than those obtained by measuring conidial production. The results from berry counts indicate that in these experiments Ortho Difolatan (BSI 'Captafol') was equal, but not significantly superior, to the standard formulation used in estate spraying. The annual rhythm of conidial production seems to have changed over the past five years. The significance of this is discussed in relation to the control of coffee berry disease.

Prior to 1961, the coffee berry disease (CBD) of *Coffea arabica* L., caused by a form of *Colletotrichum coffeanum* Noack (*Glomerella cingulata* (Stonem.) Spauld. and Schrenk), was responsible for serious crop losses in coffee areas situated above 5,500 ft (Nutman & Roberts, 1961), but since then, following abnormally heavy rains, CBD has rapidly spread into lower districts, where it now causes heavy loss of crop.

Bock (1963) reported that in the higher altitude areas copper fungicides gave a higher degree of control of CBD than twenty-five non-copper formulations. Although an organo-mercury fungicide was even more effective than copper formulations, this compound produced phytotoxic and harmful residual effects (Bock *et al.*, 1958). The present recommendations of four to six sprays per year, using either a two per cent Burgundy mixture or 10 lb per acre of a 50 per cent copper formulation, do not seem to give adequate control.

CBD caused increased losses in 1964. Although these were certainly attributable in part to wrongly-timed copper sprays (Bock, 1963) and to inefficient application, it was felt necessary to resume research on fungicides in an attempt to find a compound superior to the copper formulations. It was also desirable to find a fungicide with an eradicant action, which would destroy the mycelium of *C. coffeanum* inhabiting the bark tissue of bearing branches of the coffee tree (Nutman & Roberts, 1961).

MATERIAL AND METHODS

Laboratory screening

Five chemicals and 43 fungicides (Table 1) were tested by comparing their ability to prevent the germination of conidia of *C. coffeanum*, using the 'slide' method described by Nutman & Roberts (1962). It was fully realized that this method,

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like any other laboratory screening technique, would not necessarily give results closely comparable to any future field performance. It was, however, at least considered likely that in a relatively short period the unpromising compounds could be discarded, because of their inability to inhibit germination of *C. coffeanum* conidia *in vitro*.

Table 1. *Trade names or code-numbers of tested compounds*

Cadmium chloride crystals	pure chemicals	Dodine acetate	
Cadmium sulphide		Tricarbamix	
Cadmium sulphate		Ortho Phaltan (=Folpet)	
Mercury bichloride		Ortho Difolatan (BSI 'Captafol')	
Stannous citrate		DAC-2787	
Perenox		Olin-1763	
Shell-75		Olin-1562	
Fungex		Halophen (C-4)	
Coprantol		Venturicidin	
Colloidox		Allisan	
BCO-75		Verdasan	
Cupravit-Blue		Manzate-D	
DuTer		Zerlate (=Ziram)	
Brestan		Morestan	Bayer AG*
Dyrene		Urbacid	
Glyodin		Tuzet	
Acti-dione		Antracol	
Blasticidin		Euparen	
Fungicide 328		Fungilon	
Captan		Pomasol	
EL-211		Lonacol	
Lead arsenate		Bayer No. 5511	
Sabithane-M		Bayer No. 5467	
Dithane M45		Bayer No. 5468	

The conidia used in these tests were harvested from lesions on ripe coffee berries picked from SL-34 trees on the Coffee Research Station, Ruiru. The conidia were washed from the lesions after 24 hours incubation at 100 per cent R.H. at room temperature (21°–22°C). For each test, new ripe berries with CBD lesions were used to obtain fresh conidia of *C. coffeanum*. Thin films of potato dextrose agar, mixed with known concentrations of fungicides, were prepared on microscope slides and seeded with a 10^5 conidia/ml. suspension, applied with an 'Aerograph' airbrush. After seeding, the slides were incubated at room temperature in plastic boxes lined with wet cellulose wadding for eight hours. The conidia were then killed with lacto-phenol-cottonblue and the percentage germination assessed by counting 200–400 conidia. The fungicides were first tested in concentrations ranging from 6 to 100 p.p.m., but where a fungicide did not give marked inhibition of germination at 100 p.p.m., a further concentration range of 100 to 1000 p.p.m. was tested.

Field trials

Two suitable sites were selected in the high altitude area (Kiambu: Njuno and Kiamara Estates) in January 1965, because a spray-timing schedule for copper

* Tested by Bayer AG in separate field trials.

had already been established for this altitude (Bock, 1963; Nutman & Roberts, 1965). On both sites the trees were 40 years old, multiple stem and of the French Mission variety. Further details are given in Table 2.

Table 2. *Details of two sites selected for trials*

Estate	Altitude	Shade trees	Interplanting	Main flowering	Picking
Njuno	6000 ft	yes	no	March 1965	June 1965–January 1966
Kiamara	5500 ft	no	Blue Mountain & SL-34	October 1964 & March 1965	May 1965–December 1965

The incidence of CBD on both Njuno and Kiamara Estates was high and uniformly spread throughout the trial areas. Seven different fungicides were compared with a standard copper fungicide and with the unsprayed control on both estates (Table 3).

The field trials were designed as randomized blocks, with nine treatments, eight replications (ten trees per plot) and a single row of guard trees between the plots. Fungicides were first applied with a Drake and Fletcher 'Kingston' headland pump and later with a Holden-BSA spraying unit, using handlances at high-volume (200 gal./acre) and high pressure (200–300 p.s.i.) (Bock, 1963). The normal pest control programmes were carried out by the farm management.

Assessment of treatment effects

To assess the effect of the fungicides on the incidence of CBD in the field the following methods were used:

1. Each month a random sample of five fruit-bearing branches was taken from every plot. Internodes with the first signs of bark browning, and seven successive internodes, were treated according the technique described by Nutman and Roberts (1961) and by Bock (1963). The number of conidia produced per cm.^{2} surface area/hr under favourable conditions (approx. 22°C and 100 per cent R.H.) was subsequently assessed.
2. All ripe berries were picked once every every eight to ten days, from four marked trees per plot, throughout the picking season. Before the picking season started all black, infected and old berries were removed from the branches. The total number of picked fruit, including infected and healthy berries, was recorded.

The differences between the treatment means were tested for significance, using the Duncan Multiple Range test at a probability level of $P = 0{\cdot}01$.

RESULTS

Laboratory screening

Fourteen fungicides were selected for field trials, based on their consistent ability to inhibit germination of *C. coffeanum* conidia *in vitro* at concentrations of 100 p.p.m.

or lower. Table 3 illustrates the results of the screening of the fourteen fungicides as compared to a standard fungicide.

Table 3. *Inhibition of germination at various concentration*

	1000	500	250	100	50	25	12·5	6·25
Mercury bichloride*	−	−	−	−	−	−	−	−
DuTer	−	−	+	+	+	+	+	+
Brestan	−	−	−	−	+	+	+	+
Fungicide 328	−	−	−	+	+	+	+	+
Captan	−	−	−	−	−	−	−	−
Sabithane-M	−	−	−	+	+	+	+	+
Dithane M45	−	−	−	−	−	±	+	+
Dodine acetate	−	−	−	−	±	+	+	+
Tricarbamix				−	−	±	±	+
Ortho Difolatan				−	−	−	−	−
DAC-2787				−	−	−	−	−
Olin-1763				−	±	±	±	±
Olin-1562				−	−	−	−	±
Halophen				−	−	+	+	+
Venturicidin				−	−	−	−	−

− = no germination
± = less than 50 per cent germination
+ = more than 50 per cent germination
* used as a laboratory standard

Field trials

The data obtained from both trials, on the production of conidia per unit of twig surface per hour, indicate that twigs treated with Perenox (cuprous oxide) and Ortho Difolatan tended to have a lower conidia production than the other treatments. Although this trend was at first not statistically significant, it became more apparent in the latter part of 1965. Furthermore, it was found that on the individual sampling dates, a high degree of variation occurred in the number of conidia produced per cm.^{2}/hr between replicates of the same treatment, while a high variation was also found from month to month in the same replicate. The seasonal variation of the mean of eight replicates of the number of conidia produced cm.^{2}/hr. in the most interesting treatments are shown in Fig. 1.

The ripe berry counts on Njuno and Kiamara Estates showed that Perenox in both trials and Ortho Difolatan in the Kiamara trial gave the highest percentage of healthy ripe berries. The effect of both fungicides in reducing disease incidence became apparent in the Kiamara trial in May–June and remained consistent throughout the year. On Njuno Estate Perenox gave the highest percentage of healthy ripe berries on all picking dates. The total yield data of ripe berries picked on both estates are presented in Fig. 2.

On Njuno Estate, DAC-2787 gave the highest number of ripe berries picked corresponding to a yield of 10·3 cwt/acre, followed by DuTer (9·3 cwt/acre), but their respective proportions of healthy berry, 50 and 38 per cent, were lower than with Perenox (6·5 cwt/acre total yield and 74 per cent healthy berries). On Kiamara Estate Ortho Difolatan gave both the highest number of ripe berries

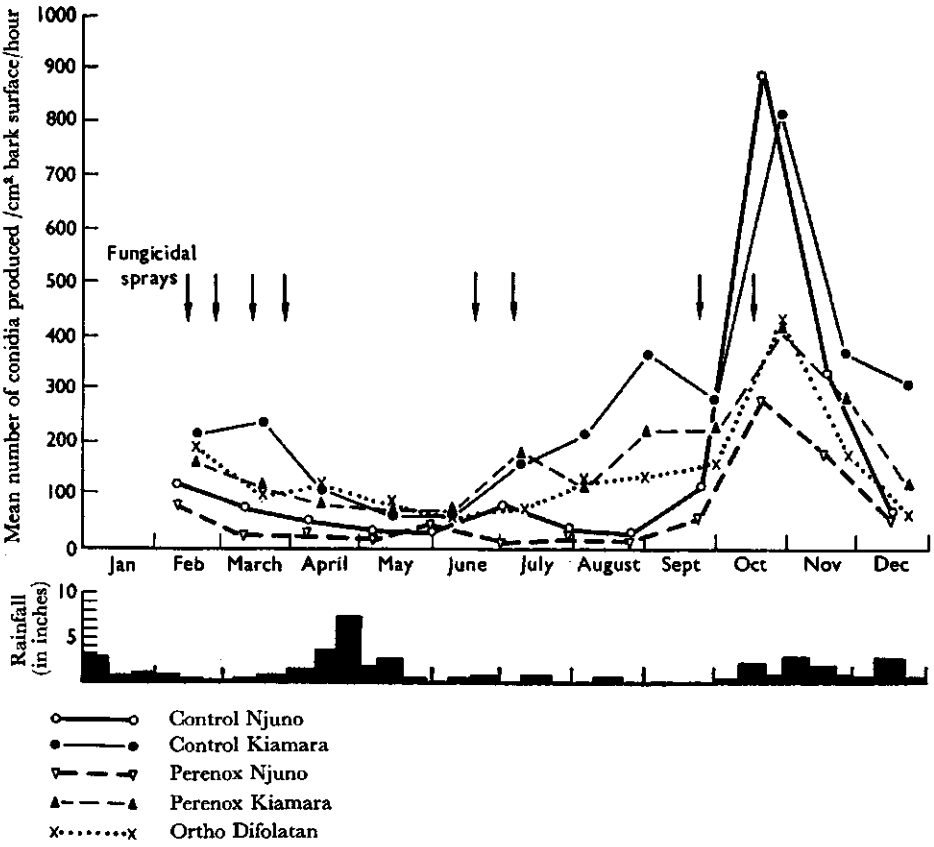

Fig. 1.—The seasonal variation of the number of conidia produced on branches per cm²/hr in some treatments in the Njuno and Kiamara trials.

picked (13·7 cwt/acre) and the highest percentage healthy ripe berries (75 per cent), followed by Perenox with a total yield of 11·9 cwt/acre and 72 per cent healthy ripe berries.

DISCUSSION

The laboratory screening of fungicides using the slide technique has inherent limitations. With this method only the inhibition of the conidia germination is being assessed *in vitro,* but the far more complex conditions in the field after spraying cannot be evaluated. It was therefore unlikely that the laboratory results, covering only one aspect of fungicidal action, would be closely comparable with field performance. In spite of this, it was, however, considered to be the only method available at short notice for selecting more promising fungicides for inclusion in field experiments.

The annual cycle of conidial production for 1965 differed very much from the cycle found by Bock (1963) for 1960. His data showed a marked increase in

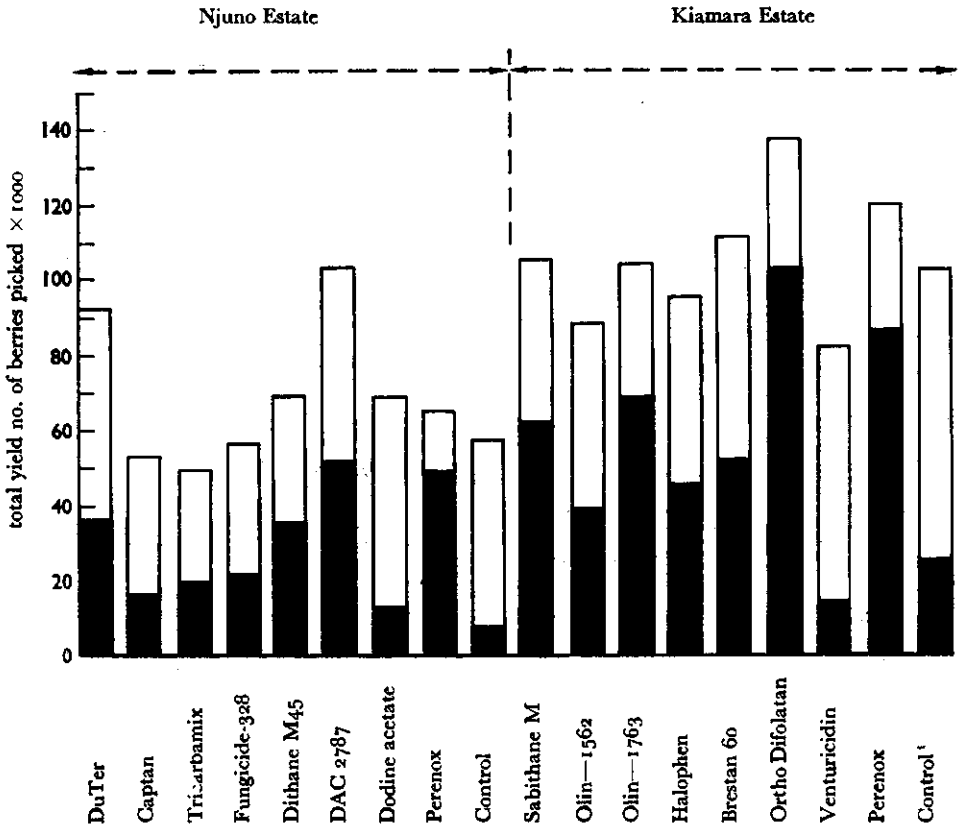

Fig. 2.—The total yield data of ripe berries picked from marked trees on Njuno and Kiamara estates. Useful yield is expressed by total column length, while the healthy berry yield is depicted by the black column only.

March–April, whereas for 1965 there was no such increase. The reason why the expected maximum conidial production in March–April did not occur is unknown. The difference in this annual conidial production pattern may be a reflection of the change in annual climatic patterns since 1961. Bock stated that there was a critical period for control, and that this was the period immediately before the March–April maximum. It has been suggested that the critical period for disease control has now shifted to December–January, and that spraying in these months would result in a greater effectiveness of the applied fungicide (Nutman & Roberts, personal communication, 1967). It can be concluded from Fig. 1 that spraying in June–July did not decrease the conidial production to a great extent, especially in Njuno Estate. This observation seems to confirm Bock's findings that the critical period for CBD-control is before the peak in conidial production, but more detailed work is necessary.

With regard to the fungicidal effect on ripe berries, Bock found 'because of the extreme susceptibility of the ripe fruit, the degree of its infection over this

comparatively long period affords a useful and accurate index of the effects of fungicide treatments'. The incidence of berry infection should be an indirect fungicidal effect on the production of conidia of the bearing branch, but from the data obtained it can be seen that berry counts gave more consistent results than those on conidial production. During the first eight months of the trial periods it was difficult to relate laboratory values on conidial production to the incidence of field infection; only in the last three months could the data be correlated.

It is possible that the differences in total yield (Fig. 2) indicate that varying amounts of fruit were lost before the picking season had ended, i.e. in the early fruit setting stage, as found by Muller and Gestin (1967). This possibility should be investigated in further field trials, by counting all berries on some marked branches to assess the losses of early fruit.

The results of the two trials indicate that, of the new fungicides tested, only Ortho Difolatan could be compared with copper in efficiency, although it was not significantly better at the applied fungicidal rate and spray-timing. The favourable side-effects of Ortho Difolatan, namely on the control of crinkle-leaf and the inducement of better growth of the coffee tree, should also be taken into account.

However, the lack of control of leaf rust (*Hemileia vastatrix* B. et Br.) with Ortho Difolatan implies that, even if subsequent trials confirm the value of this fungicide for CBD-control, some copper sprays will still be required for the control of leaf rust.

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7.2 Discussion

At the time that the results of the trials presented here were obtained, viz. 1964/1965, it was duly realized, that laboratory screening tests had a limited value, especially as the observations were only based on the inhibition of conidial germination *in vitro* (VERMEULEN, 1968). However, considering the urgent need to find at short notice fungicides more effective than copper compounds, it was then decided to use this relatively fast technique.

In subsequent experiments VINE et al. (1973a) applied for laboratory tests a screening technique based both on the ability of fungicides to depress sporulating capacity of bark and the inhibition of spore germination, thus judging the combined effects of the two factors. This screening technique of VINE and co-workers was therefore partly based on the assumption that the depression of the bark sporulating capacity would be an indication of the efficacy of the candidate compound (NUTMAN & ROBERTS, 1970). The findings of GIBBS (1969, 1972), GRIFFITHS et al. (1970a), HINDORF (1970, 1972, 1973a, b, c) and VERMEULEN (1970a) drastically changed the concept of sporulating capacity, thus shifting the aims of disease control from the suppression of the bark inoculum to that of the crop inoculum. Furthermore, it should be kept in mind that in general laboratory screening of fungicides at best offers only indications of the future disease controlling prospects of a candidate compound.

Considering the aforementioned changes in the inoculum potential concept, the sporulating capacity data presented in this chapter should be evaluated differently, as the spray application of the selected compounds in the field was based on the then current pre-rain recommendations (BOCK, 1963; NUTMAN & ROBERTS, 1961). The preliminary report, presented at the First Specialist Meeting on Coffee Research on East Africa, Nairobi, stating the favourable effects of Ortho Difolatan on CBD incidence (VERMEULEN, 1966b) attracted little attention, as this compound did not markedly decrease the sporulating capacity and therefore was not considered to be effective. The firm belief in the inoculum potential theory caused all favourable reports on a fungicide, which did not suppress conidial production on branches, to be summarily dismissed. The same fate was allotted at the 1966 Meeting on Coffee Research to the findings of MULLER (1964), reporting on the CBD controlling effects of copper sprays, applied on developing crop during the monomodal rainy season in Cameroon. During a second visit to Kenya MULLER (1968) recommended a similar spray-schedule for Kenya, emphasizing the importance of crop protection by fungicides, rather than the reduction of the conidial production on the bark, as advocated in East Africa.

In Kenya the copper sprays had to be applied before the onset of the rains in order to reduce the peaks of the S.C. of the bark and thus decreasing the incidence of CBD, originating from bark (BOCK, 1963; NUTMAN & ROBERTS, 1961). Between 1956 and 1961 this spray schedule gave adequate results in high altitude coffee growing areas in Kenya (NUTMAN & ROBERTS, 1961), but gradually evidence accumulated that CBD could often not be controlled by the application of copper sprays before the rains. The aforementioned change in concept of the sporulating capacity and subsequent field trials led eventually to the recommendation of a continuous spray programme (GRIFFITHS & GIBBS, 1969; GRIFFITHS & WALLER, 1971 and GRIFFITHS et al., 1971), similar to the Cameroon schedule (MULLER, 1964, 1968, 1970, 1973, 1978; MULLER & GESTIN, 1967).

The question how copper, in the trials between 1956 - 1961, and copper and Ortho Difolatan in the 1965 trials, applied before the rains, could still have excercised a markedly, although not statistically significant, CBD suppressing effect has been answered by GRIFFITHS and WALLER (1971), VINE and GRIFFITHS (1968) and VINE et al. (1973a). It was found that Ortho Difolatan and most likely also copper were still active after many weeks of exposure to heavy rain, due to redistribution within the tree canopy (NEELY, 1970, 1971; PATEL, 1971; PEREIRA et al., 1973). As the compound thus protected effectively the first stages of the cropping cycle susceptible to *C. coffeanum*, viz. flowers and expanding green berries (MOGK, 1970), this would explain the favourable anti-CBD effect of Ortho Difolatan in the fungicide screening trial presented in this chapter.

When VERMEULEN (1968) pointed out the CBD controlling and other favourable effects of Ortho Difolatan, he also stated that this compound did not control leaf rust, *H. vastatrix*. The application of copper fungicides to control the latter disease would still be required, also if subsequent trials would further confirm the value of Ortho Difolatan for CBD control. Later experiments (VINE et al., 1973a) have shown this to be correct and Ortho Difolatan is still the main anti-CBD fungicide in Kenya (FIRMAN & WALLER, 1977).

8. EFFECTS OF CULTURAL PRACTICES ON THE INCIDENCE OF COFFEE BERRY DISEASE ON TREES AND THE SUSCEPTIBILITY OF BERRIES

8.1 Introduction

It was claimed that certain cultural practices would decrease the incidence of CBD, especially when no copper containing fungicides had been applied (TWIST & COLTMAN, 1966). In order to investigate the CBD suppressing effects of cultural practices, a series of observations were carried out in 1967 - 1969 on three, adjacent coffee estates (Table 12: A, B and C) in the Kiambu area, East of the Rift, altitude approximately 1700 m and compared with coffee trees at the National Agricultural Laboratories, 1700 m (D). The details of the observation sites are presented in Table 12, using for the assessment of visual CBD incidence the system listed in 4.8:

The observations included the following aspects:

- the *C. coffeanum* level in the bark of single and multiple stem coffee trees grown respectively with normal and heavy fertilizers use, with and without mulch, with and without soil tillage, with and without pruning modifications and with and without copper sprays;
- the susceptibility of expanding, non-copper sprayed, green berries to standard conidial suspensions of *C. coffeanum* as compared to the susceptibility of expanding, copper sprayed green berries;
- the pathogenicity of *Colletotrichum* conidial suspensions from prunings and mummified berries left on the ground in copper and non-copper sprayed coffee fields on expanding, green berries.

This range of aspects was investigated by using the sporulating capacity technique (25 branches/site) and the assessment of the pathogenicity on green berries of the conidial suspensions obtained from bark as used in the epidemiological observations (4.7). All tests were replicated at least six times. Conidial suspensions obtained by washing bark were standardized on approximately 2×10^4 conidia per ml.

8.2 Results

Unsprayed coffee had a lower level of *C. coffeanum*, inhabiting the coffee bark, than sprayed coffee as already established by FURTADO (1969, 1970). Heavy applications of chemical fertilizers had no effect on the level of *C. coffeanum* in the bark, either in sprayed or unsprayed coffee. No effects of high levels or

Table 12: Information on the observation sites in the Kiambu area (A, B and C) and the coffee collection of the National Agricultural Laboratories (D), Nairobi, Kenya.

Code	Disease control measures	Pruning systems and modifications	Coffee cultivars	Mulching and soil cultivation	Chemical fertilizers	Herbicide and weed control	CBD incidence (scoring system in 4.8)
A	No fungicidal sprays ever applied	Multiple stem and single stem; no modifications in pruning recommendations	S.L. 28, S.L. 34 and French Mission	Recommended mulching programme; no hand or mechanical soil tillage	No chemical fertilizers applied	No herbicides applied; weed debris removed by hand	0 - 1
B	Copper sprays applied until 1966; no chemical disease control in 1967, 1968 and 1969	Multiple stem and single stem; on single stem a rigorous more severe than usual pruning, alleged to be CBD suppressing	S.L. 28, S.L. 34 and French Mission	Heavy applications of mulch, including prunings, weed debris and coffee husk; no hand or mechanical soil tillage	No chemical fertilizers applied	No herbicides applied; clean weeding, debris left under trees	1 - 2
C	Recommended copper spray schedule	Multiple stem and single stem; no modifications in pruning recommendations	S.L. 28, S.L. 34 and French Mission	Recommended mulching and soil tillage programme in most coffee; in one single stem plot high applications of mulch	Chemical fertilizer programme varying from recommended to high	Herbicides and clean weeding; removal of debris	2 - 3
D	Recommended copper spray schedule	Multiple and single stem; no modifications in pruning recommendations	S.L. 28, S.L. 34, Harar and French Mission	Recommended mulch and soil tillage programmes	Recommended fertilizer programme	Herbicides and clean weeding; removal of weed debris	3 - 4

organic material, including mulching, and variations in pruning systems on the *C. coffeanum* level in bark, could be established. Apart from the aforementioned differences between sprayed and unsprayed coffee, the other cultural measures have no other effect than improving the general condition of the coffee trees.

The CBD susceptibility of green berries obtained from unsprayed coffee trees proved to be lower after inoculation with standard suspensions of *C. coffeanum*, than the susceptibility of green berries from routinely sprayed trees (Table 13).

Prunings and berries left on the ground for some time did not yield any conidial *Colletotrichum* suspensions, capable to cause infection on green berries.

Table 13: CBD infection percentages of green berries of sprayed and unsprayed coffee trees, inoculated with a standard suspension of *C. coffeanum* conidia of 2×10^4 conidia per ml.

	1st test	2nd test	3rd test	4th test	5th test	6th test
Berries from unsprayed coffee trees	45	50	46	42	36	35
Berries from sprayed coffee trees	70	82	68	64	61	57

8.3 Discussion

Apart from the already available evidence, that copper sprayed coffee trees harbour a higher level of *C. coffeanum* in the bark, no substantial indications have thus been found that other cultural practices might affect the *C. coffeanum* level in coffee bark to any practical or economical extent. Alleged instances of lower CBD incidence on coffee trees, treated only with high levels of organic material or special cultural practices as described in Table 12, have not been substantiated in these observations. It seems that the effects of fungicidal applications have been of greater importance with regard to the *C. coffeanum* level in the coffee bark, than factors like organic manuring or cultural practices. STEINER (1972a, 1973d) found that the application of foliar fertilizers in combination with fungicides had an increased CBD controlling effect, as compared to fungicide sprays alone. NUTMAN and ROBERTS (1969e) on the contrary claimed a higher level of CBD after application of foliar fertilizers, thus challenging the results of DA PONTE (1966) indicating that foliar applications of calcium super phosphates controlled CBD in Angola. The whole matter of the foliar application of

fertilizers should be further investigated, especially in relation to their possible effects on the phyllosphere of coffee branches (GIBBS, 1972; ROBINSON & WALLER, 1966; WALLER, 1972a).

With regard to the lower CBD susceptibility of green berries from unsprayed coffee trees (VERMEULEN, 1970c) the following explanation can be offered. ADUAYI (1972) reported in his work on the effects of copper sprays on the mineral nutrient content of coffee seedlings. HINDORF (1973d) established also that young leaves on regularly copper sprayed plots, but developed after the last spray application, contained six times more copper than identical leaves on non-copper sprayed sites. A similar increase of copper content in the developing berries of sprayed coffee might thus be expected and these levels of copper may have had a stimulating effect on the germination of *C. coffeanum* (NUTMAN & ROBERTS, 1962b; RICHARDSON & THORN, 1962). This aspect needs to be investigated in more detail in order to establish whether or not only copper fungicides tend to induce a higher level of CBD susceptibility in developing berries.

9. GENERAL DISCUSSION AND CONCLUSIONS

Research presented in this publication constitutes a part of the entire research efforts since 1964 directed at coffee berry disease (*Colletotrichum coffeanum*), the major coffee problem in Kenya. The results obtained are therefore discussed in conjunction with an evaluation of relevant research of other workers at that time.

From the literature review in Chapter 3 and also from the information presented in the other chapters, it becomes clear that the research findings obtained in the mid-1950's have had a considerable impact on the research carried out in the period 1961 - 1968.

The adherence to the 1956 - 1961 findings was based on the firm belief, that the spread of CBD into the low altitude coffee areas was caused by a change in climate, mainly by an increase in rainfall (NUTMAN & ROBERTS, 1969d; WALLIS, 1964), but that otherwise no essential changes had occurred. In the years 1964 - 1968 the policy of the Coffee Research Station towards CBD remained therefore founded on the old control concepts, i.e. application of pre-rain copper sprays, which were then still accepted as valid and effective by the farmers and the responsible government officials. Most of the CBD research in the early and mid-1960's has to be considered and evaluated against this background.

The international coffee contacts improved after the FAO-Technical working party on coffee production and protection in Brazil in 1965. Coffee authorities in Kenya became aware of the fact, how little was known about the CBD pathogen. In addition it became known that the disease was not only confined to East Africa and the neighbouring Zaïre (Belgian Congo), but was also present in Angola and Cameroon. Thus CBD was lifted out of the rather limited - and exclusive - Kenyan context and international help and know-how could bring about a break-through of the old CBD concepts and an deepened insight of CBD research aspects in only a few years.

The results presented in Chapter 5 illustrate how the questions around the composition of the *Colletotrichum* complex and the epidemiological role of *G. cingulata* were jointly answered at the end of the 1960's, largely as a result of the research activities of FURTADO (1969, 1970), GIBBS (1969), HINDORF (1970, 1973) and VERMEULEN (1970a, b). It remains unclear, however, why no link could be found between *C. coffeanum* and *G. cingulata* in Kenya, while this relationship had been reported from Tanzania (HOCKING et al., 1967; JOHANNS, cited by HINDORF,

1972). Without any doubt it is necessary to initiate genetic work with *Colletotrichum* isolates of coffee in order to establish their pathogenic variability in relation to heterocaryosis (STEPHAN, 1968; STROBAL, 1960). The work of WHEELER (1954) on the genetics of *G. cingulata* should be incorporated in this new research programme.

The research findings during the late 1960's also led to a completely different evaluation of the 'inoculum potential' theory of NUTMAN and ROBERTS, which had hitherto been generally accepted. The inadequacy of the usage of the total sporulation capacity data as a means of predicting disease incidence, is illustrated by the pathogenicity tests carried out with conidia obtained from individual internodes, as presented in Chapter 6. The actual rhythm of conidial production of *C. coffeanum* and the periods of pathogenicity of these conidia from bark are defined both in time (during or after the rains) and location (internodes with the first sign of maturation) by the tests on green berries with the conidial suspensions from bark.

In conjunction with the detailed studies of WALLER (1972a) these findings provide an indication of the time when the bark inoculum will be available to infect the susceptible stages of the crop (MOGK, 1970; MOGK & HINDORF, 1975). Considering the peaks in pathogenicity of the bark population of *C. coffeanum* and the findings of WALLER it may then be concluded that the shift in S.C. composition of the bark towards *C. coffeanum* will occur in the middle of the rainy season, when the very susceptible expanding green berries are present on the branches. Once berry inoculum is available it will quickly surpass the bark inoculum, especially when sufficient susceptible crop is present (GIBBS, 1969; GRIFFITHS & WALLER, 1971; GRIFFITHS et al., 1971; MULINGE, 1971a; WALLER, 1972a). The reported higher incidence of *G. cingulata* on copper sprayed coffee trees (NUTMAN & ROBERTS, 1969c; VERMEULEN, 1970b) seems to be contradictory to the shift of the *Colletotrichum* population towards *C. coffeanum*. Most likely the increase of *G. cingulata* might be compared with the influence of decreasing temperatures on the initiation of perfect stages of other fungi in the temperate climate. Moreover, perithecia of *G. cingulata* have never been found on the restricted bark area, occupied by *C. coffeanum*.

The subject of bark colonization by *Colletotrichum* spp. needs to be investigated more closely. Our knowledge of the reasons why coffee bark becomes colonized, especially by the CBD fungus is still very limited and it is also unknown why *Colletotrichum* species occupy strictly defined bark areas only. Evidence that *C. coffeanum* would re-infect bark from berry inoculum (GIBBS, 1972; NUTMAN & ROBERTS, 1969d) is insufficiently documented.

Experimental evidence pertaining to the epidemiology of CBD through Kenya, the adjacent East African countries and ultimately Ethiopia, Zaïre, Cameroon and Angola is also limited. For Kenya, Uganda and Tanzania, the general accepted theory (NUTMAN & ROBERTS, 1961) was that the disease was transported as bark inoculum in the branches of seedlings. This might have been true in a number of cases in Kenya, Uganda or Tanzania, but it cannot fully explain all CBD outbreaks in East Africa. In particular the theory does not account for the outbreaks in Angola, Cameroon and even Ethiopia, as it considers East Africa the original centre of infection.

The results of experiments in African smallholder coffee areas (Chapter 6), were in agreement with those obtained in the areas which were previously researched by NUTMAN and ROBERTS (1961). Accounting for the different rainfall in Meru, where the main precipitation is six months behind that of the other areas studied, all results were similar.

The applications of fungicides during the rains in 1969 had a markedly disease controlling effect, as predicted for Kenya by MULLER (1968) and already indicated by the Kitale-Meru spray timing trials. The change from pre-rain sprays to spraying during the rains was slow in Kenya. It took the CRS some time to realize that the pre-rain spraying schedule was too unreliable and largely ineffective (GRIFFITHS & WALLER, 1971), before spraying during the rains became their policy (GRIFFITHS et al., 1971). The incentive for this change in the spray programme was undoubtedly provided by MULLER (1964, 1968).

One of the first fungicides, which looked promising in screening trials, was Ortho Difolatan; its effectiveness in CBD control has since then been confirmed in additional trials. The compound is still widely applied after being used already for a period of some twelve years despite the introduction of systemic compounds such as Benlate (VINE et al., 1973a). The decreasing effectiveness of Benlate and related compounds, because of the build-up of fungal resistance (OKIOGA, 1976), has diminished the disease controlling prospects of these systemic fungicides. Ultimately future CBD control will, however, most likely rely more on systemic rather than non-systemic compounds, in line with the nature of the bark colonization by *C. coffeanum*. Copper fungicides will remain part of the spraying schedule, as they are still the most effective compounds for leaf rust control, unless specific rust controlling fungicides will become available. GRIFFITHS (1972) already pointed out the urgent need to know more about the interactions between fungicides, coffee and the pathogens on coffee, especially because of the increased applications of anti-leaf rust fungicides in the major coffee producing countries (WALLER, 1972b). Considering the experience in Kenya with

fungicides in general and the results presented in this publication, the urgency to investigate the aforementioned interactions is still present.

The alleged CBD suppressing effects of cultural methods have already been discussed by RAYNER (1952). The results of the comprehensive observations, presented in this publication, do not show any difference in disease incidence between a wide range of reputedly effective measures. The already mentioned higher bark incidence of *C. coffeanum* in coffee sprayed with copper fungicides is again evident, regardless of the cultural practice applied.

The increased susceptibility of copper sprayed berries suggests, that in the years 1956 - 1961, when pre-rain copper sprays were successfully applied, the quantities of copper on and in coffee tissues would decline gradually during the rains until a point where the remaining copper level would actually favour the infection by *C. coffeanum* (NUTMAN & ROBERTS, 1962b; RICHARDSON & THORN, 1962). As this would happen during the most susceptible stages of the cropping cycle (MOGK, 1970) it might explain the considerable losses incurred during the period 1962 - 1966 when the distribution of the rainfall differed from that in 1956 - 1961, thus affecting the redistribution and level of deposits of copper fungicides in the coffee trees (GRIFFITHS & GIBBS, 1969; GRIFFITHS & WALLER, 1971).

In relation to the aforementioned aspects of CBD research finally the important findings of STEINER (1973b) with regard to the relationship between rainfall quantities and new CBD infections provided the first experimental evidence about the optimal application of fungicidal sprays. Also related to these aspects was the work of GASSERT (1976, 1978) in Ethiopia, indicating the great human interference in the CBD epidemiology and the favourable disease controlling prospects of 'clean picking', viz. removing all affected berries by hand at regular intervals, thus preventing the escalation of the CBD outbreaks through 'berry-to-berry' infection (GIBBS, 1969; GRIFFITHS & WALLER, 1971; GRIFFITHS et al., 1971).

The research data presented here provide information on the following aspects of CBD:

a. The location and frequency of occurrence of some components of the *Colletotrichum* population on the bark in relation to fungicide applications.
b. The role of *G. cingulata* in the disease epidemiology and the absence of a relationship with *C. coffeanum* has been established.
c. The sporulating capacity of bearing branches in terms of pathogenicity on green berries as a function of rainfall and fungicide application.
d. The pathogenicity of the bark inoculum which increases during the rains, largely because:
   - the number of saprophytic *Colletotrichum* conidia decreases, also due to pre-

rain fungicide applications;

- the *C. coffeanum* containing tissues increase with the growth flushes at the onset of rains;
- the bark surface of the internodes with the first signs of bark maturation offers less opportunity than on older internodes for fungicides to remain attached, thus providing the fungus with suitable sporulation surfaces.

e. The effectiveness of Ortho Difolatan as an anti-CBD fungicide.

f. The higher susceptibility of berries sprayed with copper fungicides as compared with non-copper sprayed berries.

g. The ineffectiveness of cultural methods in CBD control.

The following topics are suggested as priorities in future research:

- a world-wide classification of *Colletotrichum* spp., inhabiting the bark of arabica coffee in order to predict possible outbreaks of *C. coffeanum*;
- continued investigations into the effects of copper and other fungicides on the composition of the *Colletotrichum* bark populations;
- detailed studies of the anatomical preference of *C. coffeanum* for certain bark areas of coffee twigs;
- a study of the genetics of *Colletotrichum* spp. and their possible relationship with *G. cingulata*;
- the selection of effective fungicides, with special reference to systemic compounds and the build-up of fungal resistance to these fungicides;
- the continuation of breeding for CBD resistance.

Although the CBD problem in Kenya is certainly less calamitous now than it was in the mid-1960's, it still requires continuous attention; the development of more resistant coffee cultivars seems of particular importance. The same applies to other African coffee countries, where CBD inflicts crop losses.

Since *H. vastatrix* was found in Brazil in 1970, rigid control measures are carried out in the South and Central American coffee growing countries (WALLER, 1972b; WELLMAN, 1970). Considering the effects in Kenya of fungicidal sprays in favour of *C. coffeanum*, careful attention should be given in the aforementioned countries to symptoms similar to those caused by CBD in Kenya. The reported field incidence of CBD in Brazil (FIGUEIREDO, personal communication to Dr. D. MULDER), is alarming as this would endanger vast areas of coffee not only in Brazil, but also in the other South and Central American countries. The assumed inability of CBD to spread quickly and inflict heavy losses under the climatic conditions in those countries, should be viewed with great caution, as especially the climatic and cultural conditions in the montane areas of Central America might be conductive for CBD outbreaks. International cooperation not only in the field of

leaf rust eradication, but also in controlling coffee berry disease is necessary and requires the gathering of detailed information on the *Colletotrichum* bark colonization from all arabica coffee growing countries in the world. This co-operation should also include a programme for testing of CBD resistant material, as initiated in Kenya.

10. SAMENVATTING

Door het ontbreken van natuurlijke grondstoffen is de economie van Kenia voor een groot deel afhankelijk van de landbouw. Uitgestrekte gebieden zijn beplant met arabica koffie, *Coffea arabica*, en de export van kwaliteitskoffie is een belangrijke bron van inkomsten.

Vooral ná de 2e Wereldoorlog is het aandeel van de kleine boer in de koffiecultuur toegenomen. Tegenwoordig is bijna de gehele cultuur in handen van Afrikaanse landbouwers en -ondernemers en is de eertijdse dominantie van Europese koffieplanters en firma's verdwenen.

De ontwikkeling van de koffieteelt in Kenia is - behalve door de economische terugslag in de jaren dertig en de 2e Wereldoorlog - ook belemmerd door het optreden van twee schimmelziekten: de koffieroest, veroorzaakt door *Hemileia vastatrix* en de koffiebesziekte, veroorzaakt door *Colletotrichum coffeanum*. Deze laatste ziekte, waarbij bloemen en bessen in de verschillende ontwikkelingsstadia worden aangetast, is het onderzoek van deze publikatie, waarin gepubliceerde en nog ongepubliceerde onderzoekgegevens uit de periode 1964 - 1969 worden gepresenteerd.

De publikatie begint met een kort overzicht van het gehele onderzoek verricht sinds de eerste waarneming van de koffiebesziekte in West Kenia in 1922. In de periode 1922 - 1950 werd vastgesteld, dat de schimmel *C. coffeanum* op groene koffiebessen ziektesymptomen veroorzaakt. In experimenten bleken fungiciden niet of weinig effectief te zijn. Ook de proeven met kunstmest toonden aan, dat verhoogde kunstmestgiften geen invloed hadden op het voorkomen van de ziekte. Slechts het gebruik van ziekte-resistente rassen leek rond 1950 perspectieven te bieden.

Gedurende de periode 1950 - 1966 werd meer inzicht verkregen in enkele aspecten van de ziekte. Zo werd vastgesteld, dat het pathogeen in de bast van koffietakken leeft en van daaruit de infectie van bessen veroorzaakt. Toen bespuitingen met koperhoudende fungiciden vóór het begin van de regens gunstige resultaten opleverden, naar men aannam door een vermindering van het bastinoculum, leek de ziekte blijvend te kunnen worden onderdrukt.

Het optreden van de koffiebesziekte was aanvankelijk beperkt tot de hooggelegen koffiegebieden (boven 1680 m) van West- en Midden Kenia. Ná 1962 kwam de ziekte echter in toenemende mate voor in lager gelegen gebieden. Aan het eind van de zestiger jaren waren alle koffiegebieden in Kenia aangetast. De hierdoor veroorzaakte verliezen waren buitengewoon hoog, hetgeen ertoe leidde, dat in 1966 het

onderzoek op het terrein van de koffiebesziekte werd geïntensiveerd. Hierdoor werd ook op internationaal niveau steun gezocht en in 1967 werden onderzoekers en fondsen beschikbaar gesteld door de regeringen van Engeland, West Duitsland en Nederland.

In samenwerking met Keniaanse onderzoekers werd als gevolg hiervan snel ná 1966 een grote vooruitgang gemaakt met betrekking tot de kennis van de epidemiologie, biologie en bestrijding van de ziekte. De nieuwe inzichten maakten het o.a. mogelijk de ziekte op economisch verantwoorde wijze chemisch te bestrijden. De gunstige resultaten in Kameroen hebben daarbij het onderzoek in belangrijke mate gestimuleerd.

De in deze publikatie gepresenteerde resultaten betreffen enerzijds reeds gepubliceerde gegevens, anderzijds een vrij grote hoeveelheid informatie, die nog niet eerder was gepubliceerd. In het hoofdstuk over de anatomische en mycologische aspecten wordt ingegaan op het voorkomen van het pathogeen in de cortex van de bast van koffietakken ten tijde van de vorming van de eerste kurklaag daarin. De takgedeelten waar in de cortex reeds meer kurklagen zijn gevormd, bevatten alleen saprofytische *Colletotrichum* soorten. *Glomerella cingulata*, het perfecte stadium van de saprofytische *Colletotrichum* soorten, speelt in Kenia waarschijnlijk geen rol in de epidemiologie van de koffiebesziekte. Daarentegen zijn er aanwijzingen, dat de schimmel in Tanzania van het *Glomerella* stadium in *C. coffeanum* kan overgaan.

Voorts werd aangetoond, dat in Kenia meer *C. coffeanum* voorkomt op koffiebomen, die regelmatig worden bespoten met koperhoudende fungiciden dan op onbespoten bomen. Dit wijst op een verschuiving in de *Colletotrichum*-populatie ten gunste van *C. coffeanum* onder invloed van de fungiciden behandeling.

Eveneens werd gevonden dat op met kopermiddelen bespoten koffietakken de vruchtlichamen van *G. cingulata* in veel grotere aantallen voorkwamen dan op takken niet bespoten met fungiciden. Het verhoogde aantal perithecia van *G. cingulata* is echter nooit aangetroffen op de bastgedeelten met de eerste tekenen van bastrijping, waarin het pathogeen van de koffiebesziekte, *C. coffeanum*, voorkomt.

De epidemiologische gegevens werden verzameld in twee gebieden met uitsluitend kleine koffietuinen van Afrikaanse boeren. De waarnemingen omvatten de sporulatie-capaciteit van het *Colletotrichum*-complex van de bast van takken in relatie tot de tijd, de regenval en het gebruik van fungiciden. Voorts werd nagegaan hoe de sporulatie-capaciteit van de koffietakken samenhangt met de pathogeniteit van dit bastinoculum op groene bessen.

Uit deze gegevens bleek opnieuw dat *C. coffeanum* alleen voorkomt in de jongere takgedeelten waar de rijping van de bast pas is ingezet. Slechts de conidiën verkregen van deze takgedeelten konden na incubatie onder vochtige om-

standigheden infecties op groene bessen veroorzaken. Daarnaast werd weer vastgesteld, dat de populatie van de parasiet, *C. coffeanum*, duidelijk groter was in met kopermiddelen bespoten velden dan in onbespoten koffie. Het moment, waarop bastinoculum van de ziekte aanwezig is, valt samen met het meest vatbare stadium in de vruchtvorming. Dit is het stadium waarin de koffiebes snel uitgroeit en waarbij de klimatologische omstandigheden, t.w. hoge relatieve vochtigheid en gunstige kiem- en infectie-temperaturen, optimaal zijn voor infectie. Als koffiebessen zijn geinfecteerd, wordt de uitbreiding van de ziekte spoedig versneld door een continue, hoge produktie van conidiën op bessen.

In het hoofdstuk over de keuze van geschikte fungiciden en het bepalen van het juiste tijdstip voor de toepassing ervan wordt ingegaan op onderwerpen, die in de zestiger jaren in Kenia zeer controversieel waren. Enerzijds was men in die tijd werkelijk overtuigd van het gunstige effect van bespuitingen met kopermiddelen toegepast vóór de regens. Anderzijds werden steeds weer stemmen gehoord, die niet alleen de juistheid van het tijdstip van spuiten in twijfel trokken, maar ook de effectiviteit van deze fungiciden voor de bestrijding van de koffiebesziekte betwijfelden. In het raam van deze ambivalente situatie moet de opzet van dit fungicidenonderzoek worden gezien. Gedurende de uitvoering van deze proeven bleek reeds spoedig dat de gangbare gehanteerde inoculum-potentiaaltheorie en de consequenties, die uit de inoculum-potentiaalgegevens werden getrokken met betrekking tot de verliezen veroorzaakt door de koffiebesziekte, niet werden ondersteund door de ziektewaarnemingen in het veld. Uitgaande namelijk van de inoculum-potentiaalresultaten werd het middel Ortho Difolatan door andere onderzoekers aanvankelijk niet beschouwd als een effectief fungicide, alhoewel de resultaten in het veld veelbelovend waren. De uiteindelijke resultaten, welke met Ortho Difolatan werden bereikt, waren hoopgevend, hetgeen vooral werd bevestigd bij latere toepassingen gedurende de regentijd. Dit middel is tegenwoordig nog steeds één van de belangrijkste fungiciden voor de bestrijding van de koffiebesziekte in Kenia.

Wat het tijdstip van toepassing van bespuitingen betreft, bewezen de proeven de juistheid van de resultaten eerder bereikt in Kameroen, waar de fungiciden niet werden toegepast ter vermindering van het 'bastinoculum', maar voor het aanbrengen van een beschermende laag rond de vruchten.

De veronderstelling, dat bepaalde cultuurmethoden een gunstige invloed zouden hebben op het optreden van de koffiebesziekte, kon niet worden bevestigd door waarnemingen in het veld en in het laboratorium. In velden met hoge giften kunstmest, stalmest, organische stof en/of intensieve snoeisystemen werd géén lager niveau van de ziekte aangetroffen. De eerder gesignaleerde verschillen in het *C. coffeanum*-niveau traden alleen op tussen bespoten en onbespoten koffie. Voorts bleken bessen

van onbespoten koffiebomen minder vatbaar te zijn dan bessen van bespoten bomen.

In aansluiting hierop dient het werk van een Duitse onderzoeker in Kenia te worden genoemd, waardoor kon worden vastgesteld na welke hoeveelheid regen opnieuw moet worden gespoten met fungiciden om nieuwe uitbarstingen van de koffiebesziekte te voorkomen. In Ethiopië bewees een andere Duitse onderzoeker, dat de mens een belangrijke rol speelt bij de verspreiding van de ziekte. Voorts werd aangetoond, dat het 'schoonplukken' van de bomen, d.w.z. het regelmatig verwijderen van aangetaste koffiebessen, een belangrijke factor was in de ziektebestrijding. Deze onderzoekgegevens zouden, méér dan voorheen, dienen te worden ingepast in een breed opgezet programma voor de effectieve bestrijding van de koffiebesziekte, niet alleen in Kenia maar ook in andere koffieverbouwende landen.

In deze publikatie wordt enkele keren gewezen op het verschuiven van de *Colletotrichum*-balans in Kenia in de richting van *C. coffeanum* tengevolge van langdurig spuiten met fungiciden. In Zuid- en Midden Amerika, waar men sinds 1970 de koffieroest, *H. vastatrix*, bestrijdt met koperhoudende middelen, zou een dergelijke verschuiving in de *Colletotrichum*-populatie eveneens kunnen optreden. Recente berichten uit Brazilië wijzen er inderdaad op, dat *C. coffeanum* daar in het veld op koffiebessen schijnt te zijn aangetroffen.

Wat het toekomstige onderzoek betreft wordt aangedrongen op een internationale samenwerking tussen koffieproducerende landen op het terrein van de inventarisatie van bastbewonende *Colletotrichum* spp. om zodoende te kunnen vaststellen of *C. coffeanum* aanwezig is en dus potentieel een gevaar vormt. Daarnaast zal het toetsen van hoogwaardige cultivars op resistentie tegen de koffiebesziekte ook internationaal moeten worden voortgezet.

11. SUMMARY

Data are presented on research in Kenya in 1964 - 1969 on anatomical, mycological, epidemiological, chemical control and cultural aspects of coffee berry disease, *Colletotrichum coffeanum* Noack, of *Coffea arabica* L. The pathogen causes flower and berry losses and was found in branches where it occupied clearly defined areas of the cortex just before or after formation of the first phellogen. Saprophytic *Colletotrichum* spp. inhabit bark areas with more periderms in the cortex. No relationship could be found in Kenya between *Glomerella cingulata* (Stonem.) Sp. & Schr., the perfect stage of most of the saprophytic *Colletotrichum* bark components, and *C. coffeanum*. The seasonal fluctuations in pathogenicity in the bark population of *C. coffeanum* could be assessed and compared with the total sporulating capacity of the bark population of all *Colletotrichum* spp. Formerly the level of this total sporulating capacity, or 'inoculum potential' as it was then called, was used as an indication when pre-rain copper sprays had to be applied and how effectively the fungicide had reduced the bark inoculum. Based on these data the recommendations for chemical control were changed from pre-rain fungicide applications, to a spraying regime well into the rainy period, the accent being on protection of the berries rather than on a reduction of the bark inoculum. The fungicide Ortho Difolatan proved to be more effective than copper based compounds. Cultural practices, like the application of high levels of fertilizers, manure and mulch and rigid pruning practices, had no effect on the level of *C. coffeanum* in branches. Copper containing fungicides pushed the *Colletotrichum* balance in favour of *C. coffeanum*. Berries from non-copper sprayed coffee fields were less susceptible to standard conidial suspensions of *C. coffeanum* than berries from copper sprayed trees. A similar effect of fungicides should be considered in South and Central American coffee growing countries, where the application of fungicides has increased tremendously since the occurrence of *Hemileia vastatrix* Berk. et Br. in Brazil.
(13 tables; 12 figures; 5 reprinted papers; 223 references).

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Niets is volmaakt, niets is ten einde,
elke verstarring is bedrog.

Fragment uit: Apolloon tegenover XVII,
Herwig Hensen.

CURRICULUM VITAE

Hans Vermeulen werd op 14 maart 1932 te Bandung, Indonesië geboren. Het belangrijkste deel van zijn middelbare schoolopleiding vond plaats op het Baarns Lyceum, alwaar hij in 1952 het einddiploma H.B.S.-B behaalde. Daarna volgde de studie aan de Landbouwhogeschool te Wageningen, die hij op 7 april 1960 afsloot met het behalen van het ingenieursdiploma in de richting tuinbouwplantenteelt met als hoofdvak de tuinbouwplantenteelt en als bijvakken de fytopathologie, de virologie en de tropische plantensystematiek.

Van 1960 tot eind 1962 werd in Suriname een onderzoek verricht naar de verwekker van de zeefvatenziekte van liberia koffie, onder auspiciën van de Stichting Wetenschappelijk Onderzoek Suriname en de Nederlandse Antillen (WOSUNA). Na terugkeer in Nederland volgde begin 1963 een aanstelling bij de Plantenziektenkundige Dienst te Wageningen.

In augustus 1964 aanvaardde hij een betrekking als fytopatholoog aan het koffieproefstation te Ruiru, Kenia. In 1966 werd besloten het verblijf in Kenia te verlengen, in het raam van de bilaterale samenwerking tussen Kenia en Nederland. De detachering bij de Oostafrikaanse land- en bosbouwkundige onderzoekorganisatie (East African Agriculture and Forestry Research Organization) te Nairobi volgde in januari 1967 met als opdracht het in Ruiru begonnen onderzoek verder voort te zetten. Dit onderzoek werd in september 1969 afgesloten.

In 1969 werd hij opgenomen in de pool van landbouwdeskundigen van het Ministerie van Landbouw en Visserij in Nederland. Na terugkeer uit Kenia volgde in 1970 uitzending naar Indonesië, opnieuw in het raam van een bilateraal, agrarisch samenwerkingsproject. Gedurende ruim zeven jaren werkte hij aldaar als adviseur met als voornaamste opdracht een afdeling gewasbescherming op te bouwen binnen het Indonesische instituut voor tuinbouwkundig onderzoek.